ORAL PRESENTATION

S1
Abstract withdrawn
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S2
Conformational Changes in the HIV/SIV Envelope Glycoprotein
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The crystallographically determined structures of unliganded, fully glycosylated SIV gp120 core (Chen et al, 2005) and of HIV gp120 core with bound CD4 and Fab 17b (Kwong et al, 1998) together allow us to visualize the conformational changes that occur in gp120 upon binding of receptor and co-receptor.

S3
Coreceptor Dependent Signaling in Individual Primary Resting CD4+ T-cells Mediated by Low Levels of HIV Binding
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In order to enter into the target cell, HIV requires functional contact with CD4 and CCR5 or CXCR4. The last two are G-protein coupled receptors that when activated with chemokines, or HIV envelope can initiate a wide range of biological responses, including Ca²⁺ mobilization, cytoskeletal rearrangements and cell migration. To determine the specificity of X4-tropic gp120-mediated signaling through CXCR4, we have chosen a microscopy-based approach to observe the response at the level of individual cells providing greater sensitivity. Target cells were able to activate a signaling cascade in response to both monomeric recombinant gp120 and virion-bound trimeric gp120. Ca²⁺ elevation was a direct measurement of CXCR4 engagement because it was dependent on the tropism of the envelope, engagement of CD4, and sensitive to the CXCR4 antagonist AMD-3100. Signaling required much lower levels of envelope when virion associated. Imaging analysis allowed the correlation of the pattern of virion-mediated Ca²⁺ fluxing with the exact number of viral particles bound to cells. This analysis revealed that an average of four virions, and as few as two virions associating with a primary resting T cell could mediate Ca²⁺ mobilization. The ability of several virions to stimulate signaling in primary resting T cells is physiologically relevant and has important implications for AIDS pathogenesis. Funded in part by DHHS NO1-CO-12400 and RO1-AI052051.

S4
Time-Resolved Imaging of Single Retrovirus-Cell Fusion
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Membrane fusion induced by retroviral envelope glycoproteins (Env) has been studied by imaging early events during single virus entry. Viral particles were visualized by incorporating a membrane dye and by entrapping GFP within the virus interior. Lipid and content (GFP) transfer from viruses to cells was simultaneously monitored by confocal microscopy. Content mixing usually occurred after lipid transfer, suggesting that fusion proceeded through a hemifusion intermediate. Our data also revealed that small pores connecting the viral and cell membranes are the key intermediate of retrovirus fusion. These pores can persist for several minutes before they either irreversibly close, aborting virus entry, or fully enlarge, leading to nucleocapsid delivery and infection.

S5
HIV-1 Escape From Small Molecule CCR5 Inhibitors In Vitro
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We have generated HIV-1 isolates that are resistant to several small molecule CCR5 inhibitors by serial passage in vitro. We
have investigated the molecular basis of this resistance through genetic and phenotypic studies. We find that different genetic pathways leading to small molecule inhibitor resistance can be taken by the same starting virus. Additionally, these viruses can exhibit different levels of cross resistance to RANTES derivatives. These studies provide insight into potential mechanisms of CCR5 inhibitor resistance.

S6
Kinetic Factors Control Efficiencies of Cell Entry by HIV-1
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In order to understand HIV-1 inactivation by entry inhibitors and neutralizing antibodies, we quantitatively examined the factors that limit viral entry into cultured cells. Previous results suggested that only a minute proportion of HIV-1 virions (c.a., $10^{-3}$–$10^{-4}$) are infectious and that the remainder are defective. Experiments will be described showing that this is incorrect and that newly made HIV-1 virions are almost completely infectious. Following their attachment onto cells that contain CD4 and appropriate coreceptors, a race ensues between successful viral entry by membrane fusion and a competing process(es) of viral inactivation. Many entry inhibitors reduce virus titers kinetically, simply by slowing the pathway for membrane fusion and thereby enhancing the efficiency of spontaneous inactivation. In contrast, neutralizing monoclonal antibodies appear to cause a true viral inactivation by a non-kinetic mechanism. The membrane fusion pathway requires assembly of a reversible complex containing multiple coreceptors, which lowers the activation energy barrier for a slow rate-limiting conformational change in the gp41 envelope subunits. Evidence will be described showing how this slow step entry is affected by mutations in coreceptors and by compensating adaptive mutations in HIV-1 envelope glycoproteins. This kinetically determining step of entry is critically influenced by the gp120 V3 loop and by a gp120 N-linked oligosaccharide. In addition to its proposed role in association with coreceptors, our results suggest that the gp120 V3 loop has a major kinetic influence on the slow step in entry. We propose that this may explain why viral adaptations in cell cultures including resistances to entry inhibitors frequently involve changes in the V3 loop.

S7
HIV Entry and Its Inhibition: How Do Chemokines Fit In?
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HIV entry, HIV requires a chemokine coreceptor, either CCR5 or CXCR4, in addition to CD4 to enter target cells. A recently proposed model, based on apparent structural homology between the V3 region of the envelope glycoprotein and chemokine beta-hairpin loops, suggests that the V3 region may direct coreceptor choice through structural mimicry of the chemokines that bind to either coreceptor. Some of our recent research based on available chemokine structure-activity data has produced evidence that challenges this model.

HIV entry inhibition. The native ligands of HIV coreceptors prevent viral entry via a combination of steric blockade and removal of receptors from the cell surface (receptor sequestration). Our work on synthetic and semi-synthetic chemokine analogues has led to the discovery of potent HIV entry inhibitors such as PSC-RANTES, which owe their activity to a greatly enhanced capacity to sequester CCR5. PSC-RANTES has shown promise as a microbicide candidate, but as a totally synthetic protein it is likely to be too expensive for use in the developing world. We are now focused on discovering similarly potent molecules that are either partly or wholly accessible by biosynthesis and thus much cheaper to produce.

S8
Mechanisms of HIV Suppression by Various Microbes in Human Lymphoid Tissue
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Various pathogens enhance HIV replication and disease progression in coinfected individuals. However, we, and others, have provided examples of microbial interactions that suppress HIV-1 infection both in vivo and ex vivo. Here, we report on the mechanisms of interactions of HIV-1 with several such microbes in the context of ex vivo infected human lymphoid tissue. Blocks of human tonsils maintained ex vivo were coinfected with R5 or X4 HIV-1 and with another microbe, measles virus (MV), vaccinia, herpessivirus 6 (HHV-6), herpessivirus 7 (HHV-7), cytomegalovirus (CMV), or a parasite, Toxoplasma gondii (TG). In ex vivo tissues, all the above-listed microbes, except CMV, inhibited replication of R5 HIV-1 whereas replication of X4 HIV-1 was affected mildly. In spite of similarity of the effects, the mechanisms of R5 inhibition by coinfecting microbes are strikingly diverse. HHV-6 and MV upregulate CC-chemokines, in particular RANTES to the levels sufficient to inhibit replication of R5 HIV-1; Toxoplasma gondii seems to secrete its own soluble factor, cyclophilin, that also binds to CCR5 coreceptor, HHV-7 downregulates the expression of CD4 and T cells.

In conclusion, soluble factors encoded by microbes, including their components, or secreted by infected and bystander cells in response to microbial invasion (in particular cytokines/chemokines) constitute a universal network through which microbes interact with human body creating a microenvironment that is beneficial for them. However, what is beneficial for one microbe can be detrimental for another. Deciphering the molecular mechanisms by which pathogens alter tissue microenvironment so that it becomes detrimental to HIV, may significantly contribute to the development of efficient anti-HIV therapies.
S9
The Fitness Cost to Human Immunodeficiency Virus Type 1 of Escaping From a Small Molecule CCR5 Inhibitor
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Replicative fitness of an HIV-1 isolate generally tracks to the region of the genome that is under the most selective pressure. During HIV-1 infection, significant selection pressure is applied by the immune system causing sequence changes in the env gene. The clinical use of entry inhibitors will add to this pressure. Drug selection pressure can often force the virus to compromise its fitness in favor of acquiring resistance. This loss of fitness may lead to lower viral loads in vivo despite high levels of drug resistance. If the virus has already undergone compromises to survive in the face of the immune response, the additional changes in env may cause a significant reduction in replicative capacity. This presentation will focus on in vitro evaluation of the replicative fitness of HIV-1 isolates resistant to entry inhibitors and possible implications of such findings.

S10
The Immunomodulatory Agent Rapamycin Potentiates the Antiviral Activity of the Fusion Inhibitor T20 Against R5 Strains of HIV-1
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The fusion inhibitor T20 marks the beginning of a new era in the management of HIV-1 disease. By inhibiting viral entry, T20 suppresses viral replication in patients carrying strains resistant to reverse transcriptase or protease inhibitors. However, its antiviral activity is compromised by mutations in gp41. Based on our previous work demonstrating that Rapamycin (RAPA) inhibits R5 HIV-1 by downregulating CCR5 surface expression, we now show that RAPA and T20 synergize in antiviral activity against R5 strains. Synergy studies using the Median Effect Analysis revealed that the IC50 values of RAPA and T20 in the RAPA/T20 combination were reduced 9- and 3-fold, respectively. Three-Dimensional modeling confirmed the observed synergy (synergy volume of 253.85; 95% CL : 91–147). We also show that the RAPA/T20 combo, but not T20 alone, prevented the emergence of T20 resistance upon continuous passage of R5 HIV-1 ADA on PBMCs for 24 weeks under subinhibitory concentrations of T20. In addition, R5 ADA and YU-2 clones carrying T20 single mutations 36D, 38 M or 43 K (4–10 fold resistance) or the double mutation 36D/38 M (65-fold resistance), were all inhibited in the presence of RAPA. In conclusion, our results demonstrating that the RAPA/T20 combination has synergistic antiviral activity, prevents the emergence of T20 resistance, and inhibits T20 resistant strains, suggest a novel therapeutic approach to enhance the antiviral activity of T20 in patients carrying R5 strains of HIV-1.

S11
The Discovery and Exploratory Development of Maraviroc (UK-427,857): A Novel CCR5 Antagonist for the Treatment of HIV
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Maraviroc is a novel CCR5 antagonist and is the most advanced clinical candidate in Pfizer’s CCR5 discovery and development programme. It is exquisitely selective for the CCR5 receptor and demonstrates potent activity in vitro against both lab-adapted and primary clinical HIV isolates spanning all of the clades, including viruses that are resistant to current classes of HIV agents. Maraviroc has been evaluated in >400 volunteers and in 66 HIV patients where it is well tolerated at doses in excess of those required to block the CCR5 receptor and those providing free drug levels above the in vitro concentrations for potent antiviral activity. Consistent with this, Maraviroc has demonstrated encouraging short-term (10 day), single agent efficacy as measured by reductions in viral loads in asymptomatic HIV patients; doses of 300 mg QD and 300 mg BID resulted in mean maximum HIV RNA reductions of 1.60log10 and 1.84log10, respectively. Studies both with CYP 3A4 inhibitors and inducers have demonstrated that Maraviroc will have manageable drug interactions when used in the setting of HIV patients receiving HAART. In summary, Maraviroc has potency, pharmacokinetic and toleration profiles that merit its further evaluation as a new therapy for patients with HIV/AIDS.

S12
Vicriviroc Is a Novel, Potent CCR5 Inhibitor With Outstanding Pharmacodynamic, Pharmacokinetic and PharmaCodynamic (PK/PD) Properties
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The CCR5 chemokine receptor is a promising target for antiretroviral therapy because of its role as a coreceptor for HIV entry and propagation of infection. Vicriviroc, a small molecule CCR5 inhibitor being studied in clinical trials, has shown potent in vitro activity against HIV (IC50<13 nM). In addition, the pharmacokinetic and PK/PD properties of vicriviroc appear to distinguish it from other compounds in clinical development. Vicriviroc is highly water-soluble and demonstrates oral bioavailability of >89% in rats and monkeys. The compound is modestly human plasma protein-bound (=84%) and widely distributed in the extra vascular space. Absorption and exposure in humans are linear and dose-proportional, with a terminal phase half-life >24 hours supportive of once daily...
dosing. Variability in absorption is modest (20–40%). Exposure to vicriviroc, a substrate for CYP3A4, is “boosted” by CYP3A4 inhibition with ritonavir (RTV), without being affected by other metabolizing enzymes or pGp. Because of the high oral bioavailability and highly predictable exposure, particularly when boosted with as little as 100 mg RTV, potent HIV suppression of 1.5 log10 is anticipated with as little as 10 mg vicriviroc daily with RTV. Dosage adjustments are not expected in combination regimens.

Vicriviroc shows particular promise as an HIV therapeutic due to pharmacologic, PK/PD properties that support its potent activity and convenient once daily dosing.

**S13**
Update on Aplaviroc: An HIV Entry Inhibitor Targeting CCR5
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Aplaviroc (873140) is a novel spirodiketopiperazine CCR5 antagonist that binds specifically to human CCR5 and allosterically inhibits HIV entry. Aplaviroc has exhibited potent in vivo antiviral activity (1.66 log decrease in viral load at nadir) following 10 days of monotherapy. In vitro studies of antiviral activity demonstrate that aplaviroc is active against HIV isolates from a variety of clades as well as those resistant to current HIV therapies targeting RT, PR, and gp41. In vitro studies suggest prolonged CCR5 receptor occupancy (RO) by aplaviroc with an offset half-life of >100 hours. In vivo studies following short term aplaviroc administration using CCR5-specific mAb demonstrate substantial and prolonged CCR5 RO (>50%) by aplaviroc, when plasma drug levels were undetectable, observed for approximately 5 days.

In the 10 day monotherapy study of aplaviroc in HIV+ subjects, one subject whose virus was R5-tropic at baseline and day 5 showed that R5X4-tropic (dual/mixed) virus was present at Day 10. Subsequent analysis revealed reversion to an R5-tropic only phenotype by day 24, with no decrease in sensitivity to aplaviroc (Fold IC50). The change in tropism at the population level observed on day 10 was the result of the emergence of pre-existing dual-tropic virus(-es) that were below the limits of detection on day 1. Viruses present in a subject’s quasispecies that are below the limits of detection with currently available tropism assays may become detectable following monotherapy with a CCR5 antagonist; however, whether similar changes may occur on combination therapy with a CCR5 antagonist remains to be determined.

Aplaviroc has demonstrated potent anti-HIV activity in vitro and in vivo. Furthermore, aplaviroc exhibits a unique allosteric interaction with CCR5 and demonstrates prolonged receptor occupancy. CCR5 antagonists show promise for inhibiting entry of CCR5-using viruses in the clinical setting.

**S14**
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**S15**
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**S16**
GATA3 and STAT5 – Critical Inducers of the Th2 Fate
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GATA3 has been implicated as a key factor in commitment of CD4 T cells to the Th2 phenotype. Naïve CD4 T cells cultured without exogenous cytokines induce GATA3 and IL-4 transcription only when stimulated with low concentrations of cognate peptide in the presence of IL-2. Naïve CD4 T cells from mice with a conditional deletion of Gata3 fail to induce IL-4 in response to TCR engagement as do cells cultured in the presence of anti-IL-2. High concentration inhibition of GATA3 and IL-4 expression and of Stat5 signaling is rescued by MEK inhibitors implying that inhibition is mediated through erk action. Infection of cells cultured under Thnull conditions with retroviruses containing GATA3 and constitutively active Stat5a induce a Th2 phenotype and result in full accessibility of the Il4 gene. Further, ChIP analyses reveals that in Th2 cells, Stat5a is bound to DNase I hypersensitive sites in the second exon of the Il4 gene. Thus, both GATA3 and STAT5 are key factors in “opening” the Il4 gene and in inducing the Th2 phenotype. Furthermore, GATA3 proved to be important in Th2 growth. In vivo deletion of Gata3 using OX40-Cre eliminated Th2 responses and allowed the development of IFN-g-producing cells in mice infected with *Nippostrongylus brasiliensis*. Thus, GATA3 serves three functions in Th2 biology: it induces the Th2 fate, represses the Th1 fate and it promotes selective outgrowth of Th2 cells.

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**S19**
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**S20**
HIV Entry Inhibitors: Entering the Treatment Paradigm
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There are 21 antiretroviral drugs approved for the treatment of HIV infection. Current drugs fall into 3 mechanistic classes: the 2 traditional classes – HIV reverse transcriptase inhibitors and...
HIV protease inhibitors and the newest class – HIV entry inhibitors. Current antiretroviral regimens are effective in suppressing viral replication, enhancing immune function, and preventing clinical progression of HIV disease. However, current antiretroviral drugs may be compromised by suboptimal antiretroviral activity; drug resistance and cross-resistance; complexity; acute, chronic and life-threatening toxicities; and drug-drug interactions. Newer antiretroviral agents, such as the HIV entry inhibitors, are needed to improve antiretroviral activity (particularly against drug-resistant strains), avoid the selection of drug resistance, improve convenience, improve tolerability and reduce toxicity, and minimize drug-drug interactions. With demonstrated safety and effectiveness against drug-resistant viruses, HIV entry inhibitors quickly may become part of treatment regimens for treatment-experienced patients. With additional safety, tolerability, convenience, and antiretroviral activity data, HIV entry inhibitors could become part of standard treatment regimens for treatment-naïve patients.

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S22
Visualizing Induction of Lytic Gammaherpesvirus Infection
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EBV and KSHV both are associated with malignancies. In tumors the latent form of infection predominates and there is little or no expression of lytic genes. FIAU is a nucleoside analogue that is selectively phosphorylated by EBV and KSHV thymidine kinases. We have used a variety of pharmacologic agents to induce lytic infection in xenografts of Burkitt’s lymphoma and primary effusion lymphoma cell lines in SCID mice. Using 125I labeled FIAU, we have been able to visualize lytic gene reactivation.

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S25
HERV-K113: The Newest and Most Lively Member of the Human Endogenous Retroviruses
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Although retroviruses are usually spread horizontally by infecting new somatic cells, entry into cells of the germ line may result in endogenization and hence vertical transmission from generation to generation. Once endogenized, the incorporated proviruses are likely to undergo intra-genomic amplification resulting in high copy numbers. Endogenous Retroviruses can cause significant harm by disrupting or disregulating essential genes. Of considerable interest and a topic of intense investigation is the possible role of endogenous retroviruses in the etiology of malignancies, autoimmune and neurologic diseases. In recent years, striking evidence has accumulated indicating that some proviral sequences and HERV proteins might even serve the needs of the host. In contrast to several other mammals, human endogenous retroviruses (HERVs) are believed to have lost the ability to replicate but HERV-K, the youngest and most conserved family, is able to generate virus-like particles. Following integration almost all known HERVs have suffered extensive deletions and mutations. One exception is HERV-K113 located on chromosome 19p13.11. This young provirus is not yet fixed in the human population and shows an ethnicity dependent allelic prevalence of about 5–30%. A HERV-K113 provirus with preserved open reading frames has been successfully cloned and its transcripts, protein expression and replication potential has been studied.

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S28
Rational Design of AIDS Vaccines
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Development of an effective vaccine against HIV has posed significant challenges to the scientific community. Lack of knowledge about the molecular pathogenesis of this disease, and the absence of naturally occurring, protective immune responses, which is attributed in large part to the diversity of the viral envelope and multiple escape mechanisms, have made it difficult to design successful candidates. Recent studies of the envelope have suggested that it is possible to enhance immunogenicity of the envelope by improving the breadth of the neutralizing antibody response and by stimulating cell-mediated immunity. This relies on an understanding of the structure of HIV Env and the use of site-specific mutation to create novel immunogens. Based on structural and functional data, our lab has developed DNA and adenoviral vectors which express envelope proteins containing alterations to regions in the variable loop to better control tropism and improve immunogenicity. These modified HIV genetic vaccine candidates have been tested in combination with Gag, Pol, and Nef immunogens, and shown to improve the potency of cellular and humoral immune responses in primates. An initial Phase I trial (VRC 004) dose escalation study to test a DNA vaccine composed of a 4-plasmid combination of clade B
gag/pol/nef with clades A, B, and C envelope was recently completed in 50 healthy adults in the U.S. CD4+ and CD8+ T cell responses were detected in the majority of vaccinees using IFN-γ (ELISPOT) and flow cytometric detection of intracellular IL-2 or IFN-γ. HIV-specific antibody response was detected in about one third of vaccinees. Combination modality regimens using a DNA vaccine prime followed by a viral vector boost have shown promise in non-human primate models of HIV infection. Phase I clinical trials to test the safety and immunogenicity of an adenoviral vaccine expressing proteins similar to the DNA, and a DNA prime, adenoviral boost regimen are in progress. The initial adenoviral vector vaccine uses an Ad5 serotype vector. However, pre-existing immunity can mitigate the efficacy, so alternate serotypes and modifications to the adenoviral fiber regions are being explored.

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S30
AIDS Vaccine Research and Development: Past, Present and Future
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Today, nearly twenty-five years since the first AIDS cases were identified, the AIDS pandemic is recognized as a global public health priority. With 14,000 new HIV infections every day, the best hope for stemming the insidious spread of HIV and for ending the pandemic remains the development of a safe and effective AIDS vaccine. The search for an AIDS vaccine can be viewed from past, present, and future perspectives. Since the identification in 1983 of HIV as the etiologic cause of AIDS, the field has gained significant knowledge on the pathogenesis of HIV relevant for vaccine development, several vaccine approaches have been designed and tested in clinical trials, and infrastructure has been established both in developed and developing countries to assess HIV incidence, molecular epidemiology, host immune response to early infection, and to conduct Phase I, II and III trials. Yet despite current global investment of nearly $650 million per year, the HIV vaccine pipeline remains inadequate. Vaccine candidates designed by empiric approaches and tested thus far in human efficacy trials have failed to prevent HIV infection or suppress HIV viral load. Current candidates approaching human efficacy trials have shown some benefit in certain monkey models but not in others. There is considerable potential that these current candidates will achieve no more than limited success if any, since they have provided little or no protection from pathogenic SIV challenge in monkeys, are markedly impeded in their capacity to elicit cell mediated immune responses in humans due to anti-vector immunity, and have not been designed to elicit effective neutralizing antibodies. In order to significantly accelerate global efforts in AIDS vaccine development and shorten the timetable for a licensed and widely accessible AIDS vaccine, the following issues should be addressed. First of all, key scientific problems, known to the field for more than a decade, need focused and direct efforts to inform rational vaccine design. These problems include: the lack of understanding of how best to design vaccines to elicit broadly neutralizing antibodies to HIV; the lack of safe and suitable candidates for clinical development which mimic the protective efficacy thus far only achieved by live attenuated SIV vaccines; and the lack of candidates in the pipeline which adequately address the hypervariability of HIV. Secondly, an “industrial model” for applied research and product development needs to be established to encourage innovative product development and accelerated clinical testing of AIDS vaccine candidates without compromising safety.

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S38
HIV Vaccine Research Agenda: Perspectives of an HVTN Site Investigator
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The HIV Vaccine Trials Network has as its central mission, the implementation of clinical trials of candidate vaccine products
(immunogens, vector, delivery, adjuvants) that induce immunologic responses relevant to preventing HIV infection and/or controlling HIV disease progression. The research agenda targets evaluation of candidates at all stages of product development from phase I first in human trials to proof of concept and efficacy trials. Innovative trial designs are driven by investigators and statisticians to efficiently advance products and information that seeks fundamental insight into correlates of protective immunity. HVITN laboratories quantitate adaptive and innate immune responses employing highly reproducible and standardized endpoint assays that meet GLP validation standards for core assessment as well as exploratory assays to understand correlate mechanisms. Currently 24 HIV vaccine candidates are slated for evaluation over the next 24 months including proof of concept and expanded phase 2 evaluation in preparation for larger efficacy trials in the 2007–2008 time period.

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S40
Kaposi Sarcoma Herpesvirus: Update
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The incidence of HIV-related cancer before and during the ART era indicates that oncogenic viruses continue to contribute to the majority of these cancers and they are therefore considered opportunistic malignancies. ART has lead to a definitive decline in the incidence of certain AIDS-defining cancers including Kaposi sarcoma (KS) and non-Hodgkin’s lymphoma. Before ART, non-AIDS-related malignancies accounted for 1% of all causes of death in this population, this has now raised to over 25% because of the sharp decline of competitive risks. The relative frequent coinfection in this population with the oncogenic viruses Hepatitis B or C, the aging of HIV-infected population and the possible direct contribution to oncogenesis by HIV-1 ART. Due to the HIV-pandemic, KS is now one of the most common tumors overall in sub-Saharan Africa. Kaposi sarcoma-associated herpesvirus (KSHV or HHV8) is essential in the etiopathogenesis of KS. The global seroprevalence of KSHV largely reflects the incidence of KS. In the vast majority of infected individuals KSHV persists without harm to its host. When the balance between pathogen and host immunity is disturbed, KSHV reactivation and outgrowth of KSHV infected cells occur. The transcriptome of KS tumor cells is closest to that of lymphatic endothelial cells (LEC). We determined the global effect of KSHV infection of LEC on genes involved in immunity. We compiled a group of 834 genes, classified into six functional clusters, including antigen presentation, inflammation (cytokines and chemokines), apoptosis, interferon response, cell adhesion, and cell signaling. Over 30% of genes were significantly deregulated after infection of LEC (q < 0.005). The inflammation, antigen presentation, and interferon response profiles of infected LEC correlated to that seen in KS. We determined the expression levels of surface proteins involved in antigen presentation by FACS, and cytokines by protein arrays. Infection led to the downregulation of key proteins involved in antigen presentation. We also observed an expression pattern of chemokines associated with T cell, monocyte, and dendritic cell migration and conducted chemotaxis assays to assess the functional relevance of these data. Overall, we characterise the immune profile of LEC and show its profound regulation by KSHV, resulting in multiple immune evasion pathways.

S41
Kaposi’s Sarcoma and HIV-Tat: Challenges to Antiangiogenesis Research
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Kaposi’s sarcoma (KS) is characterized by an abnormal growth of blood vessels. KS was found mainly in older men of Mediterranean or African origin (classic KS) or in patients after organ transplantation (iatrogenic KS). However, in the early 1980s, an aggressive epidemic form, linked to AIDS, was noticed and was one of the first clues to the existence of HIV-1 pandemy. The link between KS occurrence and HIV has raised multiple hypotheses. The drastic reduction of KS after the introduction of HAART, suggests HIV as a powerful co-factor for KS progression. We and others have contributed to the elucidation of KS cell nature and the possible involvement of extracellular HIV Tat. Tat is proangiogenic and is a true promoter of KS lesions acting as a VEGFR2 ligand both on KS and endothelial cells, in addition Tat is able to bind and activate chemokine receptors on monocytes and granulocytes causing a proinflammatory status. Evaluation of the effects of extracellular Tat on KS cells by microarray analysis after 24 h of incubation shows an interesting clustering of gene products involved in signal transduction, especially GTP-ase, Kinase and cAMP activity, confirming that Tat acts extracellularly by ways that are probably unrelated to its nuclear activity. KS occurrence is reduced by HAART but still present and in Africa is one of the most frequent oncologic disease. To find suitable drugs with low toxic impact on KS patients, we have tested several drugs and gene therapy approaches in in vivo models.

S42
Roles of EBV in Haemopoetic Malignancies
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In addition to the B-cell derived Burkitt lymphoma and the immunoblastomas of immunodefective patients, most of the rare nasal NK lymphomas and about half of the Hodgkin’s lymphomas (HL) carry EBV. In the 2 latter malignancies, the neoplastic cells are embedded in granulation tissue (with evidence for the contribution of the microenvironment to the maintenance of the disease). Their viral expression pattern is similar, EBNA-1 and LMP-1 pos and EBNA-2 neg (type II). In contrast to the EBV-driven immunoblastomas, EBV cannot be held directly responsible for the growth of these 2 malignancies that also differ among themselves.
**Hodgkin’s lymphomas:** The few existing HL derived cell lines are EBV negative. When from one line a forcibly converted EBV positive subline was established, it expressed EBNA-1 only (Type I). LMP-1 could be induced by exposure to CD40L and IL-4. Conceivably, the in vivo phenotype (Type II) is imposed by the cytokines that abound in the granulation tissue. According to one present view EBV is important in the early stage of HL development, in that it rescues B lymphocytes with non functional Ig-rearrangements from apoptosis.

**Nasal NK/T lymphomas:** Normal NK/T cells do not carry EBV receptors. The mechanism of infection of the NK/T cells is not known. EBV carrying cell lines exist and they express the Type II pattern, corresponding thus to the in vivo phenotype. They require IL-2 for in vitro proliferation. Conceivably IL-2 can be provided by activated T cells in vivo. Treatment with cytokines (IL-10, IFN-γ) upregulates LMP-1 expression that leads to more efficient growth response to IL-2.

**S43 HHV8 Regulates Vascular and Lymphatic Endothelial Cell Specific Genes Producing Distinct/Mixed Profile in Kaposi’s Sarcoma**

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Kaposi’s sarcoma represents a vascular proliferative process. Characterization of genes expressed in vascular endothelial cells in particular arterial and venous endothelial cells when examined in KS indicate that KS displays profile that resembles arterial endothelial cells. These include some Notch receptors and their ligands including Delta like 4 (dll4), neuropilin-1, ephrinB2, connexin 37, but not venous specific marker (ephB4). Furthermore HHV8 infection, and viral proteins vGPCR, leads to the induction of several proteins that preferentially induce genes expressed in artery endothelial cells (ephrinB2) but not venous specific (EphB4). KS gene expression profile also shows expression of lymphatic endothelial cell specific markers as well. Thus the phenotype of KS is distinct from the profile in resting or anigenic response in physiological responses as in wound healing or tumor vasculature. Distinct phenotypic characteristics provide opportunities for novel targeting and therapeutics. For example inhibition of EphrinB2 expression with siRNA leads to detachment of the cells from matrix, and loss of cell viability represents a prototype. Growing understanding of KS biology is thus likely to offer novel targets for therapy.

**S44 Ritonavir Inhibits NF-AT Activation Through Effects on the PI-3 Kinase/Akt Pathway**

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The HIV protease inhibitor ritonavir has been reported to have activities unrelated to inhibition of HIV protease, including anti-tumor activity in vivo and in vitro, induction of lipodystrophy in vivo, inhibition of the 20S proteasome, and inhibition of NFκB activation. Here we show that ritonavir also inhibits activation of NF-AT by PMA plus ionomycin and by the HHV-8 vGPCR. Inhibition of NF-AT activation occurs through the PI-3 kinase/Akt/GSK-3 pathway, since ritonavir treatment leads to decreased Akt phosphorylation and a resultant decrease in GSK-3 phosphorylation. Treatment with ritonavir also inhibits the expression of NF-AT-dependent pro-inflammatory factors. Inhibition of multiple signaling pathways may help to explain the anti-tumor and other effects of ritonavir that are unrelated to its anti-retroviral activity.

**S45 Targeted Therapy For AIDS-related Kaposi’s Sarcoma**

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Angiogenesis plays a critical in the pathogenesis of Kaposi’s sarcoma. Platelet derived growth factor (PDGF), vascular endothelial growth factor, fibroblast growth factor and matrix metalloproteinases (MMP) are among the angiogenic pathways that have been implicated in the development of Kaposi’s sarcoma. The introduction of specific therapies such as imatinib and Col-3 that target the PDGF pathway and MMPs, respectively, provide a mechanism to test the importance of these pathways in Kaposi’s sarcoma in vivo. In this session the rationale for targeted therapy in Kaposi’s sarcoma will be discussed. The results from recent trials involving anti-angiogenic agents and signal transduction inhibitors will be reviewed. Additionally, trials of targeted therapies that are underway or in development will be outlined.

**S46 Virus (KSHV/HHV8) Infection and Genomic Aberrations in Developing AIDS Kaposi’s Sarcoma (KS)**

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Background: AKS is the most frequent AIDS tumor and like endemic (EKS) always associated with HHV8/KSHV but it is still controversial whether KS represents a monoclonal cell proliferation with distinct recurrent genomic changes or a polyclonal, hyperplastic, reactive process.

Material and Methods: Biopsies of AKS and EKS were compared by triple immunoflourescence for possible stage related phenotypic differences in HHV8 (LANA) infected tumor spindle cells (CD34+SC) and proliferating (Ki67+) cells. Histological tumor areas were also laser microdissected and the DNA analyzed by CGH and interphase FISH for cytogenetic changes in early and late stages of tumor development.
Results: LANA and CD34 tumor spindle cells (SC) varied concordantly with stage of AKS and EKS. However among CD34+SC approximately 30–40% were LANA negative, but only a minor population of LANA cells were CD34+ (3–4%) in all KS forms and stages. Cell proliferation (Ki67+) was relative constant (4.5–11.5%) at all KS stages but usually more frequent in early AKS and EKS. Most Ki67+ cells were LANA+/CD34+ but a few were LANA+/CD34-. CGH analysis of KS tumors (n = 27) showed mostly apparent random but no recurrent chromosomal aberrations. Fewer chromosomal aberrations were observed in AKS compared to EKS.

Conclusion: Apparently there is a heterogeneity among SC for HHV8 infection or a continuous recruitment of non-infected precursor SC to the lesions. The LANA+ SC appeared to have a proliferative advantage compared to LANA- SC. Comparison of random chromosomal aberrations in AKS and EKS appears to indicate that genomic instability could be a more important factor for the development of EKS than for AKS. Most likely AKS development is also promoted by various cytokines and growth factors produced during HIV infection and by the compromised state of host immune response in HIV infection.

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S47
Studies of Human Herpes Virus-8 in Thailand
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Thailand has experienced a major epidemic of HIV/AIDS since 1988. Currently over 650,000 persons are HIV-infected and 400,000 have died of AIDS in Thailand. However, Kapo’s Sarcoma (KS) is very rare. Among the 101,945 adults AIDS cases reported between 1994 and 1998 only 0.2% had KS. We have ruled out the possibility that HHV-8 infections are rare; in a study of 992 persons at risk or positive for HIV we found an HHV-8 antibody prevalence of 24.2%. Another hypothesis to explain the rarity of KS is that endothelial cells in Thai’s are relatively resistant to HHV-8 infection. We obtained umbilical cord endothelial cell cultures from 10 Thai women who were HIV negative and analyzed their DNA for novel single nucleotide polymorphisms (SNPs) in the coding region for the promotor and 3’ UTR region of the DC-SIGN gene. These results were compared to other Asian (11), Caucasian (n = 120), and African (n = 206) samples. No novel SNPs were found in the Thai samples, however some haplotypes that differed from the Caucasian samples were found. Three Thai samples were homozygous for a complete absence of SNPs in the UTR and reduced diversity in the promoter. This was not seen in the Caucasian samples. Additional analysis of linkage disequilibrium and HHV-8 infectivity analysis of these cell cultures are in progress. It is possible that genetic differences in the endothelial cell receptors for HHV-8 or resistance of cells to HHV-8 among Thai’s could partially explain the rarity of AIDS-related KS among Asians.

S48
Human Papillomaviruses and Cervical Cancer
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Infections with high-risk human papillomaviruses (HPVs) are associated with the vast majority of cervical carcinoma. A fraction of other anogenital tract malignancies such as penile cancer in males and vulvovaginal cancers in females as well as anal carcinomas in immunosuppressed patients and some oropharyngeal carcinomas are also associated with high-risk HPV infections. These cancers generally arise as a consequence of a molecular accident whereby a small genomic high-risk HPV fragment is irreversibly integrated into a host cell chromosome resulting in dysregulated expression of the HPV E6 and E7 oncoproteins. Expression of HPV E6/E7 in epithelial cells recapitulates key steps of cervical neoplasia and cancer, which allowed for the creation of tissue culture and animal models of cervical cancer. High-risk HPV E6 and E7 proteins target important cellular growth regulatory circuits among them the p53 and retinoblastoma tumor suppressors, respectively. In addition, E6/E7 expression is a driving force for malignant progression through induction of genomic instability. Hence, high-risk HPVs are the first-ever identified, necessary and molecularly defined causative agents of a major human cancer.

S49
Epstein Barr Virus: Potential Immune Selection in Associated Cancers
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Retrovirology 2005, 2(Suppl 1):S49

The EBV latent membrane protein 1 (LMP1) is expressed in most of the EBV-associated malignancies including nasopharyngeal carcinoma (NPC), Hodgkin’s Lymphoma, and immunosuppression-associated lymphomas. have been identified by distinguishing amino acids. We have identified seven sequence variants of LMP1 that can be distinguished using a heteroduplex tracking assay and have determined that most healthy individuals are infected with multiple strains of EBV. Striking differences were found between NPC and matching blood samples with one specific variant, China 1, prevalent in NPC samples and multiple other variants of LMP1 prevalent in the blood samples. The possible selection against some strains appearing in the tumor was highly significant with a p < 0.0001. Many of the LMP1 variants had changes in predicted HLA epitopes of various restrictions. The potential negative selection of the immune system on strains detected in the blood would be reflected in the striking predominance of the China 1 strain in the tumors. In lymphoma samples, changes were also frequently detected in known HLA-restricted epitopes of EBNA3A, 3B, or 3C, in addition to LMP1. Variation in potential immune recognition could contribute to the development of EBV-associated diseases in distinct populations and individuals.
S50
The Mechanism of Epstein-Barr Virus Persistence in Vivo and its Relationship to the Origins of EBV Associated Lymphoma
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Epstein-Barr virus persists latently within resting memory B lymphocytes. To gain access to this compartment the virus first infects and activates a naive B cell and then drives it to differentiate into a resting memory B cell. To achieve this the virus uses four different viral latent gene transcription programs which are also expressed in EBV associated lymphomas e.g. the growth program in immunoblastic lymphoma, the default program in Hodgkin’s disease and the EBNA1 only program in Burkitt’s lymphoma. This suggests:
1. that the EBV associated lymphomas arise when the normal progress of a latently infected, activated, naive B cell to a resting, memory B cells is blocked.
2. the viral transcription program used by the tumor reflects the normal cellular counterpart that it is derived from.

The mechanism of EBV persistence will be described and the origins of the EBV lymphomas, predicted from this model, will be discussed.

S51
Tumor-Endothelium Interactions – Novel Pathways
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A significant manifestation of tumor-endothelium interactions is the release of the proinflammatory cytokines IL-1/β or TNFα from tumor cells. These cytokines induce the expression of E-selectin molecules on endothelial cells (EC). The expression of E-selectin on EC facilitates contact with selectin ligand-expressing cancer cells thus promoting their transendothelial migration. We reported previously that factors released from cultured head and neck squamous carcinoma cells into the culture medium, induced the release of monocyte chemoattractants from EC. In view of the potential significance of this finding to tumor progression, we asked whether colorectal cancer (CRC) cells also secrete factors capable of inducing up regulating the expression of chemokines in, and their release from EC.

A cDNA-microarray analysis of EC treated with culture supernatants of CRC cells revealed that the expression of several CXC chemokines, including CXCL-1 and CXCL-8, was up regulated in EC exposed to the tumor-derived factors. These results were confirmed by RT-PCR. The treated EC secreted higher amounts of CXCL-1 and CXCL-8 than untreated, control EC. These chemokines are involved in tumor progression. Several lines of evidence suggest that E-selectin is involved in the delivery of the CRC-derived chemokine-secretion-enhancing signals to the EC. Experiments to characterize the CRC-derived molecules that mediate these signals are under way.

S52
A New Inducible RNAi Model for Cancer Target Validation In Vivo
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Background: Human xenograft tumor models are widely used for evaluation of potential cancer targets, by assessing the anti-tumor effects of specific agents, such as siRNA. siRNA is usually stably introduced into tumor cells prior to transplantation. However, oncogene silencing results in reduced cell growth/survival in vitro and/or failure to establish tumors in vivo, thus hindering tumor response-based efficacy evaluation that is more clinically relevant. We therefore explored a new tumor response model based on regulated RNAi.

Methods: A unique RNAi vector was generated to express shRNA only after induction with doxycycline. Using this vector, we created a novel xenograft tumor model, in which tumors are established under non-induced conditions, followed by induced target inactivation upon oral dosing of the inducer. Three genes were evaluated, a known oncogene (mTOR), and two novel cancer targets (HE7 and HE26), by assessing the tumor response to their silencing.

Results: We demonstrate a significant response of staged tumor regression to silencing of all three target genes. For early staged tumors, inactivation of each of the three targets caused dramatic tumor regression (100% regressed and 50% became tumor-free for both mTOR and HE7, and 100% for HE26). Advanced staged tumors also demonstrated significant responses (100% regression for mTOR, and 75% for HE7, 85% for HE26).

Conclusion: Our results demonstrate the utility of this unique and powerful model for efficacy evaluation of cancer targets: our data also provide robust in vivo efficacy validations of HE7 and HE26 as novel cancer therapeutic targets.

S53
Integration of HIV-1 Caused STAT3-Associated B Cell Lymphoma in an AIDS Patient
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B cell lymphomas remain a significant cause of morbidity in AIDS but the pathophysiology of this disease is unclear. We report a case of B cell lymphoma in which HIV-1 integrated into the host genome. The lymphoma cells with anaplastic large cell morphology formed multiple nodular lesions in the lung of a homosexual AIDS patient. The lymphoma cells did not express KSHV-LANA and EBV-EBER or multiple nodular lesions in the lung of a homosexual AIDS patient. The provirus had a 5’LTR deletion and the 3’LTR was inserted just before the first coding exon of STAT3. Reporter gene assay demonstrated that the 3’LTR had a strong promoter activity especially when co-transfected with HIV Tat. These data suggest
HIV-I integration resulted in induction of STAT3 and possibly promoted lymphoma formation. This suggests that HIV-I insertional mutagenesis may be associated with some cases of AIDS lymphoma.

**S54 Genetic Analysis of HIV in AIDS Malignancies**

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Many HIV associated diseases such as KS have resolved or dramatically decreased since the institution of HAART. Two diseases, HIV associated dementia (HAD) and AIDS related lymphoma (ARL) continue to occur and represent two diseases where HIV infected macrophages have been implicated in disease pathogenesis. In order to test whether persistent macrophage reservoirs of HIV might in part be responsible for subsets of these diseases, a survey of tissues obtained from the AIDS and Cancer Specimen Resource (ACSR) was performed. HIV copy and HIV genetic diversity studies were carried out on DNA extracted from HAD brain, ARL and KS specimens. In this pilot study, all HAD involved sections of brain, 10/14 ARL’s and 1/11 KS tissues contained >1 copy of HIV/2000 genomic equivalents. Phylogenetic analysis of the HIV in the HAD and ARL cases demonstrated the presence of dominant/monophyletic forms of compartmentalized HIV is diseased tissues. By comparison only diverse forms of HIV were observed within unininvolved tissues from the same (HAD or ARL) patient, or within KS tissues. Further genetic analysis of HIV from one patient with both HAD and primary CNS ARL, revealed distinctly different LTR’s associated with the ARL as compared to the HAD. The ARL LTR was missing an NFk-B site whereas the HAD LTR carried both sites consistent with B-clade forms of HIV. These data suggest that a persistent macrophage based reservoir of HIV may contribute to ARL.

**S55 The Molecular and Cellular Basis of Tumor Rejection After Vaccination With Mammary Adenocarcinoma Cells Transduced With the MHC Class II Transactivator CIITA**

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CD8+ T cell responses are major players of tumor eradication in various vaccination protocols. However, an optimal stimulation of CD4+ T helper cells is required for both priming and maintenance of the effector CTL response against the tumor. In this study we show that the murine mammary adenocarcinoma cell line TS/A, a highly malignant MHC-II-negative tumor, is rejected in vivo if genetically engineered to express MHC-II molecules by transfer of the MHC-II transactivator CIITA. TS/A-CIITA cells are fully rejected by 93% of the syngeneic recipients and have a significantly lower growth rate in the remaining 7% of animals. Rejection requires CD4+ and CD8+ cells. CD4+ T cells are fundamental in the priming phase, whereas CTLs are the major anti-tumor effectors. All tumor rejecting animals are protected against rechallenge with the parental TS/A tumor. Immunohistochemical data at day 5 post-inoculation showed an higher infiltrate of CD4+ T cells in mice bearing TS/A-CIITA, than in mice bearing the TS/A tumor. Subsequently, from day 7 through day 10, TS/A-CIITA tumors showed higher number of both CD4+ and CD8+ cells, dendritic cells, together with massive necrosis. The frequency of IFN-γ-secreting splenocytes early after inoculations was also assessed by an ex vivo ELISPOT assay. Only the rejecting TS/A-CIITA animals showed an high frequency of IFN-γ-secreting cells (between 80 and 120/10^6 splenocytes). Importantly, CD4 and CD8 depletion experiments revealed that at the time of tumor resolution the major cell population recognizing the TS/A-CIITA cells was of CD4 origin. This is the first example of successful tumor vaccination by genetic transfer of CIITA. These results open the way to a possible use of CIITA for increasing both the inducing and the effector phase of the anti-tumor response.
development of novel behavioral and biological intervention strategies. Study of HIV transmission pairs should facilitate for vaccine development.

S58
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S58

S59
Development of pDNA and Recombinant VSV Vectored Vaccines for Immunization Against HIV
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Retrovirology 2005, 2(Suppl 1):S59

Vaccines based on plasmid DNA (pDNA) or recombinant vesicular stomatitis virus (rVSV) vectors elicit HIV-1-specific cellular and humoral immune responses in small animal models. However, hurdles remain with each of these vaccine modalities before they can be considered for widespread clinical use. For pDNA vaccines, early clinical studies indicate that additional measures are needed to improve immunogenicity. For rVSV-based vaccines, issues related to the potential neurovirulence of the prototype vector represent a safety concern. Adjuvant development and pDNA vector optimization are two important elements of current DNA vaccine research. Cytokine expression plasmids may function as potent adjuvants, and in fact, we have observed that plasmid-encoded IL-12 can substantially enhance immune responses in non-human primates. In addition, it is hypothesized that broad immune responses against multiple HIV antigens will be required to protect against infection and/or disease progression. Results from studies will be presented in which several pDNA vaccine designs were tested for their ability to elicit broadly reactive immune responses to multiple HIV antigens. To address safety concerns related to use of rVSV vectors in humans, a range of further attenuated vector candidates has been developed and screened for neurovirulence in small animal models and nonhuman primates. Attenuated vectors have been identified that cause minimal CNS lesions after intracranial inoculation similar to those seen in control animals. Importantly, a number of these further attenuated vectors retained levels of immunogenicity in mice equivalent to prototype rVSV vectors, and have been advanced into nonhuman primate immunogenicity studies.

S60
Novel Adenovirus Vector-Based Vaccines for HIV
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To overcome the problem of pre-existing anti-Ad5 immunity, rAd vectors are being developed from rare Ad serotypes such as Ad35. However, studies have suggested that rAd35 vectors are less immunogenic than rAd5 vectors. We therefore constructed novel chimeric rAd35 vector incorporating the Ad5 fiber knob (rAd35k5). Both rAd35 and rAd35k5 vectors proved immunogenic in mice with and without anti-Ad5 immunity. In rhesus monkeys, rAd5 vectors elicited potent Gag/Env-specific immune responses, but a homologous boost immunization failed to enhance these responses as a result of high Ad5-specific NAbs. The rAd35 vectors elicited lower antigen-specific immune responses as compared with rAd5 vectors, but these responses increased substantially following a homologous boost immunization, consistent with lower vector-specific NAbs induced in these animals. Interestingly, rAd35k5 vectors elicited antigen-specific immune responses and vector-specific NAb titers that were between those induced by rAd5 and rAd35 vectors following the initial immunization. After the boost immunization, rAd35k5 vectors elicited potent cellular immune responses that were 2–3-fold higher than those elicited by both rAd5 and rAd35 vectors. These data demonstrate the immunogenicity of rare serotype and chimeric rAd vectors in rhesus monkeys. Moreover, these studies suggest that chimeric rAd vectors can be constructed to combine the beneficial immunologic and serologic properties of different Ad serotypes.

S61
Clinical Trials of DNA and Recombinant Adenovector (rAd) Vaccines for HIV
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Background: Gene-based vaccine delivery is an important strategy for induction of T cell responses that may be critical for a successful AIDS vaccine. Despite promising results in animal models, evidence of immune responses to DNA and rAd vaccines in humans has been limited.

Materials and methods: Three Phase I studies have evaluated a series of DNA and rAd vaccine candidates expressing constructs encoding clades A, B, and C Envelope and clade B Gag and Pol with or without Nef, as fusion proteins or individually.

Results: T cell and antibody responses are detected by IFN-γ ELISpot and FACS detection of intracellular IL-2 or IFN-γ in the large majority of vaccinees. Env peptide pools elicit the strongest response, but the 6-plasmid and rAd product also induced robust responses to Gag, Pol, and Nef. Both T cell and humoral responses were dose dependent. The T cell responses induced by DNA are detectable for at least 52 weeks, and the pattern of cytokine expression evolves over time with fewer IFN-γ and more IL-2 producing T cells at one year.

Conclusion: DNA and rAd5 vaccine candidates are well tolerated and induce broad, durable immune responses. The combination will be tested in Phase II trials beginning 4Q2005.

S62
HIV-1 Specific CD4 and CD8 T-cell Responses Associated With Low Viral Load in Treatment-Naïve HIV-1 Infected Individuals
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In preparation for monitoring of vaccine-induced responses, we determined HIV-specific cell-mediated immune responses in 17
S63  
Cross-Reactive HIV-1 Neutralizing Human Monoclonal Antibodies with Unique Features:  
Structural Mimicry of CD4, Conformational Flexibility, Lack of Light Chain  
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Retrovirology 2005, 2(Suppl 1):S63  

In the giant struggle with the long chronic HIV infection the immune system has generated unique antibodies matured to neutralize a virus which has evolved to escape them. I will describe unique features of a CD4bs (m18), and two CD4i (m12, X5) antibodies selected from immune phage libraries developed from long-term nonprogressors with high levels of broadly neutralizing antibodies. The m18 H3 shows striking similarity to the Ig CDR2-like C'C'' region of the CD4 domain 1 which dominates the binding to gp120. The X5 H3 is exceptionally flexible – IgG X5 inhibits efficiently infections of cells with low surface concentrations of CCR5. M12 is the only HIV-specific antibody identified which does not express its light chain but still neutralizes the virus which has evolved to escape them. I will describe unique features of two CD4bs (m18, X5) antibodies selected from immune phage libraries developed from long-term nonprogressors with high levels of broadly neutralizing antibodies. The m18 H3 shows striking similarity to the Ig CDR2-like C'C'' region of the CD4 domain 1 which dominates the binding to gp120. The X5 H3 is exceptionally flexible – IgG X5 inhibits efficiently infections of cells with low surface concentrations of CCR5. M12 is the only HIV-specific antibody identified which does not express its light chain but still neutralizes the virus which has evolved to escape them.

S64  
Prime-boost AIDS Vaccine Strategies Based on Replication-Competent Adenovirus Recombinants  
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The potent, persistent immunity needed to prevent HIV infection might be best achieved by priming cellular immunity with replicating vectors and boosting antibodies with optimally designed envelopes. Replicating Ad vaccines infect epithelial cells on mucosal surfaces and thus also elicit mucosal immunity. In chimpanzees, compared to non- replicating Ad vaccines, at the same or lower dose replicating Ad vaccines were better at eliciting cellular immunity and priming antibody responses. Mismatched envelope boosts induced broad neutralizing activity to diverse RS viruses and cross-clade ADCC activity. Multigenic Ad-SIV vaccines and SIV envelope subunit boosts elicited strong protection in 39% of rhesus macaques challenged mucosally with SIVmac251. Durability of protection against a second challenge was established in 73% of previously protected animals, associated with persistent cellular immunity. Induction of memory cells and broad, strong functional antibodies illustrates the promise of this prime-boost vaccine strategy.

S65  
Abstract withdrawn  
Retrovirology 2005, 2(Suppl 1):S65  

S66  
Abstract withdrawn  
Retrovirology 2005, 2(Suppl 1):S66  

S67  
Orally Delivered, Plant-Produced Tat Protein Primes Mice For a Challenge DNA Vaccine Expressing Tat  
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The Tat protein has been recently explored as a prospective vaccine candidate against HIV-1 with broad, subtype non-specific action. A truncated version of Tat(Tat) with the basic loop, involved in immunosuppression, removed has been previously demonstrated as efficacious as the full-size Tat protein. We produced both full-size Tat and truncated _Tat in plants, including one edible species – spinach, thus simultaneously addressing problems of an inexpensive Tat production and a direct delivery through the mucosal route. We tested this oral delivery route in a mouse model. Tat and _Tat genes were assembled from a set of synthetic overlapping oligonucleotides, and subsequently cloned into a plant virus-based expression vector. Codon optimization allows production of up to 300–500 mg of Tat or _Tat antigen per 1 g of leaf tissue in spinach. Spinach
plants inoculated with the Tat-producing constructs were collected and fed to mice 7–14 days post inoculation with or without mucosal adjuvants. Mice were fed with the Tat-producing or control vector-inoculated spinach. After 3 voluntary feedings, 1 g per mice, no differences were detected in the growth rate or behavior of the animals fed with these two types of spinach. None of the animals developed measurable Tat antibodies. Challenge DNA vaccination with a homologous Tat-expressing construct was performed using a gene gun. Following DNA vaccination, however, mice previously receiving oral Tat with cholera toxin as an adjuvant, developed higher antibody titers to Tat than did the controls, with the titers peaking at four weeks post-vaccination. Thus, our data suggested that oral Tat primed for the development of Tat antibodies when mice were challenge-vaccinated with plasmid DNA for expression of Tat.

S68
HIV-1 Infections During Vaccine Trials: Identifying New Epitopes For Differential Diagnosis Of HIV-1 Infections In The Face Of Vaccine -Induced Antibodies
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Most of the HIV-1 vaccines under development contain multiple viral genes or proteins. As a result, many vaccine-recipients react positively in licensed HIV-1 detection assays. This will have negative impact on future efficacy trials of prophylactic HIV vaccines that require early detection of intermittent HIV infections. It will also exclude all vaccinees from blood/plasma donations, and may contribute to other social harms. Therefore, it is important to design new strategies for vaccine trial participants that will clearly discriminate between vaccine-induced antibodies and true HIV-1 infection. We identified new HIV-1 epitopes that: 1) Do not contain important neutralizing or CTL epitopes, and can be omitted from future HIV vaccine candidates, 2) Recognized by antibodies from early HIV infected individuals, 3) Highly conserved among HIV-1 clades and subtypes. Using Phage Display libraries constructed from whole HIV-1 genome, combined with panning over antibodies from early seroconvertors, we identified new immunodominant epitopes, in the gp41 intracytoplasmic tail and in p6, which conform to the above criteria. These peptides were used for the development of new HIV-1 EIA. To date the assay specificity and sensitivity are at >99%. Based on reactivity of several well-characterized panels of seroconvertors it was demonstrated that these peptides could detect antibodies within 4 weeks of HIV-1 infection. Testing of diverse serum samples (>2100) from around the world supports the utilization of our assay in detecting antibodies from infected individuals with clades A, B, C, D, E, F, and multiple recombinants. Importantly, testing of sera from HIV-1 vaccine trials gave mostly negative reactivity in our assay while scoring positive in one or more of the currently FDA-licensed HIV-1/2 EIA kits. Furthermore, our assay detected intermittent HIV infections among vaccine recipients in 4 different vaccine trials. This assay could be added to the HIV detection kits used in prophylactic vaccine trials and blood/plasma collection centers.

S69
Antigenic Comparison of HIV Envelope Complexes Containing Either sCD4, Human Anti-envelope Monoclonal Antibody A32, or CD4 Mimic Protein CD4M9
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There is growing interest in using antigens that replicate the envelope transition state structures that occur during HIV entry as vaccine immunogens. Three such immunogens have been developed – complexes between gp120 and sCD4 (CD4/gp120), gp120 and a human monoclonal antibody A32 (A32/gp120), and gp120 and a CD4 mimic molecule CD4M9, SCBaLM9. Antigenic comparisons of these immunogens revealed key differences between these complexes. Coreceptor binding is 3-fold higher with the gp120/sCD4 over gp120/A32 and gp120/CD4M9 complexes. However, the CD4 induced epitopes (CD4i) recognized by 17b and FabX5 are expressed equally between all three complexes. 19e, which recognizes an epitope that is completely dependent upon CD4 binding (CD4d), binds to gp120/sCD4 but not to A32/gp120 or SCBaL/M9 complexes. Another CD4d epitope recognized by ED47 is similarly prominent in CD4/gp120 complexes but significant less so in A32/gp120 and SCBaL/M9. These data indicate that the antigenic features of the A32/gp120 and SCBaL/M9 are more consistent with a transition structure between unligated gp120 and the CD4/gp120. Chemical crosslinking can obscure these CD4i and CD4d epitopes. These antigenic differences may also explain the differences in the neutralizing antibody profiles generated by CD4/gp120 and A32/gp120 complexes in animal experiments.

S70
Baculovirus-Derived HIV-1 Virus-Like Particles (VLP) Activate Dendritic Cells and Are Cross-Presented to Induce In Vitro T-Cell Response
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Aim: to evaluate the ability of the baculovirus-expressed HIV-VLP to induce the maturation and activation of monocyte-derived dendritic cells (MDDCs).

Results: the VLP-activated MDDCs show an enhanced Th1 and Th2-specific cytokine production and the effects of VLPs on MDDCs are, to some extent, mediated through intra-cellular...
Toll-like receptors signaling. The VLP-loaded MDDCs are able to induce a primary and secondary response in autologous T cells, using an in vitro immunization assay. Moreover, the genomic transcriptional profile of VLP-activated DCs show, by gene microarray analysis, the upregulation of several genes involved in the immune response.

**Conclusion:** Our results show that baculovirus VLPs activate MDDCs and may “cross” over to the endogenous pathway to gain access to MHC class I, inducing CD8+ cytotoxic T cells activation. The intra-cellular Toll-like receptors appear to be involved in this process; additional signaling pathways induced by VLPs in the MDDCs are currently under evaluation. These data give an insight into the mechanisms of the cellular immunity induced in vivo by VLPs, which may be extremely useful to optimize and modulate the immune response.

**S71**

**Induction of HIV-neutralising Antibodies of the 2SF5/4E10 Type**

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Neutralising antibodies recognising membrane proximal epitopes of gp41 have been isolated from HIV-infected patients. Since these epitope domains are highly conserved, the corresponding antibodies 2SF5 and 4E10 neutralise a broad range of HIV strains. Numerous attempts by several laboratories to generate 2SF5/4E10-like antibodies by vaccination have failed, obviously because the conformation of the domain is difficult to reproduce. Recently we reported induction of neutralising antibodies against the porcine endogenous retrovirus (PERV) and the feline leukaemia virus (FeLV) by immunisation with their transmembrane envelope (TM) proteins p15E. In both retroviral TM proteins two epitope regions were identified, one located in the N-terminal part (designated E1) and the other located in the C-terminal part of p15E (E2). E2 is related to the 2SF5/4E10-epitope, and is located opposite E1 when the TM envelope protein has folded. An E1 domain was identified in the C-terminal part of gp41 and two strategies were developed to induce neutralising antibodies against HIV. First, immunisation was performed with a hybrid protein based on p15E of PERV, containing the E1 domain and the E2 (2SF5/4E10 epitope) domain of gp41 of HIV-1. Second, immunisation was performed with a hybrid containing the N-terminal backbone of p15E of FeLV and only the E2 (2SF5/4E10 epitope) domain of gp41. With both strategies antibodies neutralising laboratory and primary strains of HIV-1 were induced. These strategies may be used to generate a vaccine inducing broadly neutralising antibodies to prevent or curtail HIV infection.

**S74**

**A Novel Post-transcriptional Block in Gene Expression Contributes to HIV-1 Latency In Vivo**

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HIV-1 latency represents a major barrier to eradication. We describe a novel post-transcriptional block in HIV-1 gene expression in latently infected cells. Multiply spliced HIV-1 RNAs encoding the critical positive regulators Tat and Rev exhibited strict nuclear localization in latently infected primary resting CD4+ T cells. Proteomic analysis identified polypyrimidine tract binding protein (PTB) as a HIV-1 RNA binding protein which allows cytoplasmic accumulation of HIV-1 RNAs and subsequent release of replication-competent virus by latently infected cells. Thus a post-transcriptional block in resting cells interrupts a positive feedback loop and contributes to latency. This works suggests novel approaches for reversing latency.

**S75**

**New Data on HIV Reservoirs: Implications for Therapy**

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For years now, AIDS researchers have suspected that most investigations have been focusing on the wrong site, i.e. blood. In fact, it has been well known for over a decade that lymphoid tissue is the key viral replication site from the start of infection, and also a major reservoir and source of virus at all stages of the disease. Lymphoid tissue studies in humans have been impeded by difficulties in obtaining material and, first and foremost, in reiterating sampling. Recent findings in the SIV-infected macaque model pinpoint the intestinal mucosal immune system as a key site of viral replication/persistence and CD4 depletion, even in subjects with undetectable blood virus during therapy. These studies demonstrate that acute SIV and HIV infections are coupled with a dramatic and selective loss of memory CD4 T cells in lymphoid tissue, due to direct virus-induced cytolysis or immune-mediated mechanisms (Mattapallil JJ et al, Nature 2005; 434: 1093-7). Treatment strategies based primarily on blood viral load and circulating CD4 cell counts are hence misguided. These findings plead for HAART initiation as early as possible, and will also have implications for vaccine development.

Data have recently been published on the potential to decrease the latent HIV reservoir in humans by initiating HAART at acute infection (Strain MC et al, J Infect Dis 2005; 191: 1410-8). Although failure to recover infectious virus from these patients certainly does not reflect the elimination of latently infected cells, these data are encouraging in the search of new strategies combining HAART and immune interventions. Despite its major effect on plasma viremia, HAART initiated at the chronic stage of the disease is known to have several major
drawbacks, i.e. its inability to achieve HIV eradication due to poor targeting of the reservoir of latent but replication-competent virus, plus the inadequate diffusion of antiretroviral drugs in the various anatomic reservoirs. For example, it has been clearly demonstrated that some molecules in the combination are unable to reach the central nervous system or genital tract well enough to block viral replication, hence potentially creating sanctuaries of resistant virus. More recently, parallels have been made between the notion of cellular resistance to antiretroviral drugs and the resistance of some cancer cells to drugs. This resistance can involve efflux molecules like P-glycoprotein (MDR-1) and Multi-drug Resistance Protein (MRP). We investigated the expression of MDR-1, MPR-1 and MRP-4 mRNA levels in PBMC and lymph node cells (LNMC) in 15 HIV-infected patients on long-term effective PI-based HAART regimens (plasma viremia <20 copies/ml) versus controls (HIV-uninfected, HIV-infected HAART-naïve, HIV-infected on non-PI-based HAART). MDR-1, MPR-1 and MRP-4 mRNA levels were measured by PCR related to GAPDH expression and results given in arbitrary units. We found MDR-1 mRNA expression in PBMC to be much higher in HIV-infected but naïve patients than HIV-negative controls (46.64 vs 0.24), and higher in treated versus untreated patients, with no differences according to therapy type (no PI: 210.54, PI: 348.96). Surprisingly, MDR-1 expression in LNMC from PI-treated patients was significantly lower (0.97) than in paired PBMC. MRP-1 and MRP-4 expression showed significantly higher expression in HIV-treated versus HIV-negative patients, with no differences according to therapy type, and similar expression in HIV-infected untreated patients versus HIV-negative controls. No differences in MRP-1 and MRP-4 expression were found in HIV-infected untreated patients versus HIV-negative controls.

S76 Controlling the Virus Output Via Urokinase Receptor and Integrin Signaling

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We have described that either urokinase-type plasminogen activator (uPA) inhibits HIV expression in monocytic U937 and U1 cell lines (M. Alfano et al., PNAS, 2002, 99:8862-67). We have observed that uPA inhibited HIV expression exclusively when both uPAR and CD18/CD11b (Mac-1) were co-expressed at the cell surface. A second interactor of uPAR, FPRL1, was abundantly expressed on the surface of both unstimulated and stimulated U1 cells; however, peptide antagonists of FPRL1 did not interfere with HIV expression from U1 cells. Incubation of U1 cells with Trojan peptides expressing RhoA domains reversed the anti-HIV activity of uPA. In addition to cell line infection, uPA inhibited in vitro infection of primary monocyte-derived macrophages and virus replication from monocytes of infected individuals cultivated ex-vivo. Thus, RhoA-dependent cytoskeleton rearrangement and intracellular vesicles formation may be related to virion budding and entrapment in intracytoplasmic vacuoles. This is the first report linking integrin activation to a negative control of HIV replication, at least in monocyte/macrophages.

S77 The Molecular Epidemiology of a Heterosexual Subtype B HIV Epidemic: the Latest Results from the Caribbean

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Introduction: The HIV epidemic in the Caribbean is one of the few epidemics in the world that is dominated by subtype B and has a heterosexual transmission pattern. Close examination of the Caribbean subtype B viruses by full genome sequencing may shed light on the epidemic. Methods: Serum samples from Trinidad and Tobago (TT), Jamaica (JM), Haiti (HT) and the Dominican Republic (DO) were collected for characterization. CDNA synthesized from viral RNA was PCR amplified and sequenced with an ABI 3100 sequencer.

Results: Over 60 strains were analyzed and all but one was subtype B. Phylogenetically there were no island-specific or Caribbean-specific genetic clusters, though a majority of new TT samples retained the signature threonine deletion in the V3 loop. One sample from DR was a unique BC recombinant having gag and env from subtype C and pol from subtype B. Glycosylation patterns in the Caribbean gp160's were different from those of other subtype B strains from the epidemic.

Conclusion: The genetic isolation of the TT strains from the mid-1990's is no longer visible in the most recent samples, though the majority still retains the earlier signature in V3. Glycosylation differences between the Caribbean envs and those in North America may relate to natural selection of the Caribbean virus for heterosexual transmission.

S78 Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S78

S79 Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S79

S80 Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S80

S81 Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S81
Abstract withdrawn

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Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S83

Strategies to Prevent Dendritic Cell-driven Infection Across the Mucosa
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Early events leading to HIV infection across the mucosa likely involve HIV capture by a wide variety of molecules on the surface of epithelial cells and leukocytes followed by infection of permissive target cells within the tissues. In studying this biology, we are focusing on the contribution of dendritic cells (DCs) and T cells to these events and exploring effective ways to block these complex events in vitro and in vivo. Earlier work confirmed that there are at least two phases of DC-driven transmission of virus to T cells – one involves virus captured by (but not infecting) DCs that is handed directly over to the T cells and the other involves DC infection and the transmission of newly synthesized viruses. Virus captured by DCs is transmitted to CD4+ T cells moving rapidly across the synapse naturally created between DCs and T cells. Inclusion of the fusion inhibitor T1249 reduces the amount of virus movement to the T cells, while increasing the amount of virus accumulating in the DCs. These data highlight how only blocking certain pathways of virus-DC interactions are suboptimal in preventing DC-driven HIV spread. As a result, additional studies are being performed to test the ability of more broad-acting carrageenan-based formulations for their ability to impede the complex virus-cell interplay needed to facilitate transmission. Carrageenan-based microbicides are promising due to their wide range of activity against HIV/SIV and other sexually transmitted pathogens. Carrageenans impaire virus capture by DCs in vitro and block infection of permissive DC-T cell mixtures. Recent in vivo data revealed that macaques were protected against vaginal SHIV challenge by carrageenan-based microbicides. These data are encouraging for future application of carrageenan-based formulations in preventing HIV spread.

Retrocyclins: Novel Circular Peptides Active Against HIV-1
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Heterosexual transmission through mucosal surfaces is one of the most common routes of HIV-1 spread. Topical microbicides are self-applied prophylactic agents, used to prevent vaginal and other mucosal transmission of HIV-1, which have the advantage of empowering vulnerable receptive partners to take effective measures for their own protection. In a search for candidate topical microbicides we discovered that retrocyclin, a unique β-defensin synthetically constructed based on sequence data from its pseudogene, can potently prevent infection of CD4+ cells by both X4 and R5 HIV-1. While many studies have utilized simulants of mucosal fluid to test compounds, we are studying how whole human vaginal fluid and its functional components affect the activity and stability of peptide-based microbicides. We explored a novel, physiologically relevant approach to assess the ability of a candidate retrocyclin microbicide, RC-101, to inhibit HIV-1 infection of immunocompetent human cervicovaginal tissue. We revealed that 1) using a novel proteomic approach, human vaginal fluid contained at least 20 different cationic (poly)peptides with purported roles in innate host defense, 2) the cationic polypeptide fraction of vaginal fluid was required for innate anti-HIV-1 activity, 3) RC-101 retained full anti-HIV-1 activity in the presence of whole human vaginal fluid, and 4) when applied apically to organotypic cervicovaginal epithelium, RC-101 was retained in the tissue, and 5) RC-101 prevented HIV-1 infection of immunocompetent organotypic cervicovaginal epithelium. Collectively, we have characterized the innate antiviral host defense factors within vaginal fluid, and developed a highly relevant in vivo vaginal model suitable for peptide-based microbicide evaluation.

Vaginal Lactobacilli for Mucosal Delivery of the Anti-HIV Microbicide, Cyanovirin-N (CV-N)
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Background: Women are particularly at risk of HIV infection and there is an urgent need for female-controlled approaches to block the heterosexual transmission of HIV.

Material and Methods: Our work is aimed at the development of a simple, cost-effective, female-controlled preventative against heterosexual transmission of HIV in women, based on our previous proof-of-concept study employing a natural component of the vaginal microflora, as a delivery vehicle for the anti-HIV protein (PNAS, 2003, 100:11672-11677).

Results: A human vaginal isolate of Lactobacillus jensenii was engineered, by stable integration of an optimized expression cassette into the bacterial genome, to secrete high levels of the highly potent HIV inhibitor, CV-N. The L. jensenii-expressed
CV-N dramatically decreases infectivity of CCR5-tropic HIV<sub>bal</sub> and CXCR4-tropic HIV<sub>env</sub> in vitro. We further demonstrate that this strain is genetically stable and can transiently colonize animal vaginal mucosa, while retaining important characteristics of the native bacterial phenotype.

**Conclusion:** This live microbicide represents a novel approach in the development of an inexpensive and stable protein-based microbicide to curtail the HIV/AIDS pandemic worldwide.

**S88**

**Dendrimers As Drugs: Discovery, Preclinical and Clinical Development of SPL7013 Gel (VivaGel™), a Dendrimer Based Microbicide for HIV and STI Prevention**

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Starpharma focuses on the use of dendrimers as drugs in their own right – in contrast to dendrimers as drug delivery vehicles or diagnostics. Dendrimers offer a unique platform for exploring chemical diversity on the nanoscale and the production of dendrimer libraries covering a diverse array of macromolecular structures can be used in drug discovery and development. One pharmaceutical application of dendrimers that Starpharma is pursuing is the development of microbicides for the prevention of HIV and sexually transmitted infections (STIs). This presentation will describe the dendrimer drug discovery and lead candidate selection process from which SPL7013 emerged as a microbicide development candidate. Pivotal preclinical data will be presented that lead to Starpharma submitting an Investigational New Drug application (IND) for SPL7013 gel (VivaGel™) to the United States Food and Drug Administration (FDA) in June 2003, the first such submission for a dendrimer based drug. Finally, results of the first clinical trial under this IND will be presented.

**S90**

**Abstract withdrawn**

Retrovirology 2005, 2(Suppl 1):S90

**S91**

**Coinfection Of HIV With HBV And HVC In A Low Resources Setting**

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**Background:** Co-infection of HIV with HVB and C is well described in medical literature from all over the world. The coexistence of these infections can worsen the evolution of anyone of them, therefore a fast diagnosis and treatment can help to achieve a better prognosis. Malvinas Argentinian is a low resources seting located in NW of Buenos Aires Province. Objective: Determining the existence of co-infection with HIV and HVB or HVC in patients included in the Municipal Program Against HIV and establishing the characteristics of the infected population.

**Methods:** Prospective study of HIV+ p regarding the presence of positive serology for HVC (ELISA), HVB (determination of HbsAg) or both.

**Data obtained:** Sex and risk factor for HIV infection (heterosexual, homosexual, intravenous drug use (IDU)).

**Results:** Of 162 p, 15 were HVC+ (9.3%), 13 HbsAg+ (8%) y 2 HVC/HbsAg+ (1.2%). 13 HIV+ were male (87%) (p = 0.007), and among them, risk factor for HIV acquisition was IDU in 9 cases (60%) and heterosexual risk behaviour in 6 (RR 12 -IC: 4.83<RR<29.79; p;0.0005). 11 HbsAg+ were male (84.5%) (p = 0.005) and among them risk factors for HIV acquisition were heterosexual risk behaviour in 5 cases, homosexual risk behaviour in 4 cases and IDU in 2 cases, none of this were significant as relative risks.

**Conclusion:** Male sex was a significant relative for coinfection on HIV with HVB and HVC, maybe related to IDU in this population. IBU as a risk factor for acquisition of HIV was significant for...
co-infection with HCV. Warning about HCV co-infection should be adopted in this improve the prognosis of both infections.

**S92**

**Testing Candidate Topical Microbicides – Distinguishing Toxicity from Efficacy in Preclinical Testing**

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Topical microbicides are considered an affordable choice for the prevention of sexually transmitted diseases in women. We have developed a comprehensive testing program for preclinical microbicide development. Over the years, we have tested several thousand compounds for use as topical microbicides in a series of cell-based assays addressing HIV-1 efficacy and toxicity. Recently, we compared historical data of the spermicide nonoxynol-9 (N-9) in a multi-center study and found that the HIV-1 efficacy paralleled its toxicity. Intra-assay, inter-assay, and inter-laboratory variability for toxicity were remarkably consistent. In a recent clinical trial, N-9 was found to enhance HIV-1 infection, thus confirming the preclinical toxicity data. In addition to N-9, lemon and lime juices have been proposed and used as contraceptives and were recently shown to exhibit in vitro activity against HIV-1. Therefore, we tested freshly prepared lemon juice, lime juice, and household vinegar (concentration = 100%) for HIV-1 efficacy and toxicity and for effect on beneficial Lactobacillus species. In all assays, the therapeutic index was <10, due to toxicity of the juices and vinegar to cells (mean TC₅₀ of lemon juice = 5.6%, mean TC₅₀ of lime juice = 4.9%, and mean TC₅₀ of vinegar 0.1%). Ten percent lemon or lime juice were not toxic to beneficial Lactobacillus species, in contrast to 10% vinegar which was highly toxic. Our preclinical data indicate that candidate topical microbicides should be moved forward into clinical trials with caution.

**S93**

**Development of Vaginal Lactobacilli for Mucosal Delivery of a Topical Microbicide, Cyanovirin-N (CV-N)**

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**Background:** Women are particularly at risk of HIV infection, therefore, female initiated strategies are essential tocurtail the HIV/AIDS pandemic worldwide.

**Material and Methods:** We developed a live topical microbicide, which is an H2O2-producing Lactobacillus jensenii, a natural component of the vaginal microflora, as a delivery vehicle for the potent HIV inhibitor, cyanovirin-N (CV-N).

**Results:** The optimized CV-N expression cassette was stably integrated in single copy into the lactobacillus bacterial chromosome, and resolved from extraneous plasmid DNA and antibiotic resistance determinants. The L. jensenii-expressed CV-N dramatically decreased CCR5-trophic HIV-BaL infectivity in vitro, with an IC₅₀ of 0.3 nM. Histological examination of CD1 mice, which were intra-vaginally inoculated with L. jensenii expressing CV-N, revealed that L. jensenii was associated with keratinized epithelium present during estrus or free in the vaginal lumen and secreted full-length CV-N in vivo.

**Conclusion:** This live microbicide represents a major step towards developing inexpensive, durable protein-based microbicides to address the urgent need for female-controlled approaches to block the heterosexual transmission of HIV.

**S94**

**AlphaHGA; A New Antiviral Substance Against HIV Affecting Capsid Assembly**

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**Background:** We have previously shown that the tripeptide glycyl-prolyl-glycine-amide (GPG-amide) inhibits HIV-1 replication in vitro by affecting proper capsid assembly of HIV-1. However, GPG-amide failed in a phase II clinical trial on HIV-infected individuals. In the search for what went wrong we have now found that the tripeptide in itself does not exert the antiviral activity but is metabolised in two steps into the active compound by serum enzymes. The first step is cleavage of GPG-amide into GP and glycine-amide (G-amide) by the soluble di-peptidyl peptidase CD26. In the present study we show that G-amide is further metabolised to the active antiviral substance by an enzyme present in foetal bovine serum but not in human serum.

**Material and Methods:** Numerous methods have been employed including molecular biology, magnetic resonance (NMR).

**Results:** The second step is an enzyme mediated oxidation of G-amide into the active anti-viral compound. By NMR the molecule was found to be alpha-hydroxy glycine amide (alphaHGA). The latter is a small molecule with a molecular mass of 90. The conversion of G-amide into alphaHGA does not take place in human or rodent serum but in the serum from most species including fetal calf and pig serum. Hence, GPG-amide does not affect HIV-1 replication if the infected cells are cultured in the presence of human serum only. We have now synthesized alphaHGA and been able show that the synthesized substance inhibits HIV-1 replication in the presence of human serum only or with heat inactivated fetal calf
serum. In the presence of alphaHGA in the culture medium progeny HIV-1 particles have abnormal capsid structures and are non-infectious. 

**Conclusion:** AlphaHGA is a new promising antiretroviral substance. All preclinical studies on alphaHGA have now been performed and clinical studies on HIV infected individuals are planned to start this year.

**S95**

**The Presence of Mucin Increases the Anti-HIV-1 Activity of the Candidate Microbicide Polyethylene Hexamethylene Biguanide (PEHMB)**

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Topical microbicides that reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission must function effectively within the cervicovaginal environment where multiple factors may impact the efficacy of the active agent. Factors relevant to potential changes in microbicide efficacy include the presence of mucins within the cervical mucus. We hypothesize that polycationic PEHMB molecules will interact with the anionic mucin molecules to form a lattice-like network that serves as a physical barrier to the movement of infectious virus and HIV-1-infected cells to the cervical and vaginal epithelia. *In vitro* experiments demonstrated that the anti-HIV-1 activity of PEHMB was increased almost two logs in the presence of mucin. In contrast, the activity of anionic dextran sulfate was unaffected. These results suggest that electrostatic interactions between PEHMB and mucin molecules may augment the inherent anti-HIV-1 activity of PEHMB by facilitating the formation of a physical barrier between HIV-1 and susceptible cells. This property would be expected to increase the in vivo efficacy of PEHMB.

**S96**

**Use of the Synthetic Copolymer PSMA as a Component in a Combination Microbicide Active Against HIV-1**

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The remarkable successes achieved using combination therapy to treat systemic human immunodeficiency virus type 1 (HIV-1) infection suggest that combination microbicides, which include two or more active ingredients, may also provide a particularly effective means to prevent HIV-1 transmission. We have recently identified the compound PSMA, an alternating copolymer of polystyrene (PS) and maleic anhydride (MA), as a potential partner for our candidate microbicide polyethylene hexamethylene biguanide (PEHMB), a member of the polybiguanide family of compounds. *In vitro* studies of PSMA demonstrated that this compound is minimally cytotoxic and highly effective against both macrophage- and T cell-tropic strains of HIV-1. We hypothesize that the dissimilar mechanisms of action of PSMA and PEHMB may provide additive or synergistic activity against HIV-1. Experiments are now underway to identify optimal combinations of PSMA and PEHMB to be used in experiments to assess toxicity, anti-HIV-1 activity, and formulation strategies. These investigations will be used to confidently advance the preclinical development of PSMA and a combination microbicide containing both compounds toward human trials.

**S97**

**Incidence of Mycoplasma Pneumoniae Infection in HIV Infected Patients With Underlying Upper and Lower Respiratory Complaints and Correlation With Various Immunological and Haematological Findings**

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**Background and Objectives:** *Mycoplasma pneumoniae* has been implicated with community-acquired pneumonia and mild to severe respiratory infections in the normal population. The prevalence of this mollicute in HIV infected patients has never been reported from India. Mycoplasmas have also been reported to act as cofactors in AIDS progression.

**Aims and Objectives:**

a) To divulge the incidence of *Mycoplasma pneumoniae* and other respiratory pathogens in the respiratory specimens of HIV infected patients.

b) To compare the sensitivities of induced sputum and throat swab specimens for detecting *Mycoplasma pneumoniae*.

c) To correlate the various haematological and immunological findings with infection due to *M. pneumoniae* in the HIV infected patients tested.

**Materials and methods:** The present study has been carried out on 60 HIV infected patients presenting with underlying pulmonary complaints and whose clinical presentation was consistent with disease caused by *Mycoplasma pneumoniae*, after obtaining informed consent subsequent to approval by the Institutional Review Board (IRB) on human ethics in Chennai where the recovery rates of *Mycoplasma pneumoniae* from induced sputum and throat swab specimens of HIV infected patients were compared and the haematological and immunological findings were analysed. Patients screened were from the age groups ranging from 15 to 60 years, whose respiratory specimens were cultured on PPLO glucose agar and broth, the later with 1% methylene blue. Presumptive identification of *Mycoplasma pneumoniae* was carried out using guidelines
proposed by the Subcommittee on the Taxonomy of Mollicutes, 1979. The respiratory specimens from the HIV-infected subjects were later analysed for their recovery rates, incidence of other bacterial, fungal pathogens, AFβ, Pneumocystis carinii and their correlation features with CD4+ and CD8+ lymphocytes were also analysed and compared.

Results and Conclusion: The male to female ratio of the study population was 1:09. The mean age of the patients was 39 years. M. pneumoniae was presumptively detected from 23 (38.3%) of the HIV infected patients. Induced sputum and throat swabs yielded 82.6% and 55% of the mycoplasma isolates respectively, which suggests that induced sputum can be the better specimen compared to throat swabs. Simultaneous positivity of both specimens was detected in 13 (56.5%) cases. Besides Candida spp (80%), Staphylococcus aureus (26.6%), Streptococcus pneumoniae (21.6%), Pseudomonas aeruginosa (18.3%), AFβ (16.6%), Klebsiella pneumoniae (15%), Moraxella catarrhalis (8.3%), b-hemolytic streptococci, P. carinii, M. fermentans (6.6%), diphtheroids (5%), A. fumigatus and E. coli (1.6%) were the predominant isolates. The detection rate of M. pneumoniae was found to be high in patients with depleted CD4 levels. The mean CD4 count of the study cases was 106 cells/μl whereas the value was only 78 cells/μl among those positive for Mycoplasma pneumoniae. The study shows that CD4 depletion may enhance mycoplasma infection in the respiratory tracts of HIV infected patients.

S98
Preclinical Evaluation of Anti-HIV-1 Polybiguanide Vaginal Microbicides in a Murine Model of Toxicity and Inflammation
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The use of vaginal microbicides has gained support as a strategy for the protection of women against HIV-1 and other sexually transmitted disease (STD) pathogens. The preclinical Swiss Webster murine model has been developed to specifically measure cervicovaginal tissue integrity and inflammation following application of candidate vaginal microbicides, when potential exposure to an STD pathogen may occur. This model demonstrates both mechanistic and temporal differences in inflammatory responses following microbicide exposure. Currently, specific markers of inflammation, including pro-inflammatory cytokines, are being evaluated in the cervicovaginal mucosa. Safety profiles of polybiguanides (PBGs), which demonstrated significant in vitro efficacy against HIV-1, are being assessed in vivo. Intravaginal application of PEHMB (1%) resulted in little or no cervicovaginal toxicity after short- or long-term exposure. Collectively, these studies support the Swiss Webster model as a valuable tool for the preclinical assessment of toxicity and inflammation associated with exposure to candidate topical microbicides. Furthermore, these results strongly support further development of polybiguanide derivatives as vaginal microbicidal agents.

S99
Epidemiology and Genetic Background of HIV-1 Strains Circulating in Shandong, China
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Shandong is a relatively developed coastal province in China with 17 cities, a population of 91.23 million, and increasing numbers of HIV/AIDS cases. We investigated the epidemiology of circulating HIV-1 strains from 97 HIV-1 infected cases from 1992 to 2004 in Shandong. The fragments of HIV-1 env C2-V3, gog P17/P24, 1st exon of tat and adjacent region were PCR amplified and sequenced, followed by phylogenetic, homology and recombination analysis. We found 7 circulating HIV-1 subtypes/CRFs, which are B’ (75.3%), CRF01_AE (10.3%), CRF07_BC, CRF08_BC (i.e. B’/C, 4.1% respectively), B, C and CRF02_AG (2.1% respectively). Sub type B’ was found mostly in paid blood donors, while B’/C recombinants were primarily in injecting drug users. The remaining subtypes distributed mainly in sexually transmitted subjects. Genetic divergence and amino acid sequence analysis showed that B’ strains are closely related to B.CN. RL42; B/C strains to 97CN54A and 97CN6X6; CRF01_AE strains to 01_ae_th.90.cm2; CRF02_AG, C and B strains were related to standard strains from Cameroon, Ethiopia and America, respectively. Genetic divergence of env of B’ strain showed that it started circulating in Shandong 7–10 years ago. The large strain variation and occurrence of CRFs signify the rapidly increasing HIV-1 epidemic in Shandong, and have implications for vaccine design.

S100
Can Vaccine-induced Mucosal High Avidity CD8+ CTL Delay AIDS-viral Dissemination from Mucosa?
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Natural HIV transmission occurs through mucosa, but it is debated whether mucosal cytotoxic T lymphocytes (CTL) can prevent or reduce dissemination from the initial mucosal site to the systemic
circulation. Also, the role of CTL avidity in mucosal AIDS viral transmission is unknown. To address these questions, we used delay in acute-phase peak viremia after intrarectal challenge as an indicator of systemic dissemination. We find that a peptide-prime/poxviral boost vaccine inducing high levels of high avidity mucosal CTL can impact dissemination of intrarectally administered pathogenic SHIV-ku2 in macaques, and that such protection correlates better with mucosal than with systemic CTL and particularly with levels of high avidity mucosal CTL.

S101
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S101

S102
Detection of Novel Neutralizing Antibody Reactivities Against The Membrane Proximal External Region (MPER) of gp41 in HIV-1 Infected Humans
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Background: Previously, we employed site-directed mutagenesis to introduce HIV-1 4E10 and 2FS epitopes into the corresponding region of a functional HIV-2 envelope glycoproteins (AIDS Vaccine 2005, abstract #A210). The resulting “chimeric” viruses were used to screen HIV-1 infected human plasma for 4E10 or 2FS-like neutralizing antibodies (Nabs). Among 177 subjects infected with HIV-1 representing ten different subtypes or circulating recombinant forms, none had significant Nab titers directed toward these epitopes.

Materials and methods: Here, we tested the same HIV-1 positive plasma specimens for neutralizing activity against chimeric HIV-2 viruses in which we substituted the complete 25 amino acid HIV-1 MPER (designated clone C1) or non-overlapping amino-terminal or carboxy-terminal portions of it (designated C3 and C4, respectively) using a single-cycle infectivity assay (Nature 422:307, 2003).

Results: HIV-2 viruses containing the C1, C3 or C4 MPER, and the parental virus HIV-2/7372A2, were infectious and equally susceptible to neutralization by T1249, sCD4, the anti-HIV-2 Env mAb 1.7A, and polyclonal anti-HIV-2 antibodies. This result demonstrates that none of the chimeric HIV-2 viruses was “globally sensitive” to neutralization. Surprisingly, 60 out of 165 (36%) of HIV-1 plasma tested contained MPER specific Nabs, IC50 titers ranged from 0.005–0.02 (mean 0.006; median 0.004; standard deviation 0.004). Anti-MPER Nab reactivities were mapped to C3 (6 subjects) or C4 (14 subjects) regions of the HIV-1 MPER or to epitopes spanning them (13 subjects). None of the 60 subjects with Nabs to C1, C3, or C4 had antibodies that neutralized HIV-2 viruses containing 2FS or 4E10 epitopes only.

Conclusion: These results indicate that the MPER of HIV-1 elicits Nab responses in a substantial proportion of infected patients and that the epitopes recognized by these Nabs are distinct from those recognized by 4E10 or 2FS.

S103
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S103

S104
HIV-1-Specific T Cell Function During Acute HIV-1 Infection
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HIV-1-specific CD8+ T cells in primary infection are associated with the dramatic decline of peak viremia to the viral set point, while their antiviral activity in chronic infection is less apparent. Here, we comparatively analyzed functional properties of HIV-1-specific CD8+ T cells in primary and chronic infection, and demonstrate that the functional avidity and TCR affinity of HIV-1-specific CD8+ T cells was consistently higher in primary infection than in chronic infection. The change of TCR affinities between primary and chronic infection was linked to an almost complete switch in the clonotypic composition of epitope-specific CD8+ T cells, resulting from the preferential loss of high-avidity CD8+ T cell clones. These data suggest that the initial recruitment of high-avidity HIV-1-specific CD8+ T cell may contribute to the control of HIV-1 viremia during primary infection, while their selective elimination during the subsequent disease process contributes to the loss of immune control during chronic infection.

S105
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S105

S106
The Kiss of Death: A New Model for How Perforin Delivers Granzymes to Target Cells
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Cytotoxic T lymphocytes (CTL) induce target cell apoptosis when they release perforin (PFN) and granzymes (Gzms) into the immune synapse. PFN delivers Gzms into the target cell, but how PFN does this has been unclear. Because PFN forms pores in the plasma membrane, Gzms were originally thought to enter target cells via these pores. However, these pores were too small to allow even small dyes to disseminate into the target. Here we find that PFN dramatically perturbs the target cell membrane, creating pores that transiently allow Ca++ and small dyes into the cell. However, the membrane is rapidly resealed and the dyes are circumscribed within membrane proximal blebs. The Ca++ flux triggers a wounded membrane repair response in which internal vesicles, including lysosomes and endosomes, donate their membranes to reseal the damaged membrane. The target cell actively participates in determining its own fate during cell-mediated death. The target cell membrane repair response is necessary for target cells subjected to CTL attack to avoid necrosis and undergo the slower process of programmed cell death. However, Gzms do not pass into the cell via PFN plasma membrane pores. Instead PFN triggers the
rapid endocytosis of Gzms into large EEA-1-staining vesicles, which then release their cargo into the cytosol to trigger apoptosis.

S107
CD4+CD25high Regulatory T Cells in the Developing Human Immune System: Implications for Pediatric HIV Infection
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Background: Although human T cells enter the peripheral lymphoid tissues early during fetal development⁰, the adaptive immune system in the fetus has largely been regarded as functionally immature and unresponsive to stimulation. In adults, CD4CD25high regulatory T cells (TReg) are critical for maintenance of peripheral T cell tolerance, but their role in the developing fetus is unknown. Here, we demonstrate that a large population of human fetal FOX3CD4CD25high TReg cells, present from the earliest stages of T cell colonization of the periphery, efficiently suppresses fetal T cell responses.

Results: Depletion of CD4⁺CD25high TReg cells from fetal lymph node cells, but not adult lymph nodes, resulted in the proliferation and acquisition of effector functions in the absence of exogenous stimulation by a large subpopulation of T cells identifiable by the expression of CD69 in utero. A large population of fetal CD4⁺CD25high TReg cells also expressed CD69+ and displayed a memory/effector phenotype, as indicated by low expression of CD45RA and CCR7. However, the CD69+ and CD69⁻CD4⁺CD25high TReg cells did not differ in their suppression of T cell responses in the absence of exogenous stimulation, indicating that the activation status of these cells do not correlate with their suppressive function.

Conclusion: These studies demonstrate that the fetal T cells are, in the absence CD4⁺CD25high TReg cells, highly responsive to stimulation, indicating that human fetal T cells are active and functionally mature. Strong evidence has also been obtained for an important role for CD4⁺CD25high TReg cells in controlling T cell responses in utero. The implications of these findings for pediatric HIV infection will be discussed.

S108
Mucosal Pathogenesis of HIV Infection
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Retrovirology 2005, 2(Suppl 1):S108

Like HIV-infected humans, simian immunodeficiency virus rapidly and selectively infects, replicates in, and destroys memory CD4+ T cells co-expressing CCR5 (viral “target” cells) resulting in loss of the majority of the bodies CD4+ T cell pool within 21 days of infection. The vast majority of these cells reside in the intestinal tract and other mucosal tissues but selective loss of these target cells is detectable throughout the lymphoid system. Restoration of memory CD4+CCR5+ T cells directly correlates with improved clinical course and lower viremia, but these cells are never restored in macaques that progress to AIDS. Continuous and effective antiviral treatment initiated within days of SIV infection can rescue mucosal CD4+ T cells, but delaying therapy for a couple of weeks does not restore these vital helper memory cells, despite effective control of viremia. Similarly, monkeys that “appear” protected in vaccine challenge studies may in fact harbor smouldering infection in the intestine with continued CD4+ T cell loss, despite undetectable plasma viremia. The rapidity and severity of the loss of memory CD4+ T cell function is likely the major reason no cure or vaccine is in sight. In fact, converging evidence suggests that other primate species changed fundamental properties of their immune system, such as eliminating the need for CD4⁺CCR5+ T cells (yet maintaining CD8⁺CCR5+ T cells), rather than cope with this subversive infection. This and other data suggest that conventional immune responses simply may not be adequate to control or prevent HIV infection.

S109
Pathogenic Mechanisms of HIV Disease: The Role of Viral Replication and Immune Activation
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HIV replication and the immune response to the virus lead to a state of generalized immune activation, which drives the pathogenesis of HIV disease. HIV-induced immune activation results in increased T-cell turnover (production and destruction), increased death of T cells, a decline in the size of the CD4+ T-cell pool, and a state of activation-induced immunodeficiency. In many HIV-infected individuals, these phenomena can be reversed or mitigated with effective antiretroviral therapy (ART), which blunts HIV replication and can reduce plasma levels of viremia to undetectable levels. Recent data from our laboratory have demonstrated fundamental differences between viremic and aviremic individuals with regard to the physiologic state of the resting CD4+ T-cell reservoir of HIV and the phenotype and function of T cells, B cells and natural killer (NK) cells. In terms of the CD4+ T-cell reservoir, resting cells in viremic versus aviremic individuals differentially express certain genes associated with HIV replication and are continually poised to express virus as a result of continual activation related to viremia; thus, true latency likely does not exist in these patients. We had previously reported that much greater stability exists in aviremic individuals, suggesting the presence of a truly latent reservoir of virus in this population. However, recent data indicate that the normal physiologic process of low-level immune activation in aviremic individuals sustained the low level turnover and propagation of the latent HIV reservoir. Previously, we have demonstrated that HIV-mediated hyperactivation induces the terminal differentiation of B cells. More recently, we have determined that certain B-cell genes are upregulated in HIV-viremic individuals as compared with HIV-aviremic individuals. Many of these genes are associated with terminal B-cell differentiation and death by apoptosis. Recent data indicate that there is a balance between expression of survival and apoptotic genes in HIV-infected individuals that is impacted by the
presence and level of viremia. In viremic individuals we also have characterized a population of aberrantly activated CD56- NK cells that manifest lower-than-normal expression of Natural Cytotoxicity Receptors and a normal or increased expression of inhibitory NK receptors. This dichotomy is reflected in decreased cytotoxic function and decreased secretion of TNF-alpha and interferon-gamma. In addition, NK cells from viremic individuals express Fas on their surfaces at significantly increased levels, and are more susceptible to apoptosis upon exposure to sFASL. Furthermore, viremia impacts the NK cell-dendritic cell interactions that are critical to normal immune responsiveness. Taken together, our data indicate that HIV pathogenesis involves both direct and indirect effects of HIV and a relentless cycle of aberrant immune activation that drives the disease process in HIV-infected individuals.

S110
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S110

S111
MHC Class I-Specific Inhibitory Receptors on CD8 T Cells – Impact on HIV-specific Activity
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MHC class I-specific inhibitory receptors are expressed by a subset of memory-phenotype CD8+ T cells. Similar to NK cells, MHC class I-specific inhibitory receptors might subserve on CD8 T cells an important negative control that participates to the prevention of autologous damage but may also contribute to viral escape.

We found that the expression of CD94 and KIRs is increased on CD8 T cells from HIV patients, and the accumulation of CD94 +CD8+ T cells is driven by HIV replication. The expression of iNKR was found associated with a poor cytokine response (IFN-gamma and TNF-alpha) upon TCR triggering, not restored by IL-15.

The expression of CD94 and KIRs on HIV- and CMV-specific CD8 T cells was investigated with specific tetramer staining, and these NKR were barely detectable at the surface of virus-specific T cells, in contrast to CD85j. Characterization of the maturation stage of CD85jCD8 T cells, and of the impact of CD85j on CD8 T cell response upon TCR triggering with HIV-specific peptides is currently under investigation.

S112
RISP, a Novel Rev-interacting Protein
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Yeast-two hybrid screening of a T-cell cDNA library with Rev as bait led to isolation of a novel human cDNA product (16.4.1). 16.4.1-containing fusion proteins showed predominant cytoplasmic localization, which was dependent on CRM1-mediated export from the nucleus. Nuclear export activity of 16.4.1 was mapped to a 60 amino acid region and a novel transport signal identified. Interaction of 16.4.1 with Rev in human cells was shown in a mammalian two-hybrid assay and by colocalization of Rev and 16.4.1 in nucleoli, indicating that Rev can recruit 16.4.1 to the nucleus/nucleoli. Rev-dependent reporter expression was inhibited by overexpressing 16.4.1 and stimulated by siRNAs targeted to 16.4.1 sequences, demonstrating that 16.4.1 expression influences the transactivation function of Rev.

These results suggest that 16.4.1 may act as a modulator of Rev activity and it has been named RISP (Rev interacting shuttling protein). The experimental strategies outlined in this study are applicable to the identification and biological characterization of further novel Rev-interacting cellular factors.

S113
Targeting the Human CD3γ Gene Promoter
By HIV-1 and HTLV-1: Two Distinct Mechanisms Involving A Transcriptional Regulatory Element and Chromatin Remodeling
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Our studies show that HIV-1, HIV-2, and HTLV-1 infection all provoke a progressive defect in surface T cell receptor expression. A specific loss of CD3γ transcripts is responsible for the defect after HIV-1 or HIV-2 infection. Alternatively, while CD3γ transcripts are lost first after HTLV-1 infection, their reduction is followed several months later by a loss of CD3δ and subsequently CD3ε mRNA. Studies of CD3γ transcriptional control revealed parallels with elements regulating HIV-1 gene expression, including a downstream element reminiscent of HIV TAR. Mutant and deletion CD3γ promoter constructs delimited a 53 bp region downstream from the major transcription start site as critical for positive gene expression. EMSA experiments demonstrate that this sequence functions through an RNA rather than a DNA intermediate, which can bind three specific nuclear protein complexes. Deletion of U at +9 and +37 kills promoter activity. Alternatively, progressive silencing of the CD3 γ gene locus by HTLV-1 functions via chromatin remodeling, characterized by increased binding of Ikaros to the CD3 γ promoter and the CD3δ enhancer. Expression of the CD3 genes can be reactivated in HTLV-1 infected cells by the synergistic action of the histone deacetylase inhibitor trichostatin A and the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine. The importance of viral targeting of the CD3 genes will be discussed.
S114
Ligation of CD28 Alone by its Natural Ligand, CD86, Induces Lipid Raft Polarization in Human CD4 T-cells
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Background: Stimulation of naive CD4 T-cells with anti-CD3/CD28-coated beads leads to polarization of lipid rafts (LRs). Since neither stimulus alone can polarize LRs, it has been postulated that a major role of costimulation is to facilitate LR aggregation. CD86 is upregulated or expressed aberrantly on immune cells in many autoimmune and infectious diseases, including HIV-1 infection.

Methods: To ligate CD28, we used an Ig fusion with extracellular domain of CD86 bound to magnetic beads, or KS62 cells expressing CD86. Cell-bead conjugates were plated onto coverslips, stained with anti-GM1 or cholera toxin B, and K562 cells expressing CD86. Cell-bead conjugates were plated extracellular domain of CD86 bound to magnetic beads, or

T oligate CD28, we used an IGF fusion with

conjugates were plated onto coverslips, stained with anti-GM1 or cholera toxin B, and LR polarization was visualized by digital immunofluorescence microscopy.

Results: Ligation of CD28 by natural ligand, but not antibody, induced polarization of LRs at the cell-bead interface, in absence of TCR ligation. This correlated with activation of Vav-1, increased IC calcium and translocation of NFkB p65, but did not result in proliferation or cytokine production. Using DNA microarrays, we detected induction of a subset of genes, including the Egr1 family of transcription factors. Engagement of CTLA-4 blocked CD86Ig induction of LR polarization and new transcription.

Conclusion: Lipid raft polarization can occur without TCR triggering, driven solely by CD28/CD86. HIV virions preferentially incorporate CD86 into their membranes and lipid rafts facilitate HIV entry. These virions have been shown to trigger NFkB activation in a CD86-dependent manner. The heightened immune activation in HIV infection enhances CD86 expression, which could induce LR polarization between infected cells and resting T-cells, permitting virological synapse formation and HIV entry. The ability of CD86 to induce LR may in part explain susceptibility of resting T-cells to HIV infection.

S115
Follow-up of HIV Infected Patients Who Received a Therapeutic Anti-Tat Vaccination
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Basic and epidemiological documentation as well as non human primate experimentation prompted us to develop anti Tat therapeutic vaccine based on Tat toxoid, a non toxic but immunogenic HIV-1 Tat derivative. Phase I trial conducted at the Hemophilac Bonomi Center of Milan (Pr. Gringeri) in 1997–1998 and Phase II/II trial organized by Aventis Pasteur showed that the Tat toxoid immunogen adjuvanted with either Seppic oil (ISAS1), DcChol or Alum was safe and immunogenic on patients under HAART or not. A structured treatment discontinuation study (STI) monitored according to EU guidelines was conducted at Brussels (Pr. Clumeck) on the 31 vaccinees who received either a DcChol adjuvanted Tat Toxoid (n = 12), a DcChol placebo (n = 8) or non adjuvanted Tat Toxoid (n = 11). Anti-Tat Ab responders (n = 9) exhibiting both high serum Ab titers (>10 pg/ml) and a serum anti-Tat neutralizing capacity at the end of the vaccine trial remained significantly HAART-free. By contrast in patients in whom HAART has been prescribed during STI, serum collected prior to treatment did not exercise anti-Tat neutralizing capacity.

S116
Loss and Recovery of Vg2Vd2 T cells in HIV/AIDS
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HIV infection results in an early and profound loss of Vg2Vd2 T cells, and this is the only T cell receptor-specific depletion that is common to all individuals with HIV/AIDS. A similar pattern of Vg2Vd2 T cell depletion occurs in mycobacterium infection and during malaria. We hypothesize that the loss of Vg2Vd2 T cells, irrespective of the primary cause for this loss, results in disease acceleration during HIV+ tuberculosis or HIV+ malaria coinfections and also leads to increased incidence of cancer in the context of HIV/AIDS.

Using a macaque model for mycobacterium infection, we demonstrated the dynamics of Vg2Vd2 responses to infection with attenuated M. bovis (BCG). In this study, we confirmed that activation-induced cell death is the mechanism for Vg2Vd2 T cell depletion in vivo, and confirmed this with in vitro studies using human T cells. In vitro studies with human PBMC allowed us to understand the specificity of T cell receptor recognition of human lymphomas. Using AIDS-related and non-AIDS related B NHL, we defined the T cell receptor structures required for tumor recognition and showed they are indeed, missing in HIV-infected individuals. Lastly, we evaluated longitudinal specimens from HIV-infected individuals receiving HAART and showed that recovery of the Vg2 repertoire was occurring by the use of previously rare sequences that survived initial HIV-mediated depletion and were expanded during the treatment interval. Importantly, repertoire recovery occurred in the absence of new cell synthesis, consistent with observations on CD4 and CD8 T cell repertoire and changes during HIV infection and treatment.

The Vg2Vd2 T cell subset is an example of indirect or bystander cell killing during HIV infection, and its impact on immunity to seemingly unrelated pathogens. Destruction of Vg2Vd2 T cells and the critical elimination of the Vg2-jg1.2 expressing subset, likely accounts for the mutual acceleration of HIV, malaria and tuberculosis diseases, and may explain the specific of enhanced risk for AIDS-related neoplasia. We continue efforts to comprehend this unusual T cell subset both as a model for the
impact of HIV in host immunity, and to define new targets for immunomodulatory therapy.

S117 Approaches to Target Conserved Conformational Epitopes in HIV Envelope
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Background: HIV-1 envelope glycoprotein (Env) is the primary target for inducing neutralizing antibodies against the virus Env yet only a small fraction of antibodies elicited are directed against conserved epitopes. Thus, the antibodies produced during infection and vaccination (to date) have been limited in their ability to neutralize heterologous primary isolates. Since interactions between the virus and its receptor and co-receptor are critical for virus entry into the cell, targeting conserved functional epitopes located in or near the receptor and co-receptor binding sites may be the key for developing an effective vaccine. We as well as others have shown that Env-CD4 complexes are capable of inducing broadly neutralizing antibodies, however use of sCD4 as part of the vaccine has the potential for inducing an autoimmune response.

Materials and methods: Therefore, we are evaluating several approaches, including such as CD4 peptide mimetics (CD4M33), small molecules and novel scaffolds such as invasin and tat (onto which the CD4 binding domain is grafted). This may facilitate targeting of conserved functional epitopes on liganded forms of Env and also reduce immune responses directed towards CD4

Results: We have developed and characterized stable Env-CD4M33 complexes and evaluated them in rabbits for inducing neutralizing antibody responses. In a parallel approach, we have used BMS-853 as a filter to identify 100 structurally similar small molecules, and screened them for their ability to compete with CD4 and b12 for binding to Env as well as their ability to induce conformational changes as reflected by enhanced binding to 1b7 binding. We have so far identified three classes of small molecules that: i) compete for CD4 binding only, ii) induce conformational change in Env without competing for CD4, and iii) compete for CD4 binding and induce conformational changes.

Conclusion: We plan to use small molecules for stabilizing Env in liganded or un-liganded forms for further evaluation of immunogenicity in rabbits. These studies should yield important structural information about the apo and liganded structure of Env and the resulting exposure of conserved epitopes for vaccine applications.

S118 Characterization of gp120 and Its Single-chain Derivatives, gp120-CD4 and gp120-M9: Implications for Targeting the CD4i Epitope in HIV-1 Vaccine Design
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Single-chain derivatives of gp120 linked to the first two domains of CD4 (gp120-CD4D12) or to the CD4 analogue CD4M9 were assessed for their abilities to elicit CD4-induced neutralizing antibodies. Both complexes showed binding to a CD4i epitope as defined by the mAb 17b. Addition of exogenous CD4 did not increase 17b binding to gp120-CD4D12 but augmented binding to gp120-M9 perhaps reflecting the lower binding affinity of M9 to gp120 compared to CD4D12 or suggesting that M9 does not completely fill the CD4 binding site of gp120. Vaccination of guinea pigs and rhesus monkeys with recombinant protein or DNA prime followed by protein boosting generated broadly neutralizing antibodies only for sera generated against gp120-CD4D12. Passage of these sera over a CD4D12 affinity column removed neutralizing activities. Rhesus monkeys were also immunized with gp120-human CD4 or gp120-rhesus CD4 complex. Virus-neutralizing antisera were observed for each of these groups, but titers were much greater for the gp120-human CD4 complex. Neutralizing antibody titers showed a significant correlation to CD4 antibody titer for both vaccine groups. These data suggest that most neutralizing antibodies generated by gp120-CD4 complexes are directed against CD4.

S119 C3d Enhancement of Anti-Env Immunity Using Modified HIV-1 Envelopes
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Background: DNA vaccines expressing the HIV-1 envelope (Env) have been relatively ineffective at generating high-titer, long-lasting, immunity. Conjugating the molecular adjuvant, C3d, to HIV-1 Env enhances both humoral and cellular immunity.

Methods: BALB/c mice were vaccinated with DNA plasmids (weeks 0, 4, and 8) expressing wild-type or modified envelope proteins. Each Env immunogen was tested alone or conjugated to multiple copies of the molecular adjuvant, C3d. Both humoral and cellular immunity were analyzed.

Results: DNA vaccines expressing a fusion protein of the soluble human CD4 (sCD4) and the Env(gp120) enhanced the immunogenicity of the expressed fusion protein only when conjugated to mC3d3. Monoclonal antibodies that recognize CD4-induced epitopes on Envgp120 efficiently bound to sCD4-gp120 or sCD4-gp120-mC3d3. In addition, both molecules bound to cells expressing appropriate coreceptors in the absence of cell surface hCD4. Mice vaccinated with DNA plasmids expressing either gp120-mC3d3 or sCD4-gp...
120-mC3d3 elicited antibodies that neutralized homologous virus infection. However, the use of sCD4-gp120-mC3d3-DNA elicited the highest titers of neutralizing antibodies that persisted after depletion of anti-hCD4 antibodies. Interestingly, only mice vaccinated with DNA expressing sCD4-gp120-mC3d3 had antibodies that elicited cross-protective neutralizing antibodies. In a separate set of experiments, the unique sequence found in the crown of the V3 loop of the envelope from the CD4-independent isolate, HIV-1K2, was used to elicit cross-clade neutralizing antibodies. The codons encoding for the V3 loop amino acids, Pro-Met, were introduced into the sequences encoding the gp120ADA (RS) or gp120g9.6 (RX4). Mice vaccinated with gp120ADA-mC3d3-DNA with the Pro-Met mutation had antibodies that neutralized HIV-1 infection, but not the gp120g9.6-mC3d3-DNA.

Conclusion: Therefore, the use of sequences that expose cryptic epitopes by CD4 or found in CD4-independent viral isolates expose neutralizing epitopes that can elicit broad, cross-clade immunity.

S120
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S120

S121
Fusion Complexes and CD4-independent gp120s for the Induction of HIV-1 Neutralizing Antibodies
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Background: The narrow spectrum of HIV-specific neutralizing antibodies points to the need for new immunogens based on highly conserved epitopes. HIV-1 infects host cells by membrane fusion: during this process conserved epitopes are exposed on the viral glycoprotein gp120/41 that may be used as targets for the induction of antibodies against HIV-1. Neutralizing antibodies against different heterologous HIV-1 isolates may be obtained by immunizing mice with fusion complexes on which conserved epitopes have been stabilized by fixation, or with gp120/41s with a CD4-independent phenotype on which these conserved epitopes may be already exposed.

Methods: Fusion complexes were prepared using cells expressing gp120/41 cocultivated with cells expressing the receptors CD4-CCR5 at different temperatures, corresponding to intermediate stages of the membrane fusion process, and stabilized using different fixatives. Mice were immunized to reveal the induction of HIV neutralizing antibodies and their spleen cells used to generate hybridoma clones to be tested for the production of neutralizing monoclonal antibodies. In addition, syngeneic balb/c mouse cells expressing gp120/41 with a CD4-independent phenotype have been prepared by transfection and using viral vectors and are being used to assess their capability to induce broad spectrum neutralizing antibodies.

Results: Results obtained indicate that: 1) fusion complexes were immunogenic and induced neutralizing antibodies against RS and X4 HIV-1 heterologous isolates; 2) extensive purification of antibodies allowed the removal of any aspecific cytotoxic effect; 3) complexes prepared at higher temperatures were more immunogenic and induced higher titers of neutralizing antibodies; 4) titer of neutralizing antibodies was not affected by the fixative used; 5) neutralizing activity was retained after CD4-CCR5 antibody removal; 6) CD4-independent gp120s were expressed in syngeneic mice cells and recognized by HIV-1 positive human sera; mice immunizations are currently ongoing.

Conclusion: Results show that fusion complexes are immunogenic and induce neutralizing antibodies against heterologous HIV-1 isolates. Removal of non-specific inhibitors that confused early promising results is necessary to obtain a specific antibody response. The production and selection of neutralizing monoclonal antibodies will be useful to identify specific immunogenic structures and epitopes to be used to induce neutralizing antibodies when administered in a suitable delivery system. Antibodies obtained by immunizing with CD4-independent gp120s and antibody fragments isolated through phage display libraries panning will be evaluated for their broad-spectrum neutralizing activity against different HIV-1 isolates with and without the addition of sCD4 or CD4-like antibodies.

S122
Epidemiology and Evolution of Antiretroviral Drug Resistance
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The development of HIV drug resistance is a formidable obstacle in the long term success of antiretroviral therapeutic regimens. To date, resistance has occurred against all drugs that are in widespread use for treatment of HIV disease. Moreover, HIV drug resistant viruses can be sexually transmitted and it is now estimated that as many as 10% of new HIV infections in Western countries may carry at least one mutation associated with HIV drug resistance. In some cases, newly infected individuals may even harbour viruses that are resistant to two or even three classes of antiretroviral drugs. Studies have shown that mutations which are transmitted from one individual to another may often persist over long periods of time i.e. 2–7 years. Although many mutations may also revert to wild-type, a danger is that they will have become permanently archived in a patient’s long-lived memory T cells, and that this may preclude future therapeutic options.

At the same time, many of the mutations associated with HIV drug resistance may cause diminished “replicative capacity” and, indeed, some clinical studies have shown that at least some individuals infected with multi-drug resistant strains may have lower viral loads over periods of several years than do individuals infected with wild-type strains. It is also interesting that some of the mutations associated with HIV drug resistance...
may be less easily transmitted than others or are present at diminished frequency in newly infected hosts. In general, it appears as though thymidine analogue mutations (TAMs), associated with resistance to zidovudine and stavudine, as well as mutations associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs), may be transmitted fairly efficiently while mutations associated with resistance to other nucleosides, e.g. 3TC, TDF (M184V and K65R), may be transmitted much less frequently.

This subject has relevance in view of the widespread use of co-formulated nevirapine/3TC/stavudine (TRImune) as a first line regimen favored by the World Health Organisation (WHO) for use in many developing countries. There is a strong possibility that resistance might develop over time against several of the drugs in this regimen that have low genetic barriers for resistance, meaning that only a single point mutation in the reverse transcriptase gene may yield significantly diminished levels of antiviral activity (3TC and NVP). Nonetheless, we should support this WHO initiative (3 x 5) because this regimen is likely to have the greatest impact and save millions of lives during the next several years and, as well, will impact on rates of HIV transmission.

S123
yesThe Complexities of ART Which Prevent Durable Viral Suppression
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The complexities of ART which prevent durable viral suppression should not be under estimated, and include the need for absolute adherence, the rapid development of resistance, and the impact of cross resistance, unequal potency of ARV’s, pharmacokinetic limitations, and drug toxicities. Providers of HIV care in the U.S. are faced with the increased complexities of care as they encounter growing numbers of patients with drug resistance. Reliance on frequent use of viral load monitoring and genotypic analysis has become the recommended norms.

As the world prepares for antiretroviral scale up programs in resource limited nations, the need to achieve durable antiretroviral therapy (ART) success across multiple different patient populations and to decrease the reliance on frequent viral loads and genotypes to gauge treatment outcomes has become more apparent. Treatment programs in clinics which serve patients in resource limited settings are trying to address these complexities to improve long term treatment success.

S124
Chemoprophylaxis and HAART Therapy in Botswana
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Botswana has one of the highest rates of HIV infection in the world, with 37.4% of adults infected in 2003 according to UNAIDS. To address the problems of widespread infections, the government of Botswana instituted aggressive programs for the use of antiretroviral (ARV) drugs in both chemoprophylaxis for pregnant women and HAART therapy for treatment of clinical AIDS. At present, about 25% of AIDS patients receive HAART, and about 40% of HIV positive pregnant women receive chemoprophylaxis. After the country adopted an “opt out” policy for HIV testing in early 2005, these numbers are rapidly rising. Both the chemoprophylaxis regimen and the first line regimen for AIDS, include nevirapine (NVP). This is likely to cause problems with drug resistance when mothers with young infants need therapy, as about half of the mothers who receive NVP during labor reveal genotypic resistance when analyzed. In a recent trial we showed that NVP given only to newborn infants is as effective as when given to the mother during labor and the newborn infant, at least when used on a background of zidovudine (ZDV). This may provide a mechanism to avoid the establishment of NVP-resistance in HIV-positive mothers. HIV-1C, the subtype of southern Africa, shows higher rates of NVP-resistance as compared to HIV-1A or HIV-1D. Despite the potential for high levels of resistance, and advanced stages of disease, AIDS patients were very successfully treated with HAART. With initiation of drugs at median plasma viral loads of about 400,000 and median CD4 counts below 100, 87% of patients treated with ZDV+ 3TC+ NVP had undetectable RNA at 24 weeks after treatment, and 79% had undetectable RNA at 48 weeks. CD4 numbers increased by 149 at 24 weeks and 204 by 48 weeks. ZDV and 3TC containing regimens gave lower rates of viral resistance and less toxicity than regimens containing DDI and D4T.

S125
Minimal Requirements for an Effective Antiretroviral Treatment in Burma
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The availability of the Antiretroviral (ARV) generic formulations since 2000 has increased dramatically the demand to access care and ARV drugs in the developing countries. Most of the subsaharian African and Asian countries which reported an epidemic have started to benefit from the various international supports.

Although there is a global consensus among the developed countries to make the ARV access easier for the developing countries, the frequent lack of sustained structures and well trained medical teams in addition to the lack of facilities are at high risk of emergence of resistant viral strains and compromisement of the expected benefits.

In Burma the ARV implementation and scaling up have considered the possible mechanisms to decline the risk of emergence of a rapid resistance to the ARVs.

Burma one of the poorest country of the world has 50 millions of inhabitants. The UNAIDS estimates that the prevalence of the HIV infection is between 2 and 3% of the global population. It is the third country of the South East Asian region (SEAR) in term of prevalence after Cambodia and Thailand.
The mix of high level of poverty, low awareness, poor health care, low political involvement, and total lack of governmental funds dedicated to this infection led the few skills to collaborate closely to set up a comprehensive programme aimed at fighting HIV/AIDS in this country prevented from accessing to most of international supports because of the political sanctions.

The French government signed an agreement in 1996 with the Burma’s medical school to train MDs in France for HIV/AIDS. 6 out of them have already been trained. The French oil company Total joined this programme in 2004 to provide medical scholarships. The 7th MD that the Total company has supported is the first Burma’s paediatrician trained especially for HIV/AIDS in children in Paris. After their return, these medical leaders have been posted in the 2 referral HIV/AIDS departments implemented in the 2 main cities (Yangon and Mandalay) with the technical support of WHO.

The Yadana Company operated by Total in Burma has provided the financial support for the purchase of the ARVs which are ordered by WHO to avoid any custom fees, to ensure a regular supply and avoid shortages. The recipient for the Total funds is the Union: an Int’NGO well implemented in numerous developing countries in charge of Tuberculosis (TB). TB is the most frequent infection encountered in HIV patients and occurs at any time of the disease progression. This choice is a major contribution, since it has provided for the first time the opportunity to detect and treat HIV patients at an earlier disease stage. Up to now the government allowed to perform the HIV testing only in patients with evident clinical diagnosis of AIDS. The benefit of the ARV treatment started at a late disease stage is very limited compared to the one when started at an earlier stage. This earlier HIV testing possibility is the first step toward the voluntary testing in asymptomatic people who just wish to know their own status.

The HIV testing center has been implemented for the first time outside the walls of the AIDS center in the TB center. The test kits are provided by UNAIDS. It is the first time that the test is done anonymously in a public place.

The setting for the programme is the General Hospital in Mandalay, the second city of the country. All the criteria required to start the programme were met at this place. The patients are monitored by the MDs trained in France. The organization of the management of the patients has been set up by the Union which delivers the drugs to the hospital, supports the cost of the laboratories facilities as well as the cost of the management of the opportunistic infections. 2MDs from this Int’NGO specialized in TB and in HIV in the developing countries are responsible for this program.

This is the first free access to the ARVs and to the care for HIV infection in a public hospital in this country.

The anonymous testing (approved by the government), the monitoring of the patients by the well trained MDs, the management of the programme performed by the MDs of the Union, the funds provided by Yadana company, and the support of WHO is an example of a global commitment.

One major objective of the programme is to limit the resistant viral strains which should start in Burma in patients followed up in the private sector where the drugs prescribed: monotherapy, bitherapy or tripletherapy are related to the shortages and supplies, to the variable medical knowledge and to the patient’s financial resources. The success of this programme should drive the few thousands patients followed up in the private sector to the public sector providing since recently confidentiality and free access to the ARV treatment and care.

This programme is in the frame of the WHO 3 × 5 initiative and contributes to the sustained development. This rare example of a synergistic and close collaboration between different structures should help to find additional funds to join the programme and expand it to other hospitals in Burma.
versions of the ARVs were made available to many African countries. Thus, by 2001, albeit at a painfully slow and glacial pace, access began to improve in low and middle-income countries. Today, massive injection of funds have begun to flow and generic version of the ARVs are far more accessible, yet overall, less than 20% of PLHIV in low and middle-income countries have access to ARV-based treatment.

One resource-poor country that is changing the poor access to ARVs is Guyana, one of the poorest Caribbean countries. In 2004, the World Development Report ranked Guyana, in terms of the HDI, at 103. Guyana has an HIV prevalence of between 3.5 to 5.5%. It is estimated that about 3,000 persons are in need of ARVs. By 2001, no formal ARV-based treatment program existed. Today, approximately 1,000 persons are on ARV treatment program. Guyana benefits from funding through the Global Fund, the World Bank and is one of the PEPFAR countries. There are also technical assistance through CIDA, PAHO/WHO and UNICEF.

Guyana also has offered universal treatment, including ARVs and laboratory testing for CD4 to everyone living with HIV and who reaches the criteria established for the initiation of treatment. However, roll out to reach everyone who needs to be on ARV treatment has been slow due to constraints of capacity. Still, the treatment program is considered to be one of the success stories in the fight against HIV/AIDS. Thus, far, the ARVs used in the treatment of HIV/AIDS patients have been generic ARVs produced by a local company, the New GPC. There are 15 locally produced formulations and combination therapy with LSN can be obtained at a cost of $US140 per patient annually.

S131
Abstract withdrawn
Retrovirology 2005, 2(Suppl 1):S131

S132
Abstract withdrawn
Retrovirology 2005, 2(Suppl 1):S132

S133
Improved Immunological Values in HIV/AIDS Patients on Combined ARV/Antihelminthic Therapy
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Retrovirology 2005, 2(Suppl 1):S133

Background: The immune activation induced by enteric pathogens play a significant role in HIV/AIDS pathogenesis with greater potential in disease progression especially in resource limiting countries where incidence of helminthic infestation is high. These results describe the outcome, tolerance and safety of a combined ARV/anthelmintic therapy in HIV/AIDS patients in Lagos Nigeria.

Methods: Eighty-nine HIV/AIDS patients, 62% males, mean ages 52 ± 17 were randomly selected at three HIV/AIDS treatment centres in Lagos, Nigeria. Study period was between October, 2004 and April 2005. Three serial stool samples were collected from these patients and screened for helminthic pathogens. Ascaris lumbricoides, Trichuris trichuria and Hookworm representing 41%, 28% and 21% respectively were the most predominant helminths. Viral load, CD4 cell count, weight, haematological and chemistry parameters were noted at baseline. These parameters were again determined at 3 and 6 months after combined therapy of ARV and anthelmintic drugs.

Results: A general correlation exists between HIV plasma viral load and the number of excreted worm eggs in stool of studied candidates. In group ‘A’ individuals with complete eradication from helminths, there were significant reduction in HIV/AIDS related symptoms notably, pallor, abdominal pains, diarrhoea and mean increase in CD4 count. Also, a mean reduction of 0.32log10 copies/ml of viral load, weight-gain, increase haematological and decrease chemistry parameters were generally observed at 3 and 6 months follow-up from baseline. A median increase of 0.14log10 copies/ml of viral load was observed among group ‘B’ candidates who were either persistently helminth-negative or helminth-positive at 3 and 6 months follow-up from baseline. Two of the patients under study were lost to full-blown AIDS among group ‘B’ candidates.

Conclusion: Results of this study showed, that the combine use of ARV and antihelmintic drugs in the treatment of HIV/AIDS patients is safe, well tolerated and offers effective viral load suppression with attendant increase in immunological values. This therapeutic approach is of great relief for patients in developing countries when cost and accessibility to ARV drugs are considered.

S134
Anti-Retroviral Therapy (ART) Monitoring and the Development of the Oligonucleotide Ligation Assay for Detecting Critical Drug-resistant Mutations in HIV-2 Patients in Preparation of the Global Fund Initiative in The Gambia
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Retrovirology 2005, 2(Suppl 1):S134

Background: Data on resistance mutations in HIV-2 infected patients are limited. With the Global Fund Initiative a large number of HIV-2 patients will receive ART. It is important to monitor the response of HIV-2 infected patients to ART and to study their resistance profiles. Developing cheaper and more sustainable assays are a priority, especially in resource-poor settings.

Materials and methods: A cohort of 8 treatment-naive HIV-2 infected patients received ART and was studied longitudinally for about 7 years; clinical, immunological, virological and data were collected. The entire HIV-2 protease and RT was amplified, sequenced and analysed for several time points. An Oligonucleotide Ligation Assay (OLA) was developed for the detection of resistance mutations.
Results: The mutations M184V (7/8), Q151M (1/8), K65R (1/8) mutations were observed. HIV-2 OLA was successfully developed for M184V, a classic Lamivudine mutation and for Q151M, a multi-drug resistance mutation.

Conclusion: We identified important HIV-2 mutations and developed a simple, economical and sustainable HIV-2 OLA for the detection of these resistance mutations.

S135
Outcome of Treatment of Tuberculosis in HIV Infected Persons in the Era of Highly Active Antiretroviral Therapy (HAART) as Seen in the Second City Tuberculosis Hospital in Saint Petersburg, Russia
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Background: Tuberculosis (TB) is the leading cause of morbidity and mortality among persons with HIV/AIDS in highly affected regions including Russia. Despite the fact that Russia has the infrastructure for the treatment of TB unlike developing countries, authorities are not yet paying attention to provide antiretroviral treatment for these category of people who are mostly IDU users, former prisoners and jobless. As such the benefit of highly active antiretroviral therapy (HAART) in the treatment of patients co-infected with tuberculosis (TB) and human immunodeficiency virus (HIV) has never been explored in this country.

Objective: To assess the risks and benefits of administering highly active antiretroviral therapy (HAART) during the treatment of tuberculosis (TB) in HIV-infected patients.

Materials and methods: 53 HIV-infected persons diagnosed with active TB were recruited in the special HIV and TB unit of the second city tuberculosis hospital in Saint- Petersburg, Russia into an observational, prospective study aimed to evaluate tuberculosis treatment outcomes between Dec.2003 and March 2005. Only 15 patients (28.3%) with a median CD4 cell count of 235 cells/mm$^3$ were on ART (AZT+3TC+EFV), while the rest 38 (71.7%) with a median CD4 cell count of 267 cells/mm$^3$ received antituberculosis medications only. Clinical and immunologic responses were assessed within the two groups by comparing incidence of new AIDS-associated opportunistic illnesses (OIs), adverse events and CD4 cells dynamics.

Results: Among antiretroviral patients, CD4 cell count increased to 297 cells/mm$^3$ versus a decrease to 212 cell/mm$^3$ in patients on anti-tuberculosis treatment alone (p = 0.10). The risk for HIV progression to new OIs was lower among antiretroviral group (3.5 versus 24.5%; relative risk (RR) = 0.14. Adverse events (AE) progression to new OIs was lower among antiretroviral group (3.5 vs. 24.5%; relative risk (RR) = 0.14). Among patients with CD4 cell counts < 100 cells/mm$^3$ have a high event risk during the intensive phase of anti-TB treatment. These pilot data should be taken into account when deciding to initiate HAART in co-infected patients with CD4 cell counts < 100 cells/mm$^3$.

Figure 1 (abstract S135)

CD4 cell dynamics in HAART

S136
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S136

S137
The Cell Biology of HIV-I Entry
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The interaction of HIV-1 envelope glycoproteins with CD4 and coreceptors triggers a barrage of conformational changes in HIV-1 gp120 and gp41, which lead to the gp41 six-helix bundle formation that drives the membrane merger and eventual fusion. Although significant progress has been made in understanding HIV fusion, little is known about the cell biological processes that impact on HIV entry. Manipulating lipids has yielded important insights into the role that membrane of the host cell plays in regulating the HIV-1 entry process. In this talk I will describe how lipid manipulation affects HIV-1 entry by 1) altering the disposition and lateral mobility of HIV-1 receptors, 2) endosomal re-routing, and 3) cytoskeletal remodeling. Manipulation of lipid metabolism may therefore constitute a promising avenue for the development of antiretrovirals.

S138
Regulation of Cellular and Virion APOBEC3G (A3G) Complexes
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A3G is detectable in both high molecular mass (HMM) and low molecular mass (LMM) complexes in different cells. Enzymatically active LMM A3G complexes are present in resting CD4 T-cells and blood derived monocytes. These cells are not permissive for HIV
infection because LMM A3G functions as a potent post-entry restriction factor for HIV and possibly other retroviruses (Chiu et al. Nature 435:108–114, 2005). The antiviral activity of LMM A3G is exerted at the level of reverse transcription but does not appear to involve extensive cytidine deamination of nascent minus strand HIV DNA. When T-cells are activated by mitogens or naïve T cells enter lymphatic tissues where IL-2 and IL-15 are produced, LMM A3G is recruited into an enzymatically inactive HMM ribonucleoprotein complex. This change in A3G complex size is associated with the acquisition of permissiveness to HIV infection. Interestingly, HIV D✈if virions incorporate the HMM form of A3G assembled with HIV genomic RNA. Accordingly, a mechanism for activation of this latent A3G complex must come into play. Recently, we have assembled preliminary evidence supporting a key role for Rnase H in the activation of the latent HMM A3G complex. Thus, Rnase H not only prepares the substrate for mutagenesis, but also activates the enzyme.

S139 T Cell Complicity in HIV Spread
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Background: The human immunodeficiency virus type-1 (HIV-1) can spread between target cells via release of cell-free virions or by direct cell-cell transmission across a virological synapse. Virus is released from T cells in a polarized manner from regions of the plasma membrane rich in raft-associated lipids and proteins.

Materials and methods: We have established a model system in which conjugate formation between HIV-1-infected (effector) and uninfected (target) T cells results in the assembly of a virological synapse (VS) at the intercellular interface.

Results: Env-receptor and adhesion molecule interactions are required for functional VS formation. Gag transits to the plasma membrane in a CD63/CBD1+ compartment. Polarization of HIV-1 Env and Gag on effector cells and subsequent viral release depends on actin and tubulin remodelling and lipid raft and PI(4,5)P2 integrity.

Conclusion: We hypothesize that viral infection triggers pre-existing T cell programs that activate elements of the T cell secretory apparatus to deliver Gag, allowing efficient virus assembly.

S140 Long-lasting Decrease in Viremia In Chronically SIVmac251-infected Macaques After Therapeutic Immunization With Combinations Of DNA Vectors
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We explored therapeutic immunization of ART-treated SIVmac251 infected rhesus macaques using a new generation of optimized DNA-based vaccine vectors that produce either secreted or intracellularly degraded SIV antigens. Macaques infected for 15–70 wks were treated with a combination of 3 drugs (ART) for 13–23 wks. During this time, the animals were immunized via the IV route with the SIV DNAs and then released from ART. Macaques receiving DNA showed a significant decrease in viral load for long periods after therapy termination compared to controls (p < 0.001). DNA vaccination, but not ART alone, led to substantial decreases in viremia. Half of the animals (6/12) continue to control viremia levels for 2 years. Cellular immune responses were immediately boosted strongly by DNA vaccination and persisted despite lower virus loads. Thus, the combination of novel forms of DNA vaccines administered during ART treatment induced an immune response able to persistently decrease viremia after removal of ART. These DNA vectors used as therapeutic vaccine may be beneficial either alone or in combination with other vaccine modalities as an addition to antiretroviral treatment.

S141 Nef and HIV/SIV Replication
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Nef is an accessory protein of primate lentviruses that is required for high levels of viremia and progression to AIDS in infected individuals. Our studies on Nef have centered around its effects on budding and release of optimally infectious virions from cells. To these ends, we have characterized interactions between Nef and the transframe portion of the GagPol polyprotein, AIP1 and cholesterol. Specific sequences in Nef were identified that mediate these interactions. For example, the flexible loop in Nef binds p68 that connects Gag and PR. It is via this interaction that Nef helps to aggregate viral structural proteins in lipid rafts and is itself incorporated into progeny virions. A sequence in the core of the protein binds AIP1 that helps HIV-1 form multivesicular bodies and be released from cells. Indeed, fusing Nef with a mutant Gag that lacks the late domain allows for the release of VLPs into the supernatant. Finally, at its very C-terminus, Nef binds newly synthesized cholesterol, which is incorporated into viral particles that are more infectious. To determine which one of these interactions was more important for high levels of viremia and progression to AIDS in the rhesus macaque, multiple mutations were engineered into the nef gene in SIVmac239. Of interest, before high levels of viral replication could be observed in monkeys infected with the mutant SIVmac239, both the binding to GagPol and AIP1 had to be restored in the mutant Nef protein. From these studies, it appears that Nef also plays a critical role in the later phases of the viral replicative cycle and ensures that optimally infectious virions are released from infected cells.

S142 Induction of 8-oxoguanine DNA Glycosylase 1 Gene Expression by HIV-1 Tat
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In order to identify the cellular gene target for Tat, we have performed gene expression profile analysis and found that Tat
the Vif-APOBEC Interaction

**S143**
**Abstract withdrawn**

*Retrovirology* 2005, 2(Suppl 1):S143

**S144**
**Natural Resistance to HIV Infection: the Vif-APOBEC Interaction**

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Recent work has demonstrated that members of the APOBEC family of cellular polynucleotide cytidine deaminases – most notably APOBEC3G and APOBEC3F – are potent inhibitors of HIV infection. In the absence of the viral Vif protein, these two DNA editing enzymes are encapsidated by budding virus particles and then catalyse the deamination of cytidine (C) to uridine (U) in negative sense reverse transcripts. This results in guanosine (G) to adenosine (A) hypermutation of viral plus stranded cDNA and reduced accumulations of viral DNA. Unexpectedly, recent structure-function analyses of APOBEC3G have revealed that substantial anti-viral phenotypes can be achieved in the absence of cytidine deamination. The relative contributions of these editing and editing-independent activities to the inhibition of infection in the absence of Vif remain to be defined. One view of the opposing functions of Vif and the APOBEC3G/F proteins during natural infection is that they are in are in “conflict” with each other. Accordingly, increases in APOBEC protein function or levels, or interference with Vif function, could culminate in enhanced anti-viral function and/or mutation rates. Whether such shifts in the Vif/APOBEC balance can influence the natural history of HIV infection is a critical future question, and supporting evidence would further indicate that perturbing this balance deserves consideration as a future therapeutic strategy.

**S145**
**Mechanisms Contributing to Control of Viremia**

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Comparison of different cohorts of SIV-infected rhesus macaques that control viremia may help to identify the mechanisms that prevent progression towards AIDS. We study 3 groups of macaques able to control viremia to various extents. Group 1 was infected with live-attenuated Rev-independent SIV able to persistently control viremia over more than 7 years. Group 2 are live-attenuated SIV-infected macaques additionally challenged with pathogenic SIVmac251 and controls the challenge to various levels (<105 copies/ml) for more than 4 years. Group 3 are SIVmac251-infected animals therapeutically immunized using DNA vectors during ART. These animals control viremia after release from ART for more than 18 months (<106 copies/ml). Animals in groups 1 and 2 developed long-lasting humoral and cellular immune responses. Animals in group 3 have persistent increases in cellular and humoral responses leading to virus containment and slower onset of disease. The understanding of the underlying mechanism leading to protective immune responses of these 3 cohorts of ‘controllers’ will be useful for rational vaccine design.

**S146**
**Abstract withdrawn**

*Retrovirology* 2005, 2(Suppl 1):S146

**S147**
**Regulation of Host Factors Involved in HIV Budding Through Autoinhibition**

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HIV-1 engages an endosomal machinery that includes the sorting complex ESCRT-III to promote virus budding. ESCRT-III components such as CHMP3 have highly acidic N-terminal and highly basic C-terminal halves, suggesting a model in which CHMP proteins are regulated through electrostatic interactions. Consistent with this model, we find that progressively longer truncations into the acidic domain of CHMP3 lead to an increasingly potent anti-HIV budding activity, whereas the full-length molecule has no dominant-negative activity. We also find that the anti-HIV activity of CHMP3 mutants correlates strictly with their ability to interact with the isolated acidic domain in GST pulldown assays. Together, our results imply that the acidic domain of CHMP3 interacts with the N-terminal basic domain in an autoinhibitory manner, and that the exposure of the basic domain accounts for the profound anti-HIV activity of mutant CHMP proteins.
S148
HIV Replication, Immune Activation, and CD4 Depletion: What the Virus Spares is as Significant as What It Destroys

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Early depletion of mucosal CD4 T cells does not appear to substantially compromise the regenerative capacity of the immune system. Analysis of in-vivo DNA labeling suggests that in the chronic phase, activated T cells mainly arise in local proliferation bursts that resemble antigen-driven responses. Most viral replication likely occurs in such bursts. Indirect evidence suggests that cytopathic effects of the virus in this context are selective and that memory cell regeneration is spared. This and other observations question the validity of proposed mechanisms that directly link disease progression during the chronic phase to early mucosal depletion. I suggest that, paradoxically, both this early depletion and activation-induced lymphocyte turnover, while contributing to the pathogenic process in the long run, may also serve to control the rates of viral replication and evolution.

S149
Virus and Host Encoded microRNAs – A Major Role in Controlling HIV Infection?

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Background and methods: MicroRNAs (MIRs) are emerging as important regulators of gene expression through posttranscriptional control. We have developed powerful bioinformatic and biologic tools for detecting new MIRs in the whole human genome (Nature Genetics in press). These tools, were applied to the study of MIRs in several viral species including HIV.

Results: Comparing infected and non-infected cells we have revealed differential expression of several host MIRs, as well as the increased expression of some predicted viral MIRs. Several of the upregulated host MIRs have binding sites on 3’UTRs of mRNAs that play a central role in HIV infection and its life cycle. Experiments are underway to determine the role of these MIRs in the regulation of the predicted target genes and thus their involvement in viral infection. Some of the virally encoded MIRs are associated with genes known to play a central role in viral replication and latency, and are therefore under intense study.

Conclusion: 1) HIV infection is associated with upregulation of several host MIRs and appearance of viral encoded MIRs. 2) At least some of the host and of the viral encoded MIRs may have a direct effect on viral replication and latency. 3) Control of MIR expression or its modulation offers a novel approach for therapy of HIV infection. 4) The changed expression of host MIRs with HIV infection, may also lend itself to new therapies affecting the host response and susceptibility to HIV infection.

S150
Abstract withdrawn

Retrovirology 2005, 2(Suppl 1):S150

S151
yesHIV Impairs Reverse Cholesterol Transport from Macrophages: A Possible Mechanism of Atherogenic Effect of HIV-1 Infection

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Both asymptomatic HIV-1 infection and AIDS are consistently associated with increased risk of coronary artery disease (CAD). The accumulation of cholesterol-loaded ‘foam cells’ (macrophages) in the walls of arteries is a characteristic feature of atherosclerosis. Here we demonstrate that HIV-1 infection of macrophages leads to impairment of apoA-I-dependent cholesterol efflux, accumulation of cholesterol and formation of foam cells. This effect is mediated by the HIV-1 protein Nef. Transfection of RAW cells with the Nef-expressing plasmid resulted in reduction of efflux and cholesterol accumulation. Nef impaired activity of ABCA1, the main transporter of cholesterol to apoA-I. The role of HIV-infected macrophages in atherosclerosis was supported by the presence of HIV-positive foam cells in atherosclerotic plaques of HIV-infected patients. These results suggest a mechanism by which HIV-infected macrophages may contribute to atherosclerotic plaque formation.

S152
Morphine Addiction Causes Pronounced Virus Replication in Cerebral Compartment and Accelerated Onset of AIDS in SIV/SHIV-infected Indian Rhesus Macaques

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Six morphine-dependent and 3 control male Indian rhesus macaques were intravenously inoculated with mixture of SHIVgag, SHIVenv and SIV/17E-Fr. These animals were followed for a period of 56 weeks for virus replication, disease progression and immune responses. Both morphine-dependent and control macaques showed precipitous loss of CD4+ T cells but CD4 recovery was found to better in more control animals than that in the morphine-dependent animals. The plasma and CSF viral load was significantly higher in morphine-dependent group than those in the control group. Four morphine-dependent succumbed to SIV/SHIV-induced AIDS at week 18, 19, 20 and 51, post-infection with neurological disorders in 3 of those 4 animals. Other 2 morphine-dependent and 3 controls were still alive at the end of 56 week observation period. All 3
viruses replicated in the blood of both morphine-dependent and control macaques, but cerebral compartment showed a selection phenomenon and only SIV/17E-Fr and SHIV/KU crossed the blood brain barrier (BBB). The morphine-dependent macaques further showed the viral migration through blood brain barrier (BBB). Three morphine-dependent macaques (euthanized at weeks 18, 19 and 20) did not develop cellular or humoral immune responses whereas other 3 morphine-dependent and 3 control macaques developed both cellular and humoral immune responses.

S153
Prolonged AIDS-free Survival for SIV-infected Macaques Treated With Anti-FasL
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Acute SIV infection of macaques is a model for human AIDS typhified by high levels of plasma viremia that decline with the onset of specific viral immunity. SIV infection limits the development of viral immunity and persists to allow for the establishment of chronic progressive disease. The destruction of CD4+ and CD4- lymphocytes argues that both direct and indirect killing mechanisms contribute to the loss of cells. Although early intervention with antiretroviral drugs reduces viremia and disease, it is still unclear whether protection is due to diminished viral cytopathicity or due to blocking a host mechanism for cell killing that is triggered by the high level of viral replication. We tested the role of FasL killing of bystander lymphocytes by injecting monkeys with a humanized monoclonal anti-FasL. Treatment with anti-FasL during acute infection reduced the level of apoptosis of circulating T and B cells, peak vRNA levels were unaffected but higher antibody and CTL responses to viral proteins lead to lower set-point viremia among treated macaques. Reduced bystander killing and increased viral immunity were associated with attenuated SIV disease and a significant increase in the life span of infected macaques after transient treatment with a monoclonal antibody against FasL.

S154
Silencing of HIV RNA by a Hairpin-loop DNA
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We describe an RNA silencing, which inhibits HIV replication by a hairpin-loop DNA. A partially double-stranded 54mer DNA oligonucleotide (ODN) was targeted to the polypurine tract, PPT of HIV. It inhibits virus replication. We demonstrate that it prevents steps before DNA provirus formation. The effect of the ODN on HIV replication in cell culture is highly sequence-specific and sensitive to changes in length and single mismatches on either strand of the DNA. An ODN against HIV-IIIB was ineffective against HIV-Ba-L and vice versa, whereby their PPT’s differ by two of 24 nucleotides. Thus, the structure and sequence of both strands of the ODN are important in cellular assays. In vitro the ODN leads to an RNA-DNA hybrid formation at the PPT, a structure which is cleaved by the RT/RNase H in permeabilized virus particles. The hybrid at the PPT is preferentially recognized by the RT/RNase H for initiation of the second-strand DNA synthesis. This recognition is its normal biological function and shown here with the ODN. A cell extract containing cellular RNase H activities or RISC proteins, is unable to induce such a cleavage. In summary, the ODN mimicks a real step in viral replication, whereby the viral RNA is cleaved prematurely before DNA transcription is completed. The mechanism is reminiscent of RNA silencing by siRNA and supported by its relationship with RNaseH. The ODN may be a basis for drug design, because of low tendency for escape mutations.

S155
Characterization of C/EBP Binding Sites Downstream of the Transcriptional Start Site in the HIV-1 LTR
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Previous studies have shown that at least one upstream CCAAT enhancer binding protein (C/EBP) site was necessary for HIV-1 LTR activity in cells of the monocyte/macrophage lineage. However, no investigation has been performed to date on C/EBP sites downstream (DS) of the start of transcription. Analyses of 115 clade B LTRs indicated there are three potential C/EBP sites within the downstream LTR region. Electrophoretic mobility shift (EMS) analyses demonstrated one of the three sites (DS3) was able to bind members of the C/EBP family. Analyses of clade A, C, and D LTRs indicated this site was highly conserved among different clades, suggesting the presence of a functionally important cis-acting element. In comparison to the clade B consensus DS3 element, EMS analysis demonstrated the DS3 7A variant exhibited a relative high affinity for C/EBP factors, while the 3C and 7G configurations exhibited lower affinities. Additional studies demonstrated specific DS3 variants exhibited differences in relative affinity for full-length and truncated C/EBPb. Transient transfection studies utilizing parental LTRs derived from LAI, YU2, and 89.6 molecular clones containing the DS3 7A, 3C, or 7G variants exhibited altered LTR activity compared to their parental strains. These results have suggested that DS3 plays a role in regulating HIV-I transcription.
**S156**

**Sequence-Specific Vpr Binding to HIV-1 LTR C/EBP Binding Sites and Adjacent Regions**

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**Retrovirology 2005, 2(Suppl 1):S156**

Human immunodeficiency virus type 1 (HIV-1) Vpr is a virion-associated protein that transactivates the HIV-1 long terminal repeat (LTR), as well as other eukaryotic promoters. Here we used the electrophoretic mobility shift assay to demonstrate the direct binding of purified Vpr (strain pNL4-3) to HIV-1 LTR sequences that span the adjacent C/EBP site I, NF-kB site II, and ATF/CREB binding site. Binding between HIV-1 Vpr and the LTR C/EBP site II was also observed. A total of 94.7% of LTRs derived from peripheral blood displayed high relative Vpr binding affinity with respect to C/EBP site I, while only 5.3% exhibited a low relative Vpr binding affinity. Virtually all LTRs derived from peripheral blood exhibited a high relative Vpr binding phenotype relative to C/EBP site II. Additional studies have demonstrated that naturally occurring sequence variation within C/EBP site I and II can dramatically alter the relative affinity of Vpr for these cis-acting elements. Studies have also suggested a competitive interaction between C/EBP factors and Vpr for this region of the LTR. These studies suggest that Vpr may regulate the interaction of members of the C/EBP transcription factor family with the viral LTR.

**S157**

**HIV-1 LTR Activity is Altered by Recruitment of Sp Transcription Factors During Monocytic Differentiation**

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Viral replication, in part, is mediated by interactions between the HIV-1 long terminal repeat (LTR) and a variety of host cell and viral proteins. Basal and activated LTR activity is dependent on interactions between the G/C box array of the HIV-1 LTR and the Sp family of transcription factors. The effect of monocytic differentiation on Sp factor binding and transactivation has been examined with respect to the HIV-1 LTR. Primary monocyte-derived macrophages (MDM), as well as monoblastoid (U-937 and THP-1) and myelomonocytic (HL-60) cell lines were utilized in both the absence and presence of chemical differentiating agents to model selected aspects of monocytic differentiation. The binding of Sp1, full-length Sp3, and truncated Sp3 to a high affinity HIV-1 Sp element was examined utilizing electrophoretic mobility shift analyses. Sp1 binding increased relative to the sum of full-length and truncated Sp3 binding following PMA-induced monocytic differentiation in the cell lines. Sp binding ratios obtained with nuclear extracts from PMA-induced cell lines were also shown to correlate with those derived from studies performed with extracts from primary MDMs. This Sp binding phenotype was shown to alter the transcriptional activation generated by the HIV-1 G/C box array.

**POSTER PRESENTATION**

**P1**

**Immunodominant Anti-Gag SLYNTVATL Responses in HIV-patients With More Than Five Years of HAART-induced Undetectable Plasma Viremia**

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**Retrovirology 2005, 2(Suppl 1):P1**

**Background:** HIV-specific CD8+ T-cells in the peripheral blood diminish in most patients after the initiation of highly active antiretroviral therapy (HAART). However, examples of de novo appearance of HIV-specific CD8+ T-cells in patients with long-term successful therapy have also been described. The aim of our study was to determine the frequency and absolute counts of Gag-specific CD8+ T-cells in the peripheral blood of HIV-patients with more than 5 years of treatment-induced undetectable viremia and compare it with non-symptomatic untreated chronically-infected persons.

**Materials and methods:** The study enrolled 15 untreated HIV-patients (median CD4+ T-cells count 323.5 cells/µL, median percentage of CD4+ T-cells 17.3%) and 15 HIV-patients who have maintained undetectable plasma viremia (<50 copies of HIV-1 RNA/mL) for more than 5 years (median time on HAART 6.7 years, range 5 to 7 years, median CD4+ T-cell count 544 cells/µL, median percentage of CD4+ T-cells 24.3%). Percentages of Gag-specific CD8+ T-cells in the peripheral blood of our patients were determined by using iTag™ MHC class I tetramers (A*0201) specific for SLYNTVATL (Beckman Coulter Immunomics Operations, USA) on FC500 flow cytometer (Beckman Coulter, USA). Absolute counts of Gag-specific CD8+ T-cells were determined by using Flow-count Fluorospheres (Beckman Coulter, USA).

**Results:** Gag-specific CD8+ T-cells were detected in 12/15 (80%) of untreated HIV-patients and in 9/15 (66%) of treated HIV-patients with > 5 years of undetectable viremia. Percentages of Gag-specific CD8+ T-cells in ranged between 0.1 and 1.1% in untreated patients and between 0.1–0.7% in treated patients. Untreated HIV-patients had between 1 and 9 Gag-specific CD8+ T-cells/µL of blood. HIV-patients with more than 5 years of undetectable viremia had between 1–6 Gag-specific CD8+ T-cells/µL of blood.
Conclusion: Gag-specific CD8+ T-cells are detectable in some patients who have been successfully treated with HAART for more than 5 years. The frequency and absolute counts of Gag-specific CD8+ T-cells in patients with more than 5 years of successful HAART are different compared with untreated patients. These findings are relevant for the analysis of immune reconstitution following long-term successful HAART.

P2
The MHC Class II Transactivator (CIITA): A “Physiologic” Drug Against HIV-1 Replication
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Optimal vaccination strategies against HIV-1 require the fulfilment of two conditions: a)- the choice of an immunogen including pathogen’s antigenic epitopes against which an immune response with neutralizing characteristics can be generated; b)- the optimisation of triggering and maintenance of the immune response against the vaccine. Our interest is focussed on the second aspect. The expression of the MHC class-II transactivator (CIITA) whose locus AIR-1 and corresponding function were discovered in our laboratory, is needed for continuous expression of MHC class-II molecules and consequent antigen presenting function by APC. Unexpectedly, and of great relevance for antiviral functions, we found that CIITA potently inhibits HIV-1 viral replication by a competitive action on the viral transactivator Tat for Cyclin T1, the cellular cofactor used by Tat to elongate viral transcripts. This effect is found in APC and, of greater importance, also in HIV-1-infected T cells. Molecular analysis has revealed that the inhibitory activity of CIITA for HIV-1 Tat maps to the N-terminal region, and particularly to the segment 200–285 included within the P/S/T region of the CIITA activation domain which is then the molecularly defined Cyclin-T1-interacting region. Thus CIITA has a dual role on HIV infection: 1)- it increases APC function for HIV-1 viral antigens; 2)- it decreases viral replication and thus viral spreading in infected individuals. Within this frame CIITA represents the necessary and ideal molecule to control both innate and adaptive immunity against the virus. Considering the functional importance of a sustained and persistent expression of CIITA in APC, the search for potential synthetic and natural mediators, drugs and biomolecules, that can act on CIITA expression at level of transcription as well as biosynthesis, will be of great importance in tailoring better vaccines against HIV-1, and in controlling and combating HIV-1 infection and spreading.

P3
Abstract withdrawn
Retrovirology 2005, 2(Suppl 1):P3

P4
Abstract withdrawn
Retrovirology 2005, 2(Suppl 1):P4

P5
The MHC Class II Transactivator (CIITA): A Physiologic Inhibitor of HTLV-2 Retroviral Infection
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Retrovirology 2005, 2(Suppl 1):P5

The transcriptional activator CIITA is the master regulator of the expression of MHC class II genes. In addition to this major role, we have found that CIITA exerts an important inhibitory effect on the HTLV-2 replication, similar to our previously described effect on the HIV-1 replication. This inhibition is mediated by the N-terminal 1–321 region where we identified a minimal fragment of 80 aminoacids that specifically blocks the activity of the viral transactivator Tax2. This fragment does not inhibit the function of Tat, the transcriptional activator of HIV-1.

To unveil the biochemical basis of the CIITA-mediated inhibition of Tax2 we first focussed on the identification of the cellular cofactors used by Tax2 to transactivate the viral promoter. DNA-binding factors (CREB, NFYB) and several co-activators involved in chromatin remodeling (CBP, p300, PCAF, BRG1) are known to interact with or stimulate the transactivation activity of both CIITA and Tax1, the HTLV-1 homologous of Tax2. Preliminary data indicate that the transactivation activity of both CIITA and Tax1, the HTLV-1 homologous of Tax2, is differently influenced by the hystone acetyltransferases CBP, p300, PCAF providing new informations on the biology of HTLV-2. Furthermore, none of these factors was able to reverse the inhibitory action of CIITA on Tax2 function. Interestingly, we found that the B and, to a lesser extent, the A subunits of the NFY complex inhibit Tax2 activity when iper-expressed in cells. On the basis of our results and of the reported physical interactions between NFY and both CIITA and Tax1, we propose a molecular model for the CIITA-mediated inhibition of Tax2 activity via the binding of the CIITA-NFY complex to Tax2. When expressed in cells CIITA interacts with the NFY complex inhibit Tax2 activity when iper-expressed in cells. On the basis of our results and of the reported physical interactions between NFY and both CIITA and Tax1, we propose a molecular model for the CIITA-mediated inhibition of Tax2 activity via the binding of the CIITA-NFY complex to Tax2. When expressed in cells CIITA interacts with the NFY complex; this interaction changes the conformation of NFY increasing its binding affinity for Tax2. Following this model the inhibition of Tax2 by CIITA it is not due to the squelching of a transcriptional positive co-activator, but instead to the recruitment of a cellular factor, NFY, with a negative regulatory action on Tax2. This as well as possible alternative models are presently under scrutiny.

On the whole these results confirm that CIITA may represent a physiologic tool for novel therapeutic strategies aimed at counteracting HTLV-2 replication and spreading.

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P7
HTLV-I Tax Protein Induces the Secretion of Th1 Cytokines and β-Chemokines from Dendritic Cells
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Retrovirology 2005, 2(Suppl 1):P7

HTLV-I-associated myelopathy/tropical spastic paraparesis is characterized by highly stimulated immune response that includes elevated levels of inflammatory cytokines/chemokines, and oligoclonal expansion of Tax-specific CD8+ cytotoxic T lymphocytes in the cerebrospinal fluid. Studies have shown that the HTLV-I transactivator protein Tax is available for immune recognition by antigen presenting cells such as dendritic cells. In this study, we have shown that the treatment of monocyte-derived dendritic cells (MDDCs) with extracellular Tax induces the secretion of Th1 cytokines (IL-12, and TNF-α) and β-chemokines (MIP-1α, MIP-1β, and RANTES). A significant dose-dependent increase was observed with IL-12 (5-, 7-, and 24-fold) and TNF-α (5-, 6-, and 9.6-fold) with Tax treatment for 24 hr at concentrations of 0.1, 1, and 10 μg/ml, respectively. All three chemokines exhibited both dose- and time-dependent increase in the presence of Tax. More specifically, after 24 hr treatment with Tax (0.1, 1, and 10 μg/ml), MIP-1α was induced by 3.7-, 5.3-, and 6-fold, MIP-1b by 2-, 3.7-, and 3.7-fold, and RANTES by 7-, 8-, and 20-fold, respectively. The mRNA expression of these cytokines/chemokines was confirmed by real time PCR and was in direct correlation with observations regarding their protein expression.

P8
Development of a Human Bone Marrow Progenitor Cell Line to Examine HIV-1 Susceptibility and LTR Activity
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Previous studies have suggested that the bone marrow compartment may play an integral role in the genesis of HIV-1 dementia (HIVD). Interestingly, CD34+/CD38- pluripotent stem cells within the bone marrow are refractive to HIV-1 infection. The CD34+/CD38+ TF-1 cell line has been selected as a model to study HIV-1 infection during the differentiation process of hematopoietic progenitor cells. A number of cytokines such as GM-CSF, M-CSF, IL-1/β, TNF-α, and IL-4 were used to induce differentiation and activation of TF-1 cells and their surface marker expression was monitored by flow cytometry. Interestingly, IL-1/β treatment, alone or in combination with TNF-α, lead to up-regulation of CXCR4 and CCR5 surface presentation, and preservation of CD4 expression possibly providing an optimal cellular phenotype for HIV-1 infection of this cell population. The surface marker expression after this treatment also correlated with a more differentiated phenotype. To begin exploring the potential of these cells to support productive HIV-1 replication, a series of stably transfected cell lines were developed. To this end, macrophage-, T cell- and dual-tropic long terminal repeats (LTRs) were coupled to the gene encoding green fluorescent protein. These cell lines were utilized to explore the functional properties of specific cis-acting regulatory elements in LTR function within the bone marrow precursor cell population.

P9
Development of a Quantum Dot-based Assay System for Detection of Specific HIV-1 LTR Sequence Variants
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Analysis of human immunodeficiency virus type I (HIV-1) long terminal repeat (LTR) sequence variation within the CCAAT/enhancer binding protein (C/EBP) and stimulating protein (Sp) transcription factor binding sites has identified variants that correlate with HIV-associated dementia (HIVD). CdSe/ZnS nanocrystals have facilitated the investigation of nano- and pico-scale biological components. Quantum dot-conjugated oligonucleotides homologous to specific variants of Sp site III and C/EBP site I, were utilized to quantitate the relative abundance of specific LTR variants. Quantum dot-conjugated oligonucleotides containing the Sp site III 5T binding site variant, were reacted with plasmid DNA containing increasing concentrations of plasmid with the homologous LTR sequence variant. The results suggest that quantum dot-conjugated oligonucleotides specific for sequence variants within the LTR can be used as reporter molecules for identification and quantitation of HIV-1 genetic variation.

P10
Factors Affecting the Attitude Toward Safe Sex and Reproductive Health Among Shiraz City Youth
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Owing to social and cultural taboos and inhibitions, sexual health research in Iran remains restricted to a few number of studies for youth, especially those who are unmarried. Social and cultural norms impose barriers to the transfer of sexual health information. Consequently, countless remain ignorant of even...
the basic knowledge required for safer sexual behavior. As this group requires having proper guidance regarding their health, which can make them beneficial for community and nation and so, they can play an innovative role for welfare and advancement of the country.

We want to have a clear idea of young adult's knowledge about reproductive health, safe sex, and STDs. Youth's attitude toward risky behavior is an interesting topic that has attracted our attention. The main reason for this is that it focuses on youth in particular rather than the public. Youth are faced with numerous problems, which are sometimes beyond their comprehension. They may have heard of the phrase "Risky behaviors", but not everyone understands the meaning of these words. For the purpose of this research project, we have decided to limit the definition of risky behaviors to those practices that may lead to unsafe sex.

This is a quantitative study which will be done by the survey method. The major source of data in this study is a self-administered questionnaire which will be used to collect data. There is a little difference between women and men questionnaires. Due to sensitive nature of some questions and to make the respondents feel at ease, female interviewers will be responsible for interviewing female respondents while the male interviewers will ask questions of male respondents. The assurance will be given to the committee representatives and respondents specially, the identification, names will not be made public or published, code numbers are given to their names. The questionnaire will be pretested to provide information on the clarity of the questions and respondents' comprehension.

P11 Cathepsin B and Cystatin A as Indicators of a Separate Apoptotic Pathway in HIV-1 Infection
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Apoptosis has been proposed to explain the dysfunction in HIV-1 infection and FAS has been given a pivotal role. However, apoptosis in lymphoid follicles has also been explained by a follicular dendritic cell (FDC) dependent pathway regulated by a cathepsin-dependent endonuclease activity in germinal centre (GC) cells. Cystatin A is present in FDCs and is a natural inhibitor of cysteine proteinase, as Cathepsin B. As yet, the Cystatin A and Cathepsin B interaction in HIV-1 infection has not been studied.

Methods: Tonsillar tissue was obtained from 20 patients at various stages of HIV-1 infection and 10 controls. Eleven of the patients received HAART for 48 weeks. Cathepsin B, Cystatin A, FAS(CD95) and HIV-1 p24 in the GC cells were analyzed by immunohistochemical staining. Cathepsin B/Cystatin A ratios were calculated for controls and for patients before and after 48 weeks of therapy.

Results: Cathepsin B/Cystatin A ratio was 2-fold higher in patients as compared to controls; 1.03 and 0.43, respectively. After 48 weeks of therapy, this ratio was normalized (0.32). In patients, Cathepsin B correlated negatively with Cystatin A ($r = -0.686$, $p = 0.002$), and both markers correlated with the p24 antigen; $r = 0.777$ ($p = 0.001$) and $r = -0.622$ ($p = 0.013$), respectively. In multiple regression analysis presence of p24 antigen could not fully explain this relationship. There was no correlation with FAS(CD 95) for these parameters.

Conclusion: A 2-fold higher Cathepsin B/Cystatin A ratio was found in patients before HAART, suggesting a HIV-1 driven cathepsin-dependent pathway of apoptosis. Thus, Cathepsin B and Cystatin A possibly represent an apoptotic pathway distinguishable from the FAS-FAS Ligand pathway.

P12 Case Study on Anti-retroviral Therapy and Deaf Clients in Taso Mulago Uganda
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Retrovirology 2005, 2(Suppl 1):P12

Background: TASO Mulago has started giving Anti-retroviral drugs to Persons with disabilities whose cd4 is bellow 200 since 2004. Unfortunately in the Department of Medical and Counseling none knows sign language all the information concerning commitments, and Adherence, is given to care takers. A case study was done by the counselor in-charge of Disability affairs to find out if deaf clients are adhering to ARVs.

Materials and methods: Follow-up of Home visits to check adherence and commitments done by care takers. Pill count and talking to family members on the progress of the client since He/ she started on Anti-retroviral drugs.

Results: Deaf clients because of language barrier they refuse to adhere to drugs especially TB drugs and Arvs because they don’t know why they are taking them. All the information was given to caretaker who is not taking the drugs. Some disabilities are caused by the HIV Virus especially blindness, deafness, and permanent mental disorders in most clients who suffer from cryptococcomengitis.

Conclusion: The Ministry of Health, HIV/AIDS Organizations to introduce sign Language in all HIV/AIDS Programs to benefit the deaf clients. Information to be brailed for the Blind. Rehabilitation programs for the Disabled Clients.

P13 Characterization of a Downstream Positive Element Involved in Transcriptional Control of the Human CD3γ Gene Promoter
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Experimental data from our laboratory has shown that TCR/CD3 surface receptors are downmodulated after HIV-1 and HIV-2 infection of CD4+ T cells due to a specific defect in CD3γ gene transcripts. In an effort to better understand the
mechanism(s) involved, our laboratory has been investigating the critical elements responsible for regulating this gene. We have shown that the CD3γ gene is transcribed from an independent but weak, lymphoid-specific TATA-less promoter and demonstrated that a cluster of transcription initiation sites is present in the vicinity of the principal core promoter with the major start site situated in a classical initiator sequence. A GT box upstream of the initiator binds Sp family proteins and the general transcription machinery, with the activity of these contiguous elements enhanced by a second Sp binding GC box ten nucleotides further upstream. We found that two previously identified NFAT motifs positively (NFATγ1) or negatively (NFATγ1 and NFATγ2) regulate expression of the CD3γ gene by their differential binding of NFATc1 plus NF-κB p50 or NFATc2 containing complexes, respectively. Analysis of various mutant and deletion CD3γ promoter constructs in a transient reporter assay revealed that a 53 bp region downstream from the major transcription start site is critical for positive gene expression. Deletion of ten nucleotides in this region results in a 50% decrease in promoter activity, while deletion of 39 nucleotides completely eliminates promoter activity. EMSA experiments using DNA or RNA probes covering the 53 bp region demonstrate that this element functions through a RNA rather than a DNA intermediate. At least three specific nuclear protein complexes bind to the RNA probe. Deletion of the U at position +9 and the U at +37 completely abrogate binding and promoter activity. Experiments are currently underway to determine whether the composition of the transcription factor(s) complexes bound to the CD3γ element contain components of P-TEFb, which binds to HIV TAR.

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P17
Long Distance Truck Driving and HIV/AIDS
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Background: One male occupation that appears to be associated with increase risk of HIV infection is long distance truck driving, a profession that requires prolonged absence from home and families.

Methods: The present study was conducted in Jamnagar, Gujrat, India. Period of data collection was from Nov 2000 to October 2001 (365 days). Out of this 260 days were taken as working days and per day on an average 6 drivers were interviewed individually. The truck drivers were contacted at Four places which are the places of entry at this city. On the basis of this sample size is calculated as = 260 × 6 = 1560 Total 1600 were studied. Further one year was taken for analysis.

Results: Majority (46.8%) were in age group of 15–25 years. 26.3% were illiterate. 41.6% had monthly income between 1001– 200 rupees. Except 16.6% drivers all others were long distance drivers. 72% were married. 58% out of total had history of visiting CSWS (commercial sex workers). 56.9% of total drivers never used condoms during sex with CSWS. 38% of unmarried drivers gave history of STD as compared to 26.6% married. It was also observed that those drivers who remained >2 weeks or more than that away from there families have visited more to CSWS. Various types of addiction habits have been noticed in which alchohal, tobacco tops the list. STD history was found among 30% of them. Only about 400 knew about AIDS.

Conclusion: They have poor knowledge and awareness regarding HIV/AIDS and so many misconceptions are also noticed so Disseminate awareness among them.

P18
Blood in Saliva of HIV Seropositive Drug Abusers: Possible Implication in AIDS Transmission
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We have studied hemoglobin concentration in saliva of anti-HIV positive and anti-HIV negative intravenous drug abusers (IVDA) and normal controls and the relationship between hemoglobin concentration in saliva and number of CD4+ cells and clinical status of AIDS in anti-HIV positive IVDA. 120 anti-HIV positive IVDA’ 112 anti-HIV negative IVDA and 116 normal healthy subjects not belonging to any risk group for HIV infection completed the study. Saliva was collected at awakening before brushing teeth and the concentration of hemoglobin was determined. Hemoglobin concentration in saliva is higher in anti-HIV positive IVDA with respect to anti-HIV negative IVDA (p less than 0.05) and controls (p less than 0.01). In anti-HIV positive IVDA hemoglobin concentration in saliva is higher in subjects with CD4+ cells less than 200/10(6) 1 with respect to subjects with CD4+ greater than 200/10 (6) 1 (p less than 0.05) and in subjects with ARC/AIDS with respect to subjects with PGL or who are asymptomatic (p less than 0.01). Subjects with ARC/AIDS have a mean concentration of hemoglobin of 19 micrograms/0.1 mL saliva (range 0–153) which corresponds to 1.3 microliters of blood/ml saliva. If 10 ml of saliva are exchanged during kissing an average of 13 microliters of blood are transferred (110 microliters of whole blood at extreme range). Blood of symptomatic patients has an HIV titer of 7 TCID/microliters which for 10 ml saliva containing an average of 1.3 microliters blood/ml saliva corresponds to an average of 90 TCID (770 TCID at the extreme range).
P19  
Probing the Fusion-Active Structures of Envelope of Human T-cell Leukaemia Virus Type-I with Conformation-Specific Monoclonal Antibodies  
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Infection of cells by human T cell leukaemia virus (HTLV-I) is mediated by the viral envelope glycoproteins. The gp46 surface glycoprotein binds to the cell surface receptor Glut-1, allowing the transmembrane glycoprotein to initiate fusion of the viral and cellular membranes. In the absence of membrane fusion viral entry into the host cell cannot occur. Thus, envelope is a prime target for the development of anti-viral vaccines and small-molecule antagonists of viral infection. Indeed, we have shown that HTLV-I infection can be blocked at all stages of the entry process including, viral attachment, primary receptor binding and the post-binding steps of viral entry. To extend our studies, we have expressed recombinant protein fragments that mimic the core-coiled-coil region and six-helix bundle of fusion-active HTLV-I envelope. Using these recombinant proteins as immunogens we have generated monoclonal antibodies (mAbs) against the fusion-active and post-fusion conformations of HTLV-I envelope. Most importantly, we have now used these conformation-specific mAbs to probe the events that culminate in membrane fusion. We demonstrate that these monoclonal antibodies can be used to detect viral envelope on infected cells and to monitor the process of cell-to-cell viral transfer. Our recent results will be presented, and the implications of our results for HTLV-I pathogenesis will be discussed.

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P21  
In Vivo Administration of Replication-Deficient Mutant HSV-1 Targets Professional APCs and Induces Efficient CD4+ T Helper Responses  
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Both neutralizing antibodies and cytotoxic T cells are necessary to control a viral infection. However, vigorous T helper responses are essential for their elicitation and maintenance. These findings have critical implications in the design of vaccination strategies aimed at triggering and sustaining antigen specific CD4+ T helper immune responses. Here we show that a recombinant replication-deficient HSV-1 vector encoding the HIV-1 matrix protein p17 (T0-p17) is capable to infect professional APCs in vitro and in vivo without interfering with the endogenous MHC class II processing of the transgene encoded antigen. Moreover, we show that injection of T0-p17 in the mouse dermis generates a strong p17-specific CD4+ T helper response preceding both cytotoxic and humoral responses. Importantly, T0-p17 infected peritoneal macrophages were capable to trigger a long-lasting expansion of p17-specific CD4+ T cells in vitro. Because of their capability to infect professional APCs without interfering with their biological functions, replication-deficient HSV vectors are appealing candidates for the development of vaccines able to trigger strong T helper responses.

P22  
A Unique Cross-reactive HIV-1 Neutralizing CD4i Human Monoclonal Antibody Containing Only a Heavy Chain: Engineering a Domain Antibody and Improvement of Its Potency and Solubility  
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Naturally occurring human antibodies containing only heavy chain are very rare. All antibodies specific for HIV identified until now contain both light and heavy chains. By screening an immune HIV phage library we have identified an antibody, m12, that expresses only a heavy chain. The Fd of this heavy chain behaves as a CD4i antibody and binds gp120 complexed with CD4 better than gp120 alone. This antibody was further engineered to a single domain antibody, which is the smallest possible antibody fragment that still exhibits binding to the antigen. The domain m12 neutralized HIV isolates from different clades but had low solubility and was difficult to express. To further improve its solubility and potency we generated a mutant library. This library is being screened against gp120 and gp120-CD4. The results will be discussed if they become available. This unique domain antibody could have applications for design of potent HIV inhibitors.

Members of our group, including You-Qiang Wu, Igor A. Sidorov, Xiaodong Xiao, and Bang Vu contributed to these results.

P23  
Insufficient Tat Production Leads to Latent HIV Infection in Astrocytes  
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Background: In the brain, HIV infects microglia and macrophages productively while astrocytes are infected limitedly. Long life span and low turnover rate of astrocytes make them suitable hosts for viral persistence. Several studies have shown that HIV replication is blocked in astrocytes. We investigated regulation of HIV infection in astrocytes.

Methods: We used human fetal astrocytes (PFA), astrocyte cell line (SVGA) and reporter cells to monitor Tat and Rev activity. HIV IIIB and NL4-3 strains were used to infect astrocytes.
Results: Following HIV infection of SVGA reporter cells, very limited infection was detected. Further, no receptor or co-receptors except CXCR4 were present on astrocytes. To see further if viral replication is blocked in astrocytes, we infected PFA or SVGA cells with VSV pseudotyped NL4-3. High levels of p24 and robust LTR-GFP or LTR-gagGFP activation were seen. VSV-HIV infected reporter cells never lost green fluorescence and green cells were negative despite of continuous low Tat and Rev production. To rule out if Tat and Rev expression were from circular unintegrated HIV-DNA, Alu PCR revealed integration of viral DNA. Further, Tat or TNF-a reactivated the latent HIV in astrocytes and the latent HIV-SVGA cells upon co-culture transmitted the infection to Jurkat cells.

Conclusion: HIV establishes latent infection in astrocytes and HIV latency is due to the low levels of Tat production.

P24
Promoting Involvement of the People Living with HIV/AIDS (PLWHA) Participate in Social/Community in Cambodia
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Issues: Cambodia has rapidly growing HIV/AIDS epidemic, one of the worst in Southeast Asia.

Project: Partnership between public health, medical institution, PLWHA groups and NGOs at operational district level, and strong referral system between the home, community and institutional care providers are necessary for development of a successful comprehensive HIV/AIDS care and support, especially those that were coverage PLWHA in Cambodia. By underpinning systematic involvement of PLWHA at national, provincial, operational district and community level, the project aims to support PLWHA groups going through their self empowerment process from dependent phase in which NGOs/hospital arrange activities and they are recipients of the services, to partnership phase in which PLWHA facilitate active participation in services delivery in Cambodia.

Results: Cambodian People Living with HIV/AIDS Network (CPN+) to assist the Ministry of Health and National AIDS Authority. The sector will be working specially to assist developing care, treatment and other support services, and to facilitate referral systems among available services.

Lesson Learned: Reinforcement of PLWHA groups and policy making bodies resulted in a successful operational of the CPN+ strategic.

P25
Human Antigen Presenting Cell Gene Array Profiling of the Effect of Human T Cell Leukemia Virus Type 1 Tax Protein on Dendritic Cells
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Retrovirology 2005, 2(Suppl 1):P25

HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by a highly stimulated immune response including the oligoclonal expansion of CD8+ cytotoxic T lymphocytes specific for viral oncprotein Tax. Studies have demonstrated that Tax may be available for immune recognition by dendritic cells (DCs). In this study, a pathway-specific human dendritic and antigen presenting cell gene array has been used to study the global transcriptional changes mediated by extracellular Tax on monocyte-derived dendritic cells (MDDCs). Of the 192 genes examined, approximately 100 genes were differentially expressed after extracellular exposure to Tax. These genes were functionally categorized as the genes involved in antigen uptake and presentation (MHC class II molecule: HLA-DMB, DC-SIGN), intracellular adhesion molecules (ICAM-1 and -2), T cell costimulatory molecule (ICOS), cell surface receptor (CD47, toll-like receptors: TLR-1, −3, −6, −9), and several cytokines, chemokines; and their receptors (IFN-γ, IL-6, IL-12, TNF-α, IL-17, CCL5, CCL20, TNFSF4). Additionally, the expression of cellular signal transduction genes: ATF4, NFkB1, RELA, RELB (members of the NFkB family), GIP2 and GIP3 (interferon inducible) was also significantly altered. The expression of DC activation markers was confirmed by real-time PCR and was in direct correlation with the microarray observations.

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P28
Impaired CCR7 Expression on Plasmacytoid Dendritic Cells in HIV Infected Children on HAART With Virologic Failure
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Background: Defects of myeloid (m) DC and plasmacytoid (p) DC are well established in HIV infection. Studies in children and adolescents are limited, and have focused mainly on IFN-α function of pDCs.

Materials and methods: Patients with perinatal HIV infection (n = 19, ages 11–18 yr, on HAART) were classified as immunologic responders (IR+; CD4>25%), and virologic responders (VR; plasma HIV RNA < 400 copies/mL). mDC (Lin−, HLA DR+CD11c+) and pDC (Lin−, HLA DR+CD11c+) were evaluated in a novel whole blood assay by flow cytometry for expression of maturation markers CD83, CD80, homing receptor CCR7 and intracellular cytokines (TNF-α and IFN-α) after short-term stimulation with a TLR7/8 agonist, resiquimod.

Results: CCR7 expression was markedly reduced in pDC of IR-VR- subjects in comparison to IRVR patients (mean 5.6% vs 43.3%).
Levels of CCR7 were intermediate (mean 29.8%) in the IR+VR-group, and were almost absent in two patients with VL>100,000 copies who had a markedly reduced IFN- production in pDC. CD83, CD80, and TNF-α were expressed in all patients and were more pronounced in mDC than in pDC.

**Conclusion:** The most striking finding was a reduced expression of CCR7, and is indicative of a defect in homing of the plasmacytoid DC to lymph nodes in HIV infected children who have ongoing active viral replication and poor immunologic control in spite of HAART.

**P29**

**Phage Display Selection of HIV Specific Conserved Mimotopes With IgG from Long-term Non-progressors**

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Retrovirology 2005, 2(Suppl 1):P29

**Background:** The aim of this study is to identify conserved epitopes of HIV-1 neutralizing antibodies in polyclonal plasma from LTNP to finally derive vaccine candidates.

**Materials and methods:** The presence of neutralizing antibodies in 9 LTNP sera was proved by in vitro neutralization assays. Phage displayed peptide libraries were screened with LTNP IgG. HIV-specific mimotopes were analyzed for homology to the gp120 structure by a software (3DEX) especially developed for this purpose. Mice were immunized with interesting phages and their sera were analyzed for neutralizing activities against HIV-1.

**Results:** After biopannings, between 19% and 75% HIV-specific phage clones were identified by ELISA. Mimotope sequences were identified and could be aligned by 3DEX to linear or conformational epitopes on gp120. A peptide specific immune response was detected in sera of immunized mice. The first immune sera tested showed neutralizing activities against HIV-1.

**Conclusion:** Mimotopes could be selected from LTNP sera that represent conformational epitopes on gp120. Those ones inducing neutralizing antibodies upon immunization potentially are suited to derive vaccine candidates.

**P30**

**Improvement of Polybiguanide-based Microbicides Using Computational Design Methodologies**

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Polyethylene hexamethylene biguanide (PEHMB), a polybiguanide compound under development as a topical microbicide effective against HIV-1, was used as a starting point for rational design strategies and novel computational methods focused on identifying similar compounds with greater safety and activity. To investigate the hypothesis that PEHMB may represent a specific 3-D conformation and a degree of chain flexibility that confers the ability to inhibit HIV-1 infection through interactions with HIV-1 co-receptors, patented molecular calculation software (Shape Signatures) was used to predict biososisteres of PEHMB. These analyses suggested that substitution of a bithiazole group for the ethylene spacers of PEHMB would provide backbone rigidity, nitrogen atom spacing, and electrostatic potentials similar to PEHMB. The resulting molecule, poly(hexamethylene-c-2, 2-diamino-5, 5-bithiazole (PHDB), was found to have similar cytotoxicity yet greater activity than PEHMB. These studies strongly support our strategy of design and synthesis of second-generation compounds based on the PEHMB motif.

**P31**

**Protein-protein Interaction Between HTLV-1 Tax Protein and the Components of the Cellular Secretory Pathway**

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Tax protein has been shown to play an integral role in HTLV-1-induced diseases including adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Extracellular Tax has been detected in the cerebrospinal fluid of HAM/TSP patients, suggesting that cell-free Tax may be physiologically involved in the progression of neurologic disease. We have previously demonstrated the secretion of full-length Tax and its co-localization with the cytoplasmic organelles relevant to secretion. The present study elucidates the mechanism of Tax secretion. To identify Tax interacting proteins within cell, we have used an antibody array spotted with antibodies directed against cellular secretory pathway proteins. Upon reaction with protein extracts from Tax-treated cells, antibody array analyses have suggested the interaction of Tax with SCAMP1, SCAMP2, SNAP23, and COPII; proteins that facilitate transport between nucleus and cytoplasm, and between endoplasmic reticulum, Golgi complex, and plasma membrane. Subsequently, these specific protein-protein interactions have been confirmed by co-immunoprecipitation and GST pull-down assays. Collectively, these studies have demonstrated the interaction of Tax with multiple proteins in the secretory pathway and that Tax may be secreted and act as an extracellular effector molecule.

**P32**

**A Model for Advocacy and Community Participation in Research**

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The Center for AIDS Information & Advocacy (CFA) is a community-based, nonprofit organization that specializes in...
information, education, and advocacy for HIV/AIDS research and treatment. Since 1995, The CFA has engaged the patient, research, and healthcare communities with unique programs, publications, and advocacy efforts designed to improve the healthcare of patients and the quality of their lives, as well as provide meaningful input into the design and process of research to help move forward the search for a cure. Through such efforts, researchers can interact with community to create awareness of clinical trials, as well as solicit input from community advisory boards organized with participation of The CFA. Several key CFA activities focus on developing ongoing relationships with the clinical and basic science research communities, both in Houston and nationally. These are: a biannual Basic Science Workshop (most recently held as a “Salvage Therapy Think Tank” cosponsored with the Forum for Collaborative HIV Research with additional support from Baylor College of Medicine); a literature-review journal, Research Initiative/Treatment Action! (RITAI), which is indexed in PubMed by the National Library of Medicine; a clinical trials directory for HIV/AIDS trials in the Houston area; and a weekly research and treatment newsletter sent by e-mail and fax to more than 600 subscribers internationally. Such activities foster important cross-communication between HIV researchers and the HIV/AIDS community and allow for better community participation in research.

P33
HIV-1 Tat Upregulates IFN-γ mRNA in Normal PBMCs In Vitro
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IFN-γ, a cytokine produced mainly by T lymphocytes, interferes with viral replication by acting as a powerful immunomodulator, and has a negative effect on HIV-1 Tat-mediated viral transactivation in vitro. The production of this cytokine, which is upregulated during acute infection with HIV-1, seems to decrease during progressive HIV-1 infection, perhaps in part by de novo methylation of its promoter, yet the full mechanism for this decrease is still unclear. The HIV-1 Tat protein has been shown to induce the production of several cytokines and interferon-inducible proteins in high quantities, triggering a toxic cascade of events on surrounding cells, yet the role of Tat in the stimulation of these gene products is not fully known. We have assessed the effect of HIV-1 Tat on the production of IFN-γ, since it may provide an explanation for the modulation of these genes and perhaps the subsequent downregulation of IFN-γ itself. Our model system consists of normal PBMCs transfected with plasmids encoding either one-exon or two-exon Tat. We measured changes in IFN-γ mRNA by real time RT-PCR as well as intracellular cytokine levels by flow cytometry. Our results indicate a small consistent increase in IFN-γ mRNA in cells treated with either form of Tat compared to control cells. However, levels of IFN-γ protein assessed by flow cytometry do not yield a consistent pattern, leaving open the possibility that Tat regulates IFN-γ expression at multiple levels.

P34
HIV Escape From Peptide Fusion Inhibitors
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HIV envelope glycoprotein (Env) mediates infection by fusing virus with cellular membranes. Fusion inhibitors, a new class of antiretroviral drugs, inhibit HIV infection by binding to gp41 to form a peptide-gp41 6HB that is fusion-incompetent. To understand resistance mechanisms to peptide fusion inhibitors that will aid development of new drugs, we generated an escape-mutant virus against an N-peptide inhibitor. We found that two mutations in gp41, one each in the N- and C- heptad repeats, confer early resistance to the N peptide. These same mutations also confer resistance to a C peptide inhibitor. This is the first report of cross-resistance among peptide fusion inhibitors. Curiously, the N mutation alone or in combination with C mutation also conferred increased sensitivity to soluble CD4 and was associated with faster growth kinetics and larger syncytia. These results suggest global changes in Env involving receptor activation and fusion kinetics. Using thermal denaturation studies, involving N and C peptides containing wild type (Nw or Cw) or resistance residues (Nm or Cm), which self-assemble into a 6HB, we showed that the N mutation improved the energetics of the viral 6HB, however, the energetics of the 6HB formed with the inhibitor and the N and C peptides is not affected. Thus, our results demonstrate a resistance pathway that appears to involve both kinetic and thermodynamic factors that regulate virus entry and work indirectly to reduce the ability of fusion inhibitors to bind Env.

P35
Antigenic Diversity of the Hepatitis B Virus Capsid
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Antibodies to the capsid (cAg, core antigen) of HBV play a central role in the immune response to this virus. Since most anti-cAg antibodies recognize conformational epitopes, ruling out characterization by immunochemical methods, we have approached the problem by cryo-EM and image reconstruction of Fab-decorated capsids, combined with molecular modelling. Recently, we characterized the epitopes for two anti-cAg monoclonals (mAb 88, mAb 842). Both Fabs engage sites on the protruding capsid spikes, but they differ in binding orientation and are distinct. Occupancies of symmetry-related potential binding sites are unequal, reflecting preferential binding to certain quasisimilar variants. Despite steric interference, it was possible to identify both epitopes. The epitope for IgG mAb 88, residues 77–87, consists of an alpha-helix on one of the polypeptide chains within the dimeric spike. That of IgM mAb 842, also conformational, consists of loops and helical regions on both polypeptide chains, residues 74–78 and 78–83 and...
duplicates a previously characterized epitope. To date, six epitopes have been characterized, one being linear and five conformational. Although accessible sites are limited to two small regions of the capsid surface, combinations of a small set of motifs generate substantial antigenic diversity. We estimate the total number of distinct epitopes to be about 20.

**P36**

**Novel Second Generation Anti-HIV shRNA Expressing vif and Decoy TAR Arrest the Virus-breakthrough Phenomenon Associated With siRNA-escape Variants**

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We describe a novel chimera RNA expressing vif short-hairpin RNA (shRNA) and decoy trans-activation response region (TAR) RNA from a human U6 Pol II promoter, which enhanced the inhibition of human immunodeficiency virus (HIV) vif small-interfering RNA (siRNA) and arrested virus breakthrough by siRNA-generated escape variants in long-term culture assays. Our strategy was based on a second-generation anti-HIV-1 shRNA vector system, in which HIV-1 vif shRNA was fused to a decoy TAR RNA by a linker UU configuration of Sp site III (C to T at position 5) and 5T configuration of C/EBP site I (C to T). Signature sequences. The 3T configuration of C/EBP site I (C to T) and 5T configuration of Sp site III (C to T). Sequence variation at these sites was also examined in LTRs derived from autopsied brain tissue of patients both with and without HIVD. The 3T C/EBP site I was identified in 25% of brain-derived LTRs from patients with HIVD, but was absent in patients without HIVD. This suggests that 3T C/EBP site I, and possibly 5T Sp site III may prove valuable in assessing the likelihood of an HIV-1-infected individual developing HIVD.

**P37**

**Use of HIV-1 LTR Sequence Variants as Prognostic Indicators of HIV Dementia**

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To date, no prognostic viral markers exist for the onset of human immunodeficiency virus type 1 (HIV-I)-associated dementia (HIVD). The long terminal repeat (LTR) regulates HIV-1 viral gene expression via its interaction with multiple viral and host factors, including CCAAT/enhancer binding protein (C/EBP) and Sp transcription factor families. We have examined sequence variation at C/EBP sites I and II, and Sp sites I, II, and III in peripheral blood (PB)-derived LTRs from HIV-1-infected patients for potential signature sequences. The 3T configuration of C/EBP site I (C to T at position 3) and 5T configuration of Sp site III (C to T at position 5) were the only variants examined that were found in low frequencies in PB-derived LTRs derived from patients at early stages of HIV-I disease, and at increasing frequencies in patients representing later stages of disease. Sequence variation at these sites was also examined in LTRs derived from autopsied brain tissue of patients both with and without HIVD. The 3T C/EBP site I was identified in 25% of brain-derived LTRs from patients with HIVD, but was absent in patients without HIVD. This suggests that 3T C/EBP site I, and possibly 5T Sp site III may prove valuable in assessing the likelihood of an HIV-1-infected individual developing HIVD.

**P38**

**Activation and Maturation of Human Dendritic Cells by Extracellular Tax Protein of HTLV-I**

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HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/ TSP) is characterized by the generation of an intense cytotoxic T cell (CTL) response directed against oncoprotein Tax. Previous studies have suggested that Tax may be available for immune recognition by dendritic cells (DCs). In this study, we have shown that purified Tax protein efficiently bound and localized to the cell membrane of monocyte-derived dendritic cells (MDDCs) and was internalized within a few hours. After uptake, Tax-induced expression of DC activation markers MHC class I and II, and costimulatory molecules as well as the DC maturation marker, CD83. Tax has also promoted the production of major immune-directing cytokines IL-12, TNF-α, and proinflammatory chemokines MIP-1α, MIP-1β, and RANTES. The inhibitors of NF-κB have abrogated Tax-induced secretion of cytokines/chemokines indicating a role for NF-κB signaling in Tax-mediated immune response. Finally, Tax enhanced the allogenic and antigen-specific T cell proliferation capability of MDDCs. These results have indicated that extracellular Tax may selectively target MDDCs, be taken up by these cells and promote their maturation and antigen-presenting functions, driving a Th1-type immune response.

**P39**

**DC-SIGN as a Receptor for HTLV-I Binding, Entry and Infection**

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Human T cell leukemia virus type 1 (HTLV-I) has been identified as the etiologic agent of adult T cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP).
Numerous studies have demonstrated that patients diagnosed with HAM/TSP exhibit rapid activation and maturation of dendritic cells (DCs) while ATL is associated with their maturation defect. In addition to T cells, HTLV-I is known to infect DCs. HTLV-I infection of DCs could alter general DC function or the specific processing and/or presentation of HTLV-I-specific peptides, potentially playing a major role in the course of HTLV-I-associated disease. In this regard, we have demonstrated that an important antigen receptor on DCs, DC-SIGN serves as a receptor for HTLV-I binding using a quantum dot-based fluorescent binding assay. We have also demonstrated that gene silencing of DC-SIGN inhibits the infection of DC in a DC/T cell co-infection system. Furthermore, expression of DC-SIGN in B cells enhances viral binding, integration, and infection. These investigations, which consider the involvement of DC surface molecules in HTLV-I pathogenesis, are the first explorations of the intricate mechanisms that underlie the interactions between DCs and HTLV-I.

P40
Abstract withdrawn
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P41
Development of a Simple and Affordable S/LS Assay to Distinguish Recent and Established HIV Infection
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Background: Sensitive/less-sensitive (S/LS) serologic assays that differentiate recent from established HIV infection may not be suitable for use in resource-limited and financially challenged countries. A more simple and affordable method is needed to address these limitations. Methods: The Serodia HIV-1/HIV-2 particle agglutination assay (PA) was modified to act as a S/LS assay. Antigen-coated gelatin particles were diluted 1:68, and sera were diluted at intervals from 1:10 to 1:80,000; HIV antibody status was confirmed at the 1:10 dilution. 37 clade B seroconversion panels from Trinidad and BBI (n = 309) were tested at each sample dilution to calibrate the PA assay; the last positive reaction (>1+) was considered the endpoint dilution (ED). The greatest sensitivity for correctly classifying recent and established infection samples was determined by ROC analyses. A subset of these panels (n = 181) was also tested by the Vironostika S/LS (DV) as a reference for comparison. Results: At a dilution of 1:40,000 and a days post SC cutoff of 190 days the PA test gave 97% sensitivity for classifying both recent and established infection samples, as compared with 82% and 53% on a subset tested by the DV; this resulted in a poor concordance of 60% and 73%. Conclusion: A low cost, simple to perform PA test was modified as a S/LS test and exhibited excellence in distinguishing recent and established HIV infection. The PA S/LS performed more accurately than the reference DV S/LS when testing samples with known times of seroconversion.

P42
Abstract Not Submitted
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P43
Cell Mediated And Humoral Immune Functions After Methanol Intoxication in Albino Rats
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Keywords: Methanol; cell mediated immunity; humoral immunity
Toxicity from methanol (MeOH), a potentially significant problem due to occupational, accidental, intentional, as well as daily ingestion of small amounts of the agent, only receives considerable attention after severe signs of intoxication have set in or death is imminent. While accidental and intentional exposures usually involve very high doses, the occupational and ingestion forms more often reflect small daily intakes. Still, even at the low levels, little is known about the potential immunotoxic implications (and less so in regard to mechanisms) from these ongoing exposures. This study has been focused on the effect of methanol on cell mediated and humoral immune function in non-immunized and immunized rats. The level of methanol used in this study was one fourth of the LD50 values (2.37 gm/kg b.wt). The cell mediated and humoral immune function tests were carried out in seven different groups of albino rats, namely control, 1 day, 15 days, 30 days and corresponding immunized groups were used. Sheep red blood cells (SRBC 5 × 10^9 cells/ ml) were used for immunizing the animals that belongs to the immunized groups. Food intake, urine output, animal and organs weight ratio, cellularity of lymphoid organs and foot pad thickness were significantly decreased when compared with respective controls. However, water intake, and leucocytes migration inhibition were significantly decreased when compared with respective controls. The cell mediated immunity in non-immunized and immunized rats were tested with HAM/TSP exhibit rapid activation and maturation of dendritic cells (DCs) while ATL is associated with their maturation defect. In addition to T cells, HTLV-I is known to infect DCs. HTLV-I infection of DCs could alter general DC function or the specific processing and/or presentation of HTLV-I-specific peptides, potentially playing a major role in the course of HTLV-I-associated disease. In this regard, we have demonstrated that an important antigen receptor on DCs, DC-SIGN serves as a receptor for HTLV-I binding using a quantum dot-based fluorescent binding assay. We have also demonstrated that gene silencing of DC-SIGN inhibits the infection of DC in a DC/T cell co-infection system. Furthermore, expression of DC-SIGN in B cells enhances viral binding, integration, and infection. These investigations, which consider the involvement of DC surface molecules in HTLV-I pathogenesis, are the first explorations of the intricate mechanisms that underlie the interactions between DCs and HTLV-I.

Conclusion: A low cost, simple to perform PA test was modified as a S/LS test and exhibited excellence in distinguishing recent and established HIV infection. The PA S/LS performed more accurately than the reference DV S/LS when testing samples with known times of seroconversion.

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P43
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P42
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P43
Cell Mediated And Humoral Immune Functions After Methanol Intoxication in Albino Rats
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Retrovirology 2005, 2(Suppl 1):P43

Keywords: Methanol; cell mediated immunity; humoral immunity
Toxicity from methanol (MeOH), a potentially significant problem due to occupational, accidental, intentional, as well as daily ingestion of small amounts of the agent, only receives considerable attention after severe signs of intoxication have set in or death is imminent. While accidental and intentional exposures usually involve very high doses, the occupational and ingestion forms more often reflect small daily intakes. Still, even at the low levels, little is known about the potential immunotoxic implications (and less so in regard to mechanisms) from these ongoing exposures. This study has been focused on the effect of methanol on cell mediated and humoral immune function in non-immunized and immunized rats. The level of methanol used in this study was one fourth of the LD50 values (2.37 gm/kg b.wt). The cell mediated and humoral immune function tests were carried out in seven different groups of albino rats, namely control, 1 day, 15 days, 30 days and corresponding immunized groups were used. Sheep red blood cells (SRBC 5 × 10^9 cells/ ml) were used for immunizing the animals that belongs to the immunized groups. Food intake, urine output, animal and organs weight ratio, cellularity of lymphoid organs and foot pad thickness were significantly decreased when compared with respective controls. However, water intake, and leucocytes migration inhibition were significantly decreased when compared with respective controls. The cell mediated immunity in non-immunized and immunized rats were tested with HAM/TSP exhibit rapid activation and maturation of dendritic cells (DCs) while ATL is associated with their maturation defect. In addition to T cells, HTLV-I is known to infect DCs. HTLV-I infection of DCs could alter general DC function or the specific processing and/or presentation of HTLV-I-specific peptides, potentially playing a major role in the course of HTLV-I-associated disease. In this regard, we have demonstrated that an important antigen receptor on DCs, DC-SIGN serves as a receptor for HTLV-I binding using a quantum dot-based fluorescent binding assay. We have also demonstrated that gene silencing of DC-SIGN inhibits the infection of DC in a DC/T cell co-infection system. Furthermore, expression of DC-SIGN in B cells enhances viral binding, integration, and infection. These investigations, which consider the involvement of DC surface molecules in HTLV-I pathogenesis, are the first explorations of the intricate mechanisms that underlie the interactions between DCs and HTLV-I.

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P46
A High Throughput Quantum Dot-based Fluorescence Assay for Quantitation of HTLV-I Binding and Attachment
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Quantum dots (Qdots) are fluorescent semiconductor nanocrystals comprised of CdSe core with a semiconductor shell of zinc sulfide coated with a polymer shell allowing particles to be conjugated to biological molecules while retaining the optical properties of the particle. We have used this unique property of Qdots to develop a high throughput binding assay to study the attachment of HTLV-I to host cells. To this end, we have biotinylated cell-free HTLV-I (biot-HTLV-I) to facilitate viral detection using streptavidin-coated Qdots. B cells (BTHP-1 and Ramos) were exposed to biot-HTLV-I with increasing concentrations of DEAE-dextran, a reagent known to enhance binding of other retroviruses. Unbound virus was removed by washing and cells were added with strep-Qdots and fluorescence readings were obtained at 605 nm. HTLV-I bound efficiently to BTHP-1 and Ramos cells and this binding was significantly increased (3-fold) by DEAE-dextran. To confirm the specificity of viral binding, a competitive inhibition assay was performed wherein increasing amounts of non-biotinylated HTLV-I was added to the binding assay along with a fixed amount of biot-HTLV-I. A dose-dependent inhibition in biot-HTLV-I binding was observed in the presence of native virus. These results suggest that the Qdot-based assay may be useful in studying virus attachment to host cells, and the screening of inhibitors for viral binding and entry.

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Abstract withdrawn

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P48
HIV-1 Infections During Vaccine Trials: Identifying New Peptides for Differential Diagnosis of HIV-1 Infections in the Face of Vaccine-generated Antibodies
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Since 1987, more than 25,000 individuals have been immunized with 65 HIV preventive vaccines. Current candidate HIV-1 vaccines are complex products containing multiple HIV genes or proteins. As a result, high proportion of vaccinees score positive in licensed HIV diagnostic kits. This will have negative impact on vaccine trials that require early detection of breakthrough infections. For vaccinees it may contribute to range of social harms (jobs, insurance, blood donors). Therefore, it is important to design new tests that will discriminate between vaccine induced reactivity and true HIV infection. Our goals were to identify new HIV epitopes that: 1) Do not contain important neutralizing or CTL epitopes, 2) Recognized by antibodies early after HIV infection. 3) Highly conserved among HIV clades. Using Phage Display libraries constructed from whole HIV-1 genomes, combined with affinity selection with antibodies from early seroconvertors, we identified new immunodominant epitopes, in gp41 cytoplasmic tail and in p6 that fit the above criteria. These peptides were used for development of new differential HIV-1 ELISA. To date, 100% specificity for gp41 and 99.4% with p6 peptide was observed with 1300 HIV seronegative samples. Analysis of 28 early HIV seroconversion panels showed that HIV-1 infection can be detected within 2 weeks following HIV-1 RNA detection by PCR. Testing of diverse HIV-1 clade panels from around the world (1660 samples) supports the utilization of our assay in detection of HIV-1 clade A, B, C, D, E, F and CRF infections. The assay sensitivity is 99.1%. In recent testing of 2780 samples obtained from six HIV vaccine trials (with complex vaccine candidates (conducted by HVTN, DOD, Vaxgen & Vaccine Research Center), all samples from uninfected vaccines scored negative in our assay, while 46% samples were positive by commercial diagnostic kits. Importantly, our assay detected all 183 breakthrough HIV infections among these vaccinees, providing a strong proof-of-concept for utility of our EIA in diagnosis of true HIV infections in the face of vaccine generated antibodies.

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HIV/AIDS Drugs Facing Africa
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Retrovirology 2005, 2(Suppl 1):P49

Africa, continent devastated by poverty and the civil wars, became one of the continents more touched by the HIV/AIDS. According to UNAIDS, Africa is home to 70% of adults and 80% of children living with HIV in the world. Not so long ago, testing positive for HIV meant an automatic death sentence. Now things have changed for the better. A combination of drugs introduced about seven years ago has turned AIDS from a death sentence to a treatable disease. These drugs are very expensive and many African governments do not have the funds to import these drugs. In Africa, fewer than 100,000 people living with HIV have access to antiretroviral treatment and everybody know that AIDS won’t wait, this means that a majority of the 25.3 million Africans infected with AIDS won’t get the best available treatment and many African HIV/AIDS patients have died and other may follow in the next five years if nothing is made to stop the progress of the AIDS. To save the African continent, there are two possibilities, the first possibility is to allow African countries, especially those most affected by AIDS to declare a state of emergency and produce affordable generic versions of HIV/AIDS drugs to provide the much needed help to their citizens. The second is to try to slow new cases through preventive education and encouraging condom use, maybe reduce transmission from mothers to babies. Hardly enough to save African
continent, so figuring out how to save the millions who are infected remains an agonizing challenge.

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P51
Considerations and Controversies of AIDS
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The statistical probability of seroconversion is proportional to the number of needlesticks incurred and the likelihood that the needlesticks will be with HIV infected blood. Careful adherence to recommended operating room practices, combined with meticulous attention to handling needles and sharps, should result in few, if any, cases of occupational HIV seroconversion among OR personnel. HIV testing is not feasible in the management of emergency patients; these are often the individuals at highest risk for HIV infection and over whom the surgical team has the least control. Non-operative treatment of HIV-infected patients is not an option; many procedures are performed either to enable the individual to lead a more comfortable, productive life or for diagnostic purposes.

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P53
HIV/AIDS Family Caregiving Experiences
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The purpose of this grounded theory study was to describe the experience of HIV/AIDS family caregiving in the palliative phase. Seven in-depth interviews were conducted and analyzed using the constant comparative method. The analysis resulted in a conceptualization of HIV/AIDS family caregiving. This paper describes the "personal work" of caregivers, including reconciling that a loved one would die, making life-and-death decisions, and letting go. The nature of support received to attend to this work is highlighted, with attention to its influences on HIV/AIDS caregiver bereavement. The findings of this study provide some insights into the HIV/AIDS family caregiver experience and reveal a significant need for interventions designed to support caregivers in establishing the mechanisms required for bereavement resolution. The need for the creation of supportive networks for HIV/AIDS caregivers cannot be overstated. Further research is required to help clarify and expand on how social support might have an effect on HIV/AIDS family caregiver bereavement. With this knowledge, health-care providers will be better prepared to anticipate difficulties faced by caregivers, plan appropriate interventions to address these difficulties, prevent future problems, and plan care based on theory and research.

P54
Inhibition of HIV-1 Entry by Inducing a Nonproductive Conformational Change in gp120
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Conformational change in HIV-1 gp120 is a dynamic process essential for HIV-1 entry. It is not clear whether the dynamic nature of gp120 could be exploited to abort the viral entry machinery. Here we show that a small molecule entry inhibitor, IC9564, induces a conformational change in gp120 and locks the envelope into fusion incompetent conformation. Binding of IC9564 to HIV-1 envelope results in the exposure of CD4i epitopes that are ally concealed in gp120. As a result of the conformational effect, IC9564 significantly enhances the neutralizing activity of 17b that binds to an epitope overlapping chemokine receptor binding site. Unlike CD4, IC9564-induced conformational change in gp120 does not trigger a conformational change in gp41. In fact, IC9564 inhibits CD4 induced conformational changes in gp41. The binding site of IC9564 remains elusive due to the fact that mutations in both gp120 and gp41 could change IC9564 sensitivity. Nevertheless, a common effect of these mutations is that conformation of gp120 is changed to conceal conserved epitopes such as CD4i. In summary, IC9564 exploits the dynamic nature of HIV-1 gp120 by inducing a nonproductive conformational change in gp120 and prevents HIV-1 from entering the cells.

P55
Secretion of the Human T Cell Leukemia Virus Type 1 Transactivator Protein Tax
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The HTLV-I Tax protein is well known as a transcriptional transactivator and inducer of cellular transformation. However, it is also known that extracellular Tax induces the production and release of cytokines, such as TNF-a and IL-6, which have adverse effects on cells of the central nervous system. The cellular process by which Tax exits the cell into the extracellular environment is currently unknown. This study characterizes the process of Tax secretion from the cell. Specifically, cytoplasmic Tax was demonstrated to localize to organelles associated with the cellular secretory process including the endoplasmic reticulum and Golgi complex. Additionally, it was demonstrated that full-length Tax is secreted from both baby hamster kidney cells and a human kidney tumor cell line. Tax secretion was partially inhibited by brefeldin A, suggesting that Tax migrated from ER to Golgi complex. The combined treatment of Tax-transfected cells with PMA and
ionomycin resulted in a small increase in the amount of Tax secreted suggesting that a fraction of cytoplasmic Tax was present in the regulated secretory pathway. These studies provide a link between Tax accumulation in the cytoplasm, the detection of Tax in the extracellular environment.

**P56**

**Identification of C/EBP Binding Sites Within the Clade C LTR**

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Human immunodeficiency virus type 1 (HIV-1) has been transmitted worldwide and regional viral clades have been designated as subtype A through K. Subtype C, which is concentrated in southeast Asia and sub-Saharan Africa, is the most prevalent subtype worldwide. To date, no studies have examined the role of CCAAT/enhancer-binding proteins (C/EBP) in LTR-directed viral gene expression in the clade C LTR. Within clade B viruses, two functional C/EBP sites upstream of the TATA box have been shown to be required for efficient viral replication in cells of monocyte/macrophage lineage. In order to assess the role of the C/EBP sites within the subtype C viral LTR, 211 HIV-1 subtype C LTR sequences were collected and aligned via the Clustal V method. From these analyses, three potential C/EBP sites were identified: two upstream binding sites and one downstream binding site. Interestingly, the putative downstream site was highly conserved between clades B and C, suggesting the presence of a functionally important cis-acting element that has yet to be characterized. Electrophoretic mobility shift analyses demonstrated that two of the three sites within the HIV-1 subtype C were able to bind C/EBP factors. Additional studies focused on examining relative binding affinities of naturally occurring variants of these two sites. Future studies will examine the roles of these sites in the regulation of LTR activity.

**P57**

**Abstract withdrawn**

*Retrovirology 2005, 2(Suppl 1):P57*

**P58**

**HIV-1 Subtype C, CCR5-using Viruses are Detected in CD4 T Cells and Macrophages in South African AIDS Patients. Analysis by Fluorescent In Situ Hybridization and V3 Loop Sequencing**

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**Background:** In South Africa, HIV-1 subtype C is the prevalent subtype and has been found to use CCR5 throughout the viral infection in most cases. In contrast, HIV-1 subtype B often switches its coreceptor usage to CXCR4 due to mutations primarily within the V3 loop of the gp120 protein. In order to assess whether subtype C is still able to infect T cells in late stages of the infection, FISH was used to localize HIV sequences within different blood cell types of AIDS patients.

**Methods and Materials:** Fresh blood and bone marrow samples obtained upon informed consent from late stage, antiretroviral therapy-naive, non-opportunistic infectious HIV patients were separated into CD4+ and CD4− fractions using Dynabeads. The cells were washed, placed onto slides, and hybridized with HIV-gag-RNA-specific probes, labelled by nick translation with spectrum green. Following overnight incubation at 37°C, the slides were washed, stained with DAPI and analyzed under fluorescent microscopy. DNA was also extracted from each sample, the HIV envelope gene was amplified using nested primers, subjected to sequencing to determine the V3 loop sequence and the resulting overall positive charges.

**Results:** In order to assess the site of HIV subtype C active infection in late stage of the disease FISH was performed on peripheral blood or bone marrow slides of 20 HIV+ patients produced positive signals in and around the nucleus of CD4+ T cell as well as monocytes/macrophages, whereas control slides made from HIV- individuals and CD4− fractions of HIV patients possessed no signals. Both CD4+ cell population exhibited high number of signals, but the T cells had a greater variation, ranging from none to over thirty. The V3 loop sequencing and analysis of the protein sequence from 11 of the 20 patients revealed the structure’s overall positive charge and amino acid placements were compatible with HIV-1 subtype C CCR5-using viruses in these patients.

**Conclusion:** Despite the use of CCR5 by HIV-1 subtype C, CD4+ T cells were found to exhibit positive signals in vivo in late stage HIV+ patients. This may suggest that CCR5 expression is upregulated on CD4+ T cells to allow the virus access, or that the virus is exploiting some other mechanism of invasion like the viral synapses that has been found to exist between infected macrophages and uninfected T cells, rather than a direct approach of infection where the use of chemokine receptors are necessary.

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**P59**

**Abstract withdrawn**

*Retrovirology 2005, 2(Suppl 1):P59*

**P60**

**HPV Related Lesions in HIV Positive Subjects on HAART: Study of Viral Markers of Cervical Neoplasia Progression**

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**Background:** As for other slow evolving tumors, the risk of HPV related ano-genital carcinoma, has increased in the era of
HAART. We studied HPV related parameters to identify markers of progression and HIV/HPV infections interaction.

**Methods:** HIV pos women (N = 410) were followed since 1995 in a longitudinal study. Each one undergo periodico (6–12 months) colposcopy, PAP smear, biopsy if needed, and cervical sampling for HPV testing. HIV related parameters (CD4, HIV-RNA, ART) are recorded and related to cervical disease. HPV typing is performed by reverse hybridisation assays, viral load by in-house real time PCR and viral expression by E6/E7 mRNA detection. The Mann-Whitney rank test for non-parametric data and the association between discrete variables by Chi-square test of Fisher exact test were applied.

**Results:** Prevalence of high risk HPV (HR-HPV) is 59.3%; high grade lesions (HgSIL) are 8.9% and low grade (LgSIL) 24%. Subjects with lower nadir of CD4 count in their HIV story, show increased rates of these values independently by the efficacious use of HAART. Sixty one pt (15%) underwent surgical resection of high grade lesions or cervical K. HPV load was prospectively studied in 16 of these cases (HgSIL) and 22 HR-HPV positive controls without cervical lesions. In the cervical brush collected at diagnosis, all cases had an HPV load significantly higher then controls (p = 0.0004). Decreasing HPV load were observed when comparing pre- and post-surgery samples (p < 0.0001). The number and type/s of HPV strains were not statistically different between cases and controls. Multiple infections were detected at baseline in 43.7% of cases and 54.5% of the controls, in 87.5% of the cone biopsies and in 56.25% of the post-treatment sample of the cases.

**Conclusion:** Persistence of HR-HPV as a key marker in the development of cervical lesions is poorly informative in HIV positive women due to its high frequency and coinfection with multiple HR-HPV types. Novel clinical biomarkers to identify subjects at true risk for the development of cervical lesions may include viral burden: both total and type specific. High level of HPV DNA is in fact detected in the lesions and is drastically reduced by their removal.

**P61 Risk Factors for CD4 Lymphopenia in Patients Treated With a Tenofovir/Didanosine High Dose-containing Highly Active Antiretroviral Therapy Regimen**

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In this study, the dynamics of CD4 cell depletion during tenofovir/ didanosine co-administration were analysed. Ninety-five HIV- positive patients were followed for 562 days, and 37 lost at least 50 CD4 cells, with a median delay of 274 days. Cox analysis showed that the CD4 cell decrease was associated with a duration of treatment by didanosine of more than 853 days and a didanosine dose of more than 5.50 mg/kg.

**P62 HIV Surveillance By Testing Saliva**

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Saliva specimens were tested for HIV antibody (anti-HIV) by an immunoglobulin G (IgG) antibody capture radioimmunoassay (GACRIA) and three sensitive commercial assays. In tests on 460 seronegative subjects and 196 seropositive subjects GACRIA was 99.8% specific and 100% sensitive. The Wellcome HIV monoclonal and Abbott recombinant DNA enzyme-linked immunosorbent assays (ELISAs) were also highly specific (99.8%, 100%) but they were less sensitive (90.9%, 82.0%). The Fujirebio particle agglutination assay was sensitive (97.8%) but its specificity was poor (84.1%). In testing saliva specimens from populations with an anti-HIV prevalence greater than 0.5%, sampling by GACRIA alone could provide a good estimate of the true prevalence. For true prevalences less than 0.5% good estimates could only be obtained if positive GACRIA reactions were confirmed by another independent salivary assay. Salivary testing for anti HIV is a convenient and potentially an accurate epidemiological tool.

**P63 Analysis of Putative Amino Acid Signals Within Human T Cell Leukemia Virus Type 1 (HTLV-1) Tax Protein Involved in Mediating Tax Secretion**

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HTLV-1 oncprotein Tax protein is known to be released from HTLV-1-transformed cells by a mechanism other than cell death; however, the mechanism of Tax secretion remains to be established. This study elucidates domains within Tax that contribute to its subcellular localization and secretion. Analysis of the amino acid sequence of Tax has revealed the presence of four putative secretory signals within the carboxy-terminal domain namely YTNI, LL, DHE and terminal V. Mutation of two putative signals (YTNI and DHE) resulted in aberrant subcellular localization of Tax, with cytoplasmatic Tax accumulating in structures corresponding to the ER and Golgi. We have also studied the effect of these mutations on the secretion of Tax in baby hamster kidney cell (BHK-21) line. The results have demonstrated that mutating YTNI to ATNI resulted in approximately a 2-fold reduction in secretion. Mutation of LL to AA abrogated the intake of Tax, therefore, no secretion was observed. Mutating DHE and terminal V did not show any effect on Tax secretion, however, a combination of DHE mutation with YTNI mutation or AA mutation resulted in an altered intake and secretion of Tax. These studies substantiate a link between cytoplasmatic Tax and its subsequent secretion and also indicate potential amino acid signals that might direct secretion of Tax.

**P64 Abstract withdrawn**

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**P65 HIV Seroprevalence and Factors Affecting Prevention of Vertical Transmission of HIV Among Antenatal Care**

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**Background:** The aim of the study was to identify the potential barriers for intervention program on preventing
mother to child transmission of HIV among pregnant mothers attending antenatal care clinic in Southwestern Ethiopia.

**Patients and Methods:** A cross-sectional survey was conducted among antenatal care attendants of Jimma university hospital from Jan. 25, 2004 to Feb 25, 2004 using a structured questionnaire. At the same time unlinked anonymous blood sample was taken during routine ANC investigations. The sera were tested for HIV antibody using rapid test algorithm. In addition, full VCT service was given for volunteers. The data was analyzed by using SPSS 12th edition and chi-square and binary logistic regression were used to see associations and p-value less than 0.05 was taken as cut of point for significance.

**Results:** On the whole, 258 women were included in the study of which four were excluded due to incompleteness of the data. The over all HIV sero prevalence was 9.8%; being 10.4 % in urban & 7.7% in rural women. Logistic regression demonstrated low income groups, illiterates, low-level workers and those who have misconception about HIV/AIDS had higher chance of HIV infection accompanied by low likelihood of accepting HIV testing. Women who have history of STD & surgery were also found to be more likely to be HIV sero positive as compared to those with out history. Though, 92.1% of mothers were indebted about the importance of HIV screening test, only 31.9 % of them received VCT service. Similarly, only 17.3% of mothers who believed breastfeeding as a potential HIV mode of transmission to the baby if they were found to be infected could afford to buy formula milk for at least six months.

**Conclusion:** The study found out higher HIV prevalence among pregnant mothers as compared to the national figure with highest infection rate in the age group 15 – 24 yrs old, reflecting recent high rate of HIV infection. In addition, provision of VCT service only would not be enough for effective prevention of mother to child transmission of HIV, as it was made known from our study, unless it is supplemented with some form of activity which would promote prevention of vertical transmission of HIV as most mothers couldn’t afford to buy as reflected in this study.

**P66**

**Abstract withdrawn**

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**P67**

**AIDS, HIV and Women, The Next Five Years**

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Acquired immune deficiency syndrome (AIDS) is caused by an RNA retrovirus (HIV-1) and is readily transmitted heterosexually. The viral receptor is a differentiation antigen on the surface of a class of immunologically active cells including T ‘helper’ lymphocytes, some macrophages and antigen presenting cells. HIV may be transmitted vertically and viral antigens have been demonstrated in the placenta. Infants of infected mothers have at least a 60% probability of acquiring HIV in utero. The normal latent period after infection is between 2 and 5 years, and it is estimated that for every case of AIDS, 50–100 people may be infected. Extrapolation of these estimates suggest 1,000,000 may already be infected and the established risk group for AIDS may not reflect the pattern of present infection. In Central and East Africa there now appears to be an epidemic of enormous proportions. Oocytes and spermatozoa are not attacked by the HIV virus but associated lymphocytes or monocytes may be infected. Screening for HIV for semen donation is mandatory and precautions for infection with HIV should follow procedures adopted for hepatitis B virus.

**P68**

**Recruitment and Follow Up of High Risk HIV Negative Volunteers in Preparation For Vaccine Trials, Kenyan Case**

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Human immunodeficiency virus (HIV) research will continue to face significant barriers unless solution to enrollment and follow up are found. 30 high-risk HIV negative individuals were recruited and followed up every three months for 9 months. Multiple approaches were used in recruitment. Community education seminars were conducted in nightclubs and the public gatherings. Posters and fliers were used to invite people to the seminars. Reading materials were provided to enhancing understanding. In every visit volunteers were counseled and information on their high-risk behaviour was collected. The study shows that the follow up was 90% for the 30 volunteers. Two of the volunteers lost follow up in their third visit. The success of the follow up was due to counseling and mobilization of the community. Challenges encountered in recruitment and follow up include low literacy levels, poverty, gender inequality, stigma, fears and mistrust about the vaccine. The volunteers could be easily recruited and retained for future vaccine studies.

**P69**

**Detection of A Shared HIV Protease-RT Deletion in Patient Plasma & Cells: A Role For ARV-mediated Selection and Viral Complementation**

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Background: A deletion in HIV pol spanning amino acid residues 59–99 of protease and 1–204 of reverse transcriptase (RT) was detected in 12/22 plasma specimens from patients receiving antiretrovirals (ARVs). It was subsequently detected in cerebrospinal fluid, blood monocytes and T-cells, brain and lymphoid tissues. It was always accompanied by an I54V mutation in protease, and L210W, R211K and L214F mutations in RT. Sequencing and other experiments confirmed that it is not a PCR artifact.

**Materials and methods:** To study the functional impact of this defect, the 738 bp deletion was inserted into an infectious macrophage-tropic molecular clone of HIVSF162 carrying green fluorescence protein (GFP).
DNA from the GFP-containing defective virus clone was transfected into 293T cells alone, or along with DNA from the wildtype (wt) SF162 clone lacking GFP. Supernatants were collected, assessed for HIV by RT-PCR, and used to infected primary macrophages and T-cells, and CEM cells expressing CCR5 (CEM-R5). Transfected and infected cells were monitored for GFP expression by microscopy, and for the presence of defective and wt genomes by PCR. To determine if ARVs can influence the selection or persistence of this mutant, long-term cultures of CEM-R5 harboring both wt and mutant viruses were cultured with these drugs.

Results: GFP-expression was detected in cotransfected 293T cells, and in all cells infected with virus from cotransfected 293T. Cotransfection supra contained mutated and wt viruses suggesting that complementation, in addition to recombination within 293T, could have occurred. Mutated genomes persisted in CEM-R5 for 51 days, but decreased with time. Increased levels of mutants were detected in persistently-infected CEM-R5 at 3 and 9 days after exposure to AZT and saquinavir, respectively.

Conclusion: Exposure to ARVs may lead to creation of particles with large deletions in pol. These defective genomes could recombine with, or be complemented by, ones with functional protease and RT genes. Their persistence may represent a form of viral latency or mode of viral escape.

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Abstract withdrawn

P71
Region-specific Distribution of HIV-1 LTR C/EBP Site II Configurations in Demented and Non-demented Patients

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We have previously demonstrated that the C/EBP site II consensus B (conB) variant was highly conserved in brain-derived HIV-1 LTRs and that LTRs containing C/EBP site II 4C and 6G variants were only found in brain tissue of patients with HIV-1-associated dementia (HIVD). Therefore, the regional distribution of LTRs containing the conB, 4C, or 6G variant of patients with and without HIVD was examined. A statistically significant difference was found in the regional distribution of LTRs containing the C/EBP site II conB, 4C, or 6G variant in brain regions derived from patients with and without HIVD. LTRs containing a low affinity C/EBP site II 4C were shown to accumulate in the cerebellum, a site of little viral gene expression, and in conjunction with a conB site I exhibited the lowest basal LTR activity of any of the LTRs examined. LTRs containing a high affinity C/EBP site II 6G variant accumulated in the mid-frontal gyrus, a site of highly productive replication which correlates with the C/EBP site II 6G variant with a conB site I exhibiting the highest basal LTR activity. In conclusion, distinct LTR populations with specific C/EBP site II configurations were found in different regions of the brain.

P72
Public Policy and AIDS Vaccine Development and Trials

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Background: Several policy and economic challenges come up in the quest for AIDS vaccine development and trial in Ghana and Africa as a whole. In resource limited countries like Ghana, individuals often must wait for several years before they have access to licensed vaccines. Key policy issues and economic challenges affecting AIDS vaccine development and trial include: providing adequate funding for public and private sector research, enforcing protection for human subjects in clinical trials as well as speeding regulatory consideration of clinical trials.

Method: This presentation will look at potential policy interventions and each stage of the product development and delivery process. There will be a review of academic writings and current policy proposals and well as considering the merits and limitations of several proposals.

Results: As far as AIDS vaccine research is concern, Policy makers have a critical role to play. A variety of policy interventions are required to achieve accelerated results.

Conclusion: For speedy AIDS vaccine research and trials, policy makers should carefully assess the merits of policy proposals and enact changes as and when it’s necessary.

P73
Cultural Management of Stigma and HIV/AIDS in a Nigerian Ethnic Group

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The study examined how cultural management of stigma influences the impact of HIV/AIDS among people living with HIV/AIDS in an Igbo ethnic group in Nigeria. The traditional ways of caring for the sick among the Igbo has been eroded by modernization and improvement in the health sector. However, a disease like HIV/AIDS is dreaded as a disease that does not have a cure and attributed to witchcraft infliction in the area. Thus, people living with the virus receive little attention from relatives and health care providers in health institutions in this area and thus increase the vulnerability of those infected.

A total of 914 respondents were interviewed in two locations in both rural and urban locations and results were corroborated by focus group discussions to increase the participation of community leaders and Development Partners in addressing cultural practices and values that promote the transmission of HIV/AIDS and limits the participation of relatives of people living with HIV/AIDS in the provision of care to the sick.
Inadequate Knowledge About Sexually Transmitted Diseases [STDs] And Risky Sexual Behaviour: The Risk Factors for Wild Spread Of STDs Among Youth in Developing Countries

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Issues: This abstract shows that the low level of knowledge and risky sexual behaviour of youth are the risk factors for the high rate of spread of STDs among Nigerian youth.

Description: A self developed validated and reliable questionnaire [r = 0.77] was used to collect the data needed for the study and percentage was used to analyze the data. The population of the study was made up of the resident undergraduate/graduate students in male hostels in the Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The sample size is 636 selected through simple random sampling technique. The demographic data is as follows: Out of 636 respondents, 11 were below 16 years old, 95 were between 16 and 20, 309 were between 21 and 25, and 223, between 26 years and above. Two well developed tables were constructed. Relative Risk [RR] calculated is 1.7, i.e. RR > 1, indicating that the factors are risk factors, and the Confidential Interval [CI] for RR at 95% Significant level is 1.61 < RR < 1.79 from the formula, CI Lower limit < RR < CI Upper limit.

Lessons Learned: In table 1 which shows the knowledge of the respondents about STDs, revealed that 36.65% responses had knowledge about the diseases. Table 2 which shows the risky sexual behaviour of the respondents, revealed that the higher education students in Nigeria do engage in one risky sexual behaviour or the other, with majority of them having multiple sexual partners without using condom during the sexual intercourse, and this makes them highly prone to STDs including HIV/AIDS.

Conclusion and Recommendations: It is clearly seen that low level of knowledge and engagement in risky sexual behaviour are the obvious risk factors for the high rate of STDs in Nigerian youth and most developing countries.

Prevalence Of Campylobacter Species Amongst Hiv/AIDS Patients In Nigeria

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Background: This study aims to establish the prevalence of campylobacter species associated with diarrhea among HIV/AIDS patients in Lagos, their prevalent biotypes, antibiotic susceptibility patterns and plasmid profiles. This work was carried out in the Nigerian Institute of Medical Research Yaba Lagos Nigeria between March 2002 to July 2003.

Method: One hundred and sixty stool and rectal swab samples were collected from confirmed HIV patients seen at HIV clinic of Lagos University Teaching hospital and Central public health laboratory Yaba, Lagos. All the patients were heterosexual with age range between 15 and 55 years. The stool samples were collected from both diarrhoea and non diarrhoea patients. Stool samples were cultured on Butzler’s virion medium at 43°C with oxygen tension of about 5–10% for 48 hours. The isolates were subjected to ampicillin, cotrimoxazole, tetracycline, erythromycin, ciprofloxacin and gentamycin. Plasmid profile analysis was carried out on Campylobacter jejuni.

Results: 35% of the respondents reported that they will be willing to join HIV vaccine trials. Greater willingness was associated with prior sexual experience (OR = 1.23, 95% CI: 1.12–1.53), involvement in high risk sexual behaviour (OR = 1.35, 95% CI: 1.05–1.62), higher levels of awareness about HIV/AIDS (OR = 1.37, 95% CI: 1.14–1.45) and tangible incentives (OR = 1.39, 95% CI: 1.02–1.42). Decreased WTP was associated with concerns about physical harm (OR = 0.62, 95% CI: 0.21–0.54), social stigmatization (OR = 0.71, 95% CI: 0.42–0.68), use of parenteral route for vaccine administration (OR = 0.78, 95% CI: 0.53–0.76) and multiple doses of vaccines (OR = 0.81, 95% CI: 0.46–0.63).

Conclusion: The level of WTP recorded indicates that much work still needs to be done in the area of educating potential subjects in HIV vaccine trials about the safety of these vaccines. Incentives for would-be subjects should also be a part of the planning to encourage greater participation in these trials.
Conclusion: Prevalence of Campylobacter species in HIV/AIDS patients in Lagos was found to be 70% in patients with diarrhea and 2.5% in patients without diarrhoea. Campylobacter jejuni biotype 1 was the most common biotype and gentamicin and ciprofloxacin proved to be the drug of choice for campylobacter species in HIV-infected patients.

P77
CCR-5 D32 and SDF-1 3' A Polymorphism in Exposed But Uninfected Partners of HIV-Infected Individuals in North India
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Background: Genetic variations in chemokine genes have been associated with protection against infection by HIV-1 or slow progression to AIDS in infected individuals. We carried out this study to determine the prevalence of polymorphisms in CCR-5 D32 and SDF-1 3’ genes in exposed but uninfected partners of HIV-infected individuals in North India.

Materials and methods: 25 exposed but uninfected (EU) individuals and 25 normal healthy controls (HC) with low risk of infection were studied. Genomic DNA was extracted from whole blood and PCR was performed for genotyping. CCR-5 D32 and SDF-1 3’A were studied by PCR-RFLP using EcoR I and Msp I enzyme, respectively.

Results: No CCR-5 D32 mutations were detected in any individual. Both EU and HC showed a wild type CCR-5 gene. SDF-1 3’A gene polymorphism was frequently detected. Variant allele frequencies were 36% in EU and 20% in HC (P > 0.5). Out of 25 EU’s, 16 (64%) wild type, 8 (32%) heterozygous and 1 (4%) were homozygous were detected while in HC, 20 (80%) wild type and 5 (20%) heterozygous were detected. No homozygous mutation was detected in the control group.

Conclusion: CCR-5 D32 polymorphism, which was common among Caucasians, was not present in our population. Increased frequency of SDF-1 3’A polymorphism was detected in both EU’s and controls. Additional factors must be operating in protecting exposed uninfected individuals in our population.

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Retrovirology 2005, 2(Suppl 1):P78

P79
CCAAT/Enhancer-binding Protein-mediated Inhibition of HTLV-I Viral Gene Expression
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Recently, CCAAT/enhancer-binding protein (C/EBP) transcription factors have been shown to form heterodimers with cAMP-responsive element binding protein 2 (CREB-2), another bZIP transcriptional regulator known to play a critical role in driving basal and Tax-mediated transactivation of the HTLV-I long terminal repeat (LTR). Herein, we describe that overexpression of C/EBPα and C/EBPβ, including the endogenous isoforms liver-enriched activation protein (LAP) and liver-enriched inhibitory protein (LIP) inhibits Tax-mediated transactivation of the HTLV-I LTR. C/EBP-mediated inhibition was not the result of competition with the viral oncoprotein Tax for recruitment of co-activators CREB-binding protein (CBP)/p300 to the viral promotor. Electrophoretic mobility shift analyses demonstrated that C/EBP proteins derived from U-937 monocytic nuclear extracts directly interacted with the Tax-responsive element I repeat III as well as Tax-responsive element 2. However, deletion of these sequences within the context of the full-length LTR did not prevent C/EBP-mediated inhibition. Disruption of C/EBPβ/CREB-2 heterodimerization by deletion of the C/EBPβ leucine zipper prevented C/EBP inhibition of Tax-mediated transactivation of the LTR. These results suggest that C/EBP binding to Tax was not a requirement for C/EBP-mediated inhibition.

P80
Redirecting the Specificity of Naturally Occurring Antibodies Using gal-alpha1, 3-gal Coupled to HIV Recognizing Peptides
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Introduction: Due to the great variability and high glycosylation of gp120, possible targets for a fusion inhibitor include the CD4 binding region of gp120. Here we describe a method by which peptides corresponding to residues 25 to 64 of the CD4 receptor have been coupled to a major antigen, the gal-alpha 1,3-gal disaccharide, towards which humans have natural antibodies. Use of these fusion-molecules should redirect the specificity of the antibodies towards gp120 and possibly help reducing the viral loads of infected individuals.

Materials and methods: Binding of human anti gal-alpha 1,3-gal antibody glycopeptide complexes to gp120 and the virus was analysed by ELISA and a neutralization assay. The latter was based on reduction of syncytia in U87 cells in the presence of inactivated serum was also used to analyse the contribution of the complement dependent cytotoxicity to the system.

Results: Binding of the molecules was confirmed both by ELISA and by neutralization using the HIV 1 IIIB virus at several concentrations of the peptides with a 1:10 or 1:20 dilution of the human serum. Furthermore, complement proteins contributed to the neutralization capacity of the human anti gal-alpha 1,3-gal antibody-glycopeptide complexes.

Conclusion: Taking advantage of the innate response by redirecting the specificity of natural antibodies could be a complement to existing anti retroviral therapy of HIV infected individuals.
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Abstract withdrawn
Retrovirology 2005, 2(Suppl 1):P82

Promoting Involvement of the People Living with HIV/AIDS (PLWHA) Participate in Social/Community in Cambodia
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Issues: Cambodia has rapidly growing HIV/AIDS epidemic, one of the worst in Southeast Asia.
Project: Partnership between public health, medical institution, PLWHA groups and NGOs at operational district level, and strong referral system between the home, community and institutional care providers are necessary for development of a successful comprehensive HIV/AIDS care and support, especially those that were coverage PLWHA in Cambodia. By understanding systematic involvement of PLWHA at national, provincial, operational district and community level, the project aims to support PLWHA groups going through their self empowerment process from dependent phase in which NGOs/hospital arrange activities and they are recipients of the services, to partnership phase in which PLWHA facilitate active participation in services delivery in Cambodia.
Results: Cambodian People Living with HIV/AIDS Network (CPN+) to assist the Ministry of Health and National AIDS Authority. The sector will be working specially to assist developing care, treatment and other support services, and to facilitate referral systems among available services.
Lesson Learned: Reinforcement of PLWHA groups and policy making bodies resulted in a successful operational of the CPN+ strategic.

Loss of IL-7Ra is Associated With CD4+ T Cell Depletion, High IL-7 Levels and CD28 Down-regulation in HIV Infected Patients
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Interleukin-7 (IL-7) is a survival factor for naïve and memory T lymphocytes and it also increases T cell proliferation during lymphopenic conditions. Elevated levels of IL-7 have been found in the blood of HIV+ patients, which was considered as a homeostatic response to peripheral T cell depletion.
We showed that HIV infection is associated with an increased proportion of IL-7Ra low/negative peripheral T lymphocytes. Down-regulation of IL-7Ra on T cells was correlated with the depletion of CD4+ T cells and also with the increased concentration of serum IL-7. The decreased IL-7Ra expression resulted in the reduced survival capacity of T cells in presence of IL-7 and was associated with low Bcl-2 expression. Mostly the memory T cells down-regulated the IL-7Ra and we found a strong association between CD28 and IL-7Ra down-regulation. Accordingly, only CD28+ T cells responded to IL-7 with strong Bcl-2 upregulation.
The positive effects of IL-7 on survival and homeostatic proliferation of T cells might be severely impaired in HIV-infected individuals due to the decreased IL-7Ra expression. Chronic T cell activation may lead to an overall decrease of IL-7 mediated survival signals in HIV-infected individuals.

Increased IFN-γ Production by NK and NKT Cells From HIV-1-exposed But Uninfected Individuals
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Background: Innate immunity is very active at mucosal surfaces, that are the main port of viral entry; it is known that several components of the innate immune response have a direct anti-HIV-1 activity such IFNs and chemokines (1). Interestingly, a report (2) has indicated a high frequency of plasmacytoid dendritic cells and high production of IFN-α in response to Herpes simplex virus infection in long-term non-progressors and long-term survivors, suggesting an important role of these innate mechanisms in the control of HIV-1 infection.
Objective: To establish a relationship between some components of the innate immune system and the phenomenon of natural resistance exhibited by individuals who are continuously exposed sexually to HIV-1 but remain seronegatives (ESNs).
Materials and methods: We evaluated in peripheral mononuclear cells the frequency of plasmacytoid dendritic cells, myeloid dendritic cells, natural killer cells (NK) and NKT cells, and the secretion of IFN-α in unfraccionated mononuclear cells stimulated with Herpes simplex virus, as well as the expression of IFN-γ by NK and NKT cells after incubation with PMA/ionomycin, in three groups of individuals: low-risk HIV-1 negative controls (n = 30), sexually ESNs (n = 30), and HIV-1 seropositive individuals (n = 30).
Results: Among the evaluated parameters of the innate response, only the expression of IFN-γ by NK and NKT cells was significantly higher in exposed-seronegative individuals when compared with controls. As previously reported, HIV-1-infected individuals exhibited a significant decrease in the frequency of myeloid and plasmacytoid dendritic cells, NK cells and invariant NKT cells.
Conclusion: Since it is well known that IFN-γ is effective against HIV-1 in vitro, and also activates dendritic cells, NK cells
and cytotoxic T lymphocytes (3), this result suggests that the production of IFN-γ might be one of the factors involved in controlling the establishment of HIV-1 infection.

**P86**

**Randomized, Double-blind, Placebo Controlled Phase III Trial of Oxymetholone for the Treatment of HIV Wasting and Lipodystrophy**

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**Purpose of the Study:** Although HAART has greatly impacted treatment of HIV infection, lipodystrophy and HIV wasting still represent unsolved problems in HIV therapy and patent care. Oxymetholone, a testosterone derivative, has been shown to promote weight gain in AIDS-associated wasting.

**Methods:** We analyzed the effects of oxymetholone (50 mg BII) and TII) in a randomized (1:1:1), double-blind, placebo-controlled phase III study with 92 subjects (all on ART) experiencing unintended weight loss >10% of ideal weight according to Broca with special emphasis on body composition measurement (80 patients; 69 men, 11 women, mean age: 38.8 years) completed the 16-week double-blind study phase.

**Results:** Mean weight gain was +3.7 ± 3.5 kg and +3.1 ± 2.7 kg in the oxymetholone groups (BII); n = 25 vs TII); n = 27 as opposed to +0.9 ± 3.4 kg in the placebo were observed in body cell mass (30.6 kg before vs 32.5 kg after therapy), lean body mass (56.3 kg before vs 59.0 kg after therapy in the BII group) and body mass index (21.4 kg before vs 22.1 kg after therapy) exclusively in oxymetholone-treated patients. The extracellular mass to body cell mass ratio (p < 0.0001). Total body fat was unchanged by oxymetholone treatment. Adverse events were mainly hepatic occurring in 14% of oxymetholone-treated patients with significant elevations of AST, ALT and GGT; 2 patients (7.4%) in the BII) arm experienced grade 3 and 4 liver toxicity compared with 6 (21.4%) in the TII arm.

**Conclusion:** Oxymetholone was found to have true anabolic effects in a double-blind, placebo-controlled phase III trial. The (BII) (100 mg/d) regimen appeared equally effective to TII) (150 mg/d) dosing while displaying reduce liver toxicity. Due to its favourable protein anabolism, it may be recommended for (BII) (100 mg/d) regimen appeared equally effective to TII) (150 mg/d) dosing while displaying reduce liver toxicity. Due to its favourable protein anabolism, it may be recommended for (BII) (100 mg/d) regimen appeared equally effective to TII) (150 mg/d) dosing while displaying reduce liver toxicity.

Activity against infectious HIV-1 was measured using viral binding/entry inhibition (VBI) assays, in which each compound was evaluated for the ability to inhibit binding or entry events between target cells and HIV-1 strains IIIB (X4 phenotype) or BaL (R5 phenotype). Finally, compounds have been screened to determine their ability to interfere with cell-to-cell (CTC) HIV-1 transmission. In all assays, dextran sulfate was used as a control because of its minimal cytotoxicity and potent anti-HIV-1 activity. Out of 477 compounds tested since May 2001, 77 compounds tested using this algorithm were shown to have high selectivity indices (little or no cytotoxicity and consistently high activity in all three viral assays). These endeavors will greatly facilitate the search for compounds and formulations that can be used globally as topical microbicides.

**P88**

**N-myristoyltransferase1 and N-myristoyltransferase2 Exhibit Differential Substrate Specificity for HIV-Gag and HIV-Nef**

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**Background:** N-terminal myristoylation of Gag and Nef plays a critical role in retroviral virulence and budding of newly formed particles. Myristoylation involves the transfer of a myristate moiety from myristoyl-CoenzymeA (myristoyl-CoA) to substrate proteins such as Gag and Nef. Two isozymes accomplish this process in humans, and are known as N-myristoyltransferase1 (NMT1) and N-myristoyltransferase2 (NMT2). We used a biochemical approach to determine myristoylation kinetics for Gag and Nef as well as preferential substrate specificity for NMT1 or NMT2.

**Materials and methods:** NBD-labeled peptides containing the myristoylation sequence for Gag or Nef were myristoylated by recombinant NMT1 or NMT2 and subsequently analyzed by HPLC analysis.

**Results:** Results of the kinetics studies (K_m, K_cat, catalytic efficiency, turnover number, etc.) indicate that both isozymes prefer Nef up to 150 times vs. Gag as a substrate. Both isozymes exhibit greater catalytic efficiency in myristoylating Nef. Interestingly, Nef is preferentially myristoylated when Gag is present in the system.

**Conclusion:** This study is the first report of a differential role of NMT1 and NMT2 in the myristoylation of retroviral proteins and provides a new target in the treatment of HIV.

**P89**

**Abstract withdrawn**

*Retrovirology* 2005, 2(Suppl 1):P89

**P90**

**Gender Mainstreaming in HIV Vaccine Trials in India**

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*Retrovirology* 2005, 2(Suppl 1):P90

**Background:** In India, in December 2000, for the first time in the history of the country, the Ministry of Health and Indian Council of Medical Research signed a memorandum with International Aids
Vaccine Initiative (IAVI) a non profit, non government international organization to construct and evaluate appropriate vaccines. Today nearly 1 percent (approximately 4.5 million) of the Indian population is infected with HIV, where one in every four cases is a woman. Only 4 out of 10 women of the reproductive age group have heard of AIDS. A large percentage of girls are married by age 16. Yet, tradition dictates that girls are not supposed to know anything about contraception or sex. When infected with HIV, women face greater stigma and rejection than men, because of the association of HIV infection with socially and morally “disapproved” behaviors in society. Taking in view the socio cultural context of the country, considerable effort and planning is required to ensure equitable number of women participate in trials and stay through the entire duration of trial. Gender specific issues are integral to and an essential pillar of a larger programme focus on ethics. This includes building a gender sensitive perspective in members of the ethics review committee and members of the team that will be conducting the trials.

Material and methods: The National AIDS Research Institute, Pune is the nodal agency conducting the phase I trials. To ensure the mainstreaming of a gender perspective as an ethics issue in the HIV vaccine trials in India, individuals and organizations involved in running the vaccine trials are being trained to incorporate a gender perspective in planning and executing every stage of the trial. This training includes those directly involved in trials as well as norm setting bodies.

Results: The participants of the training are oriented to a gender analysis framework, which enhances understanding of gender implications of the vaccine trial.

Conclusion: The framework serves as a guideline to conduct the first ever HIV vaccine trials in India, and will feed into a global blueprint guide to gender sensitive HIV vaccine trials in other countries. This training on gender issues in HIV/AIDS helps in integrating a gender perspective in the larger issue of ethics that make up the guiding principle of HIV vaccine trials.

P91
Site Specific Codon Bias in HIV-1 pol Gene
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Background: There is increasing concern that synonymous genetic polymorphisms in HIV can influence drug resistance and molecular evolution. This study investigated the distribution of synonymous codons in HIV-1 and SIV reverse transcriptase enzymes.

Materials and methods: HIV-1 and SIV pol gene sequences were translated and the resulting protein sequences used as a scaffold to align the corresponding nucleic acid sequences. Synonymous codon usage was assessed in order to find out whether the use of unfavored codons at a given site is conserved.

Results: Some aligned positions contained unfavoured codons in most or all of the sequences analysed. Some of these positions are involved in drug resistance, CTL responses and enzymatic activity of RT.

Conclusion: Synonymous codons are differentially preferred at various sites and undergo positive selection. Thus, sites at which unfavored codons are conserved could be important in adaptive evolution of HIV. Knowledge on these sites can be applied in drug and vaccine design.

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P93
CCR5 Antagonist and Agonist Binding Site Structures
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Several small molecule antagonists of the HIV co-receptors CCR5 and CXCR4 are being developed as HIV entry inhibitors, but side effects have been observed in clinical trials that are likely due to agonist activity and/or cross-reactivity with closely related receptors. In order to develop high resolution maps of the binding sites for these antagonists that can be exploited to improve the activity and specificity, we are synthesizing derivatives of CCR5 and CXCR4 antagonists which contain photo-crosslinking groups at a variety positions in the molecules and that retain high affinity and activity against the receptors. Derivatives of two CCR5 antagonists have been crosslinked to affinity-purified CCR5 or CXCR4 expressed on cells, and the interaction sites are being mapped by mass spectrometry. Techniques for purification, crosslinking, CNBr and/or trypsin digestion, and LC/MS/MS and MALDI-TOF mass spectrometry of highly hydrophobic peptides initially developed using the rhodopsin GPCR system have been applied with some success to CCR5. The peptide photo-crosslinked to a derivative of the antagonist TAK-779 has been identified, and modeling of the interaction with CCR5 suggests that it binds within the transmembrane region of the receptor and is oriented parallel to the transmembrane helices – in striking contrast to the perpendicular orientation expected for GPCR agonists.

P94
hmm.Coreceptor Perturbation as a Possible Mechanism Underlying the Immediate and Persistent Anti-HIV-1 Activity of the Microbicidal Compound PEHMB
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Microbicides, which are products capable of reducing or eliminating the risk of HIV-1 sexual transmission, are urgently needed to combat the global spread of HIV-1. Our efforts in this area are focused on the preclinical development of the polybiguanide, PEHMB (polyethylene hexamethylene biguanide). PEHMB has been shown to have low cytotoxicity both in vitro and in vivo, as well as in vitro activity against both cell-free and
cell-associated forms of HIV-1. Flow cytometric analyses have shown that PEHMB exposure results in epitope-specific alterations in the detection of viral co-receptors CXCR4 and CCR5, suggesting that PEHMB acts by interfering with events critical to viral binding and entry. Changes in co-receptor detection have also been observed up to 24 hr after removal of PEHMB. Consistent with the latter result was the demonstration of persistent protection from infection in experiments in which cells were challenged with HIV-1 after removal of PEHMB. Cumulatively, these results suggest that HIV-1 co-receptors may play roles in PEHMB-mediated protection from HIV-1 infection, and that products containing PEHMB may provide both short- and long-term protection against HIV-1 transmission.

P95
Primary HIV-1 Infection Sets the Stage for Important B Lymphocyte Dysfunctions
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Background: B lymphocytes of patients with chronic HIV-1 infection (CHI) show functional and phenotypic abnormalities. We investigated the effects of primary HIV-1 infection (PHI) on activation, differentiation and survival of B cells. The effects of antiretroviral therapies on B cell dysfunctions in PHI were also studied.

Design and Methods: B cells of 31 PHI patients (sampled at baseline, 1 month and 6 months post therapy), 26 CHI patients, and 12 healthy donors were studied for surface expression of Fas, LAIR-1, CD70, intracellular expression of Bcl-2, and spontaneous apoptosis. Four-colour FACS (IgD+IgM+CD19+CD27), and short-term PBMC cultures to analyse induction of CD25 on B cells were performed in 5 PHI patients.

Results: In PHI, naive and memory B lymphocytes were highly activated, manifested by hypergammaglobulinemia, altered expression of Fas and LAIR-1, and increased spontaneous apoptosis. Antiretroviral treatment improved the activation/differentiation status of B cells, reduced apoptosis to levels comparable to healthy individuals and restored the ability of B cells to respond to T-cell dependent activation. B cells of PHI patients on HAART recovered better compared to patients on RTI only. Data obtained on 5 PHI patients at baseline showed decreased IgM+ memory B cells and lower induction of CD25 expression on B cells upon T cell activation. These parameters were normalized after 6 months of antiretroviral treatment.

Conclusion: B cell dysfunctions in HIV-1 infection appear during primary infection and initiation of antiretroviral therapy early during infection may help preserve the B cell compartment.
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P100
Characterization of Antibodies That Inhibit HIV gp120 Antigen Processing and Presentation
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Background: The capacity of Ab to alter antigen uptake and processing resulting in enhanced or suppressed antigen presentation has been demonstrated with a number of antigens, including tetanus toxoid, β-galactoside, apo-cytochrome c, and HIV-1 envelope glycoproteins [1–6]. In the case of HIV-1, the inhibitory activity is correlated with the serum Ab titers to the CD4-binding site (CD4bs) of gp120 [7]. In fact, by screening a panel of human anti-gp120 mAb, we ascertained that this inhibitory activity is mediated by Ab to the CD4bs; Ab to V2, V3, C2, or C5 did not exhibit such effect [1]. In previous studies only high affinity anti-CD4bs mAb were examined; these mAb completely block MHC class II presentation of gp120 antigens [1, 2]. However, it is not known if all anti-CD4bs Ab equally mediate such a strong inhibition. Since gp120/mAb complex formation was shown to be critical for anti-CD4bs mAb to block gp120 processing and presentation [1, 2], we postulated that the Ab affinity could be a key determinant for their suppressive activity.

Material and methods: In the present study we selected a panel of six anti-CD4bs mAb with different relative affinities for gp120, and examined their ability to suppress gp120 presentation to CD4 T cells. In addition, we tested CD4i mAb binding to the chemokine-receptor-binding site that, similar to anti-CD4bs mAb, were previously reported to render gp120 more resistant to degradative enzymes [7]. For comparison, a mAb specific for a conformation-dependent epitope outside the receptor binding sites and a relatively high affinity anti-V3 mAb were also tested. The ability of each of these mAb to suppress class II antigen presentation to gp120-specific CD4 T cells was correlated with the mAb affinity for gp120. The uptake of gp120 by APC was also evaluated in the presence of these mAb. Furthermore, we measured the stability of the mAb-gp120 interaction at acidic pH representing the endolysosomal environment in APC and quantified the effect of the mAb on the rate of gp120 proteolytic processing by lysosomal enzymes in vitro.

Results: Anti-CD4bs antibodies that completely obstruct gp120 presentation exhibit three common properties: relatively high affinity for gp120, acid stable interaction with gp120, and the capacity to slow the kinetics of gp120 proteolytic processing. None of these antibodies prevents gp120 internalization into APC.

Conclusion: The present studies demonstrate that poorly neutralizing anti-CD4bs Ab produced by chronically HIV-1 infected patients prevent the stimulation of gp120-specific CD4 T cell responses. These Ab form relatively stable high-affinity immune complexes, which are resistant to proteolytic processing by lysosomal enzymes. The presence of such Ab in sera of HIV-1-infected patients may contribute to the dearth of helper CD4 T responses to the virus envelope antigens and consequently weaken the anti-viral immunity necessary to control the chronic HIV infection and disease.

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P102
Cross-reactive Anti-gp41 HIV-1 Neutralizing Human Monoclonal Antibodies Selected by Competitive Panning Against gp140 – an Update
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By using a methodology based on competitive panning against gp140 in presence of excess of gp120 we identified seven new human monoclonal antibodies, m42-48, which bound to gp140s from primary isolates representing different clades. Some of them also bound a gp41-Fc fusion protein but not peptides and denatured gp140 suggesting that their epitopes are conformational; they competed with the cluster IV antibody T3 suggesting involvement of membrane proximal regions. The antibody Fabs inhibited entry mediated by envelope glycoproteins from primary isolates from different clades with potency on average comparable to that of Fab Z13; one of these antibodies, m48, was much more potent in an IgG1 format. Some of the antibodies were converted to scFvs to test the possibility for steric restriction effects; the experiments are ongoing and the results will be presented. These results indicate the possibility that conformational epitopes on gp41 could be a target for broadly neutralizing antibodies and may have potential for the development of new vaccine immunogens.

Members of my group, including Ponraj Prabakaran, You-Qiang Wu, Samitabh Chakraborti, and Xiaodong Xiao, as well as our collaborators, especially C. Broder, G. Quinlan, R. Blumenthal, D. Montefiori, and their groups contributed to these results.

P103
C-Maf Cooperates With NFAT2 to Augment HIV-1 Transcription in IL-4 Producing CD4 T Cells
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The human immunodeficiency virus, HIV-1, infects human CD4 T cells, including Th1 and Th2 helper cell subsets. The virus relies on host
transcription factors, such as NFkB and NFAT for its transcription and expression. It has previously been shown that HIV-1 preferentially replicates in Th2 cells, but the mechanism of action for this finding remains unknown. c-maf is a Th2-restricted transcription factor that is critically important for differentiation along a Th2, but not Th1 lineage, and for transcription of the prototypic Th2 cytokine, IL-4. c-maf directly binds the proximal IL-4 promoter and acts synergistically with a neighboring NFAT site. We have demonstrated at the individual cell level that IL-4 positive cells (Th2) preferentially support HIV-1 replication compared to IFNg positive (Th1) cells. In studying the HIV-1 long terminal repeat (LTR)/promoter sequence, we identified a MARE (maf-recognition element) located just proximal (5') to the dual NFkB/NFAT binding sites. We show that the HIV-1 MARE binds recombinant maf protein and abuts NFAT binding as detected by DNase I in vitro footprinting. In addition, this HIV-1 MARE demonstrates identical mobility shifts compared to the IL-4 promoter in gel-shift assays using nuclear extracts from activated primary human CD4 T cells. Using chromatin immunoprecipitation, we further show that c-maf binds to the HIV-1 LTR in vivo in HIV-1 infected primary human CD4 T cells. Although we have previously shown that both NFAT1 and NFAT2 are capable of transactivating the HIV-1 LTR, we now show for the first time preferential binding in vitro and in vivo of NFAT2 over NFAT1 to the HIV-1 LTR. By comparison, the more abundant NFAT1 family member preferentially binds to the IL-2 promoter. NFAT2 has previously been implicated in Th2 cytokine expression and appears to cooperate with c-maf in binding to the HIV-1 LTR. Functionally, over-expression of c-maf in primary human CD4 T cells cooperatively increases HIV-1 transcription when co-expressed with NFAT1 and 2, and, silencing endogenous c-maf expression in primed human CD4 T cells decreases viral transcription. Similarly, over-expression of c-maf alone, or with NFAT1 or 2, increases HIV-1 replication, as measured by intracellular p24/gag expression, in CD4 T cells co-expressing GFP but not in GFP negative/c-maf-negative controls within the same transplanted population. Lastly, depletion of c-maf expression by siRNA in primed and HIV-1 infected CD4 T cells decreases p24/gag expression. In summary, the Th2-specific transcription factor, c-maf, binds the HIV-1 promoter in cooperation with NFAT transcription factors, primarily NFAT2, to augment HIV-1 transcription and replication in primary human CD4 T cells. These are the first data to mechanistically explain preferential HIV-1 transcription in IL-4 producing (Th2) cells.

P105 Educating, Engaging and Structuring Rural Women in the fight Against HIV/AIDS in Liberia Nyode Wesley1,2 1Hills, Advocate for Treatment & Care 2Monrovia, Liberia E-mail: estelnyode@yahoo.com

P104 HIV-1 Glycopeptides as Immunogens

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One challenge in HIV-1 vaccine design is to identify epitopes able to induce neutralizing antibodies. HIV-1 glycopeptides represent a partial structure of the envelope glycoproteins that contains both peptide and carbohydrate motifs. Several pieces of evidence suggest that HIV-1 glycopeptides may constitute new neutralizing epitopes: 1) certain HIV-1 glycopeptides are highly conserved and are well accessible; 2) selected N-glycans around the V3 domain have been identified as neutralizing epitope for the broadly neutralizing antibody 2G12; and 3) N-glycans can mask unwanted epitopes, redirect the immune focus, and induce conformational epitopes. To explore this new territory for immunogen design, we have focused on exploring the V3 domain glycopeptides that correspond to the so-called principal neutralizing determinant (PND) and the gp41 C-terminal glycopeptides that are involved in viral membrane fusion.

We have developed a novel chemoenzymatic method for constructing large homogeneous HIV-1 glycopeptides that are hitherto unavailable. Preliminary studies indicated that glycosylation affects the global conformations and enhances the beta-turn and/or loop structure of the V3 domain in buffer. We also observed that the N-glycans can protect the V3 domain against protease (furin and pronase) digestion. In addition, we found that glycosylation on C34 has a profound effect on its ability to form the six-helix bundles with N36. The interesting glycosylation effects observed urge further immunization studies with the glycopeptide immunogens.

P106 Making Liberia an AIDS Free Nation

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Background: In the 1990’s and before then, nearly every women that carry out HIV/AIDS awareness came from urban areas, and were educated as rural women took little interest on HIV/AIDS issues. A multisectoral consensus meeting took place in 2002 to mobilize policy makers, to express solidarity with people that are more vulnerable to the pandemic and to train people from rural areas in raising awareness among people in the country. This could be realized by maintaining permanent contact with public and private institutions engaged in the fight against HIV/AIDS.

Methods: Under the leadership of a multisectoral task force, various women met every week, and created the Rural Women Aids Network (RWAN). With technical assistance from donor agencies, RWAN formulated a series of standard operating procedures and guidelines, developed audiovisual materials for capacity building and organized nationwide workshops to train women on ethics, stigma discrimination and human rights.

Results: In spite of limited funding, 500 women have been trained on STI/HIV/AIDS, stigma discrimination and human rights. About 20 PLHA have received capacity on communication skills, self esteem and knowledge of the media environment, and three load chapters have been created. RWAN has matured into a respected independent and sustainable instutions, participates in various national and international conferences, and organizes national prevention campaign through radio advertisement and street banners.

Conclusion: Educating, engaging and structuring rural women is an invaluable asset in combating stigma and discrimination, promoting human rights; engaging women to produce quantities of quality articles and raising self-esteem among PLHA’s from victims to a profile of courage.
(LNACP) that the rate of infection on our growing population in term of those infected with the virus are increasing immensely. Many people do not really believe that AIDS is real. People are dying ignorantly from the virus. Other people just believe that something just have to kill someone. In the year 2004 the rate of infection was estimated to be 11–12 % of the approximately 3 million people in Liberia. By this time we are optimistic that there is an increase in the infection rate which is basically caused by the high rate of illiteracy, the influx of more foreigners or aliens on peace mission and business purposes, the high rate of poverty (80–90%) also cause by the high rate of unemployment leaving many people vulnerable to the virus.

Methods: A team of awareness on AIDS education took the city of Monrovia and its surroundings, congregating students from various high schools between the ages of 12–20 explicating the danger and prevention of AIDS from poster prints, tracts, and handouts for better understanding. Audio visual aids were actually needed to authenticate the information provided, but none was available. Questionnaires were distributed to evaluate their comprehensions and for statistical purpose.

Results: Of the total number of students 55% of them now believe that AIDS is real whilst 35% believe AIDS does not exist; on the other hand 10% believe that abstinance is the best method of prevention.

Conclusion: Peer education—one on one and one on group methods of awareness, engaging and structuring the press, promoting human rights, engaging journalists to produce quantities of quality articles, raising awareness among policy makers, high level government officials and the public in general are invaluable asset in combating HIV/AIDS. Capacity building workshops and conferences are most needed among the various tribes in worship centers. If these are considered and fully supported by international AIDS groups working in collaboration with local based groups Liberia will be a success story.

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P109
Considerations and Controversies of AIDS
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The statistical probability of seroconversion is proportional to the number of needlesticks incurred and the likelihood that the needlesticks will be with HIV infected blood. Careful adherence to recommended operating room practices, combined with meticulous attention to handling needles and sharps, should result in few, if any, cases of occupational HIV seroconversion among OR personnel. HIV testing is not feasible in the management of emergency patients; these are often the individuals at highest risk for HIV infection and over whom the surgical team has the least control. Non-operative treatment of HIV-infected patients is not an option; many procedures are performed either to enable the individual to lead a more comfortable, productive life or for diagnostic purposes.

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P112
Acquired ImmunoDeficiency Syndrome (AIDS) in Persons Aged Over 55 years, Living in Tropical Areas. 175 Cases in Congo
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The objective of this study has been to estimate the frequency of AIDS occurring in older age groups on the basis of hospital statistics and note the prognostic particularities in these groups. One hundred and seventy five (175) cases of AIDS reported to the University Hospital Center of Brazzaville occurring in persons aged 55 years and over were followed up retrospectively from 1 January 2003 to 31 December 2004.

The results of this study indicate that AIDS is not rare in older age groups: 4.7% of all infected subjects registered during the period of study. The sex-ratio was 1.3/1 (99 males and 76 females). The overall mean age was 60.45. Contamination seems to be the most often of heterosexual origin. Many symptoms were found. The most frequent ones were weight loss (100% cases), fever (89.7%), diarrhoea (60.5%), neuro-psychiatric disorders (49.7%), and respiratory manifestations (50.2%). Lethal evolution was rapid, with 74% deaths at the end of the 1st year and 100% at the end of 2nd year, as a consequence of delayed diagnosis as well as the natural development of the disease.

The results of this study point to the necessity of prevention strategies which include not only young, but older age groups as well.

P113
Design of RANTES-derived Peptides With Enhanced HIV-inhibitory Activity and Derivation of Resistant HIV-1 Strains
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We previously identified the major structural determinants of CCR5 binding and HIV blockade in RANTES, describing linear RANTES-derived peptides with biological activity in the low micromolar range (Nardese et al., 2001). To deepen our under-
standing of RANTES structure-function, we have extensively mutagenized the prototypic peptide, R11-29. This presents two clusters of hydrophobic residues at its termini, (corresponding to RANTES N-loop and b3-strand) connected by a positively-charged linker. Single or multiple alanine substitutions within the N- or C-terminal hydrophobic clusters resulted in a dramatic loss of antiviral activity, whereas deletion of selected residues within the hydrophilic linker had no major functional consequences. Based on RANTES 3D structure, we designed a series of modified peptides, resulting in a progressive increase in specific antiviral activity. These peptides also displayed anti-inflammatory properties blocking RANTES-elicted lymphocyte chemotaxis. Through serial passages in culture in the presence of increasing concentrations of the most effective antiviral peptides, we have derived variants of the R5 HIV-1 isolate BaL resistant to the peptide inhibitory activity. Complete sequencing of the envelope genes from such variants is currently underway. Our results provide new insights into the structure of the receptor-binding region of RANTES and identify new antiviral peptides that may be instrumental in the development of effective HIV-1 entry inhibitors.

P114 Replication of Significant Relationship Between MIP-1/B Production Following p24 Stimulation and Type C Coping
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Background: We have shown that the Type C style of coping with stress (diminished ability to recognize and express stress/distress/emotions) is related to HIV progression. We recently found in a study of 50 HIV+ patients in Baltimore, that stronger Type C coping is associated with decreased production of beta-chemokines that bind to the HIV co-receptor CCR5. Under the aegis of NIH, we have initiated a longitudinal study with a final N = 200 to evaluate the core hypothesis that lower production of the beta-chemokines MIP-1 al/β mediates the relationship between Type C coping and HIV progression.

Methods: Type C coping was assessed using Temoshok’s Vignette Similarity Rating Method. Measurement of antigen-induced chemokine production from subjects’ blood followed methods described in Garzino-Demo et al. PNAS 1999. Cells were incubated with media alone (control), p24 antigen, PHA, or candida. Supernatants were collected on day 3 and 6 for beta-chemokine measurements. Assays for MIP-1al/β were performed by commercial ELISA for the first 47 subjects.

Results: Subjects who scored high on Type C coping (3,4,5) had a significantly lower mean stimulation index for MIP-1/β by p24, compared to subjects low on Type C coping (2.7 vs. 5.0).

Conclusion: This replication in a separate sample strengthens our hypothesis about mechanisms mediating HIV progression.

P115 Vicriviroc (SCH 417690) Distribution from the Gut to Gut-Associated Lymphoid Tissues (GALT) and to Peripheral Lymphoid Tissues Following an Oral Dose
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Background: Vicriviroc (SCH 417690) inhibits HIV-1 infection by blocking the viral CCR5 co-receptor. Early HIV replication is associated with rapid depletion of CCR5+ CD4 T lymphocytes that predominate in gut-associated lymphoid tissue (GALT), an important site of early establishment of HIV infection. Given the rapid absorption of oral Vicriviroc, appreciable drug exposure to GALT is predicted, potentially protecting this important component of the immune system.

Materials and methods: An oral 5 mg/125 μg/kg dose of 14C-labeled Vicriviroc was administered to rats and drug concentrations determined by autoradiographic techniques at various timepoints up to 168 hr.

Results: Vicriviroc rapidly permeated the gut wall resulting in appreciable drug exposure to GALT. The rank-order in cumulative Vicriviroc exposure was GALT > lymph node > lungs > blood, although exposure to GALT<spleen. GALT drug concentrations up to 48 hr were 10- to 102-fold higher than the targeted IC90 concentration (IC90 = 6 nM). Differences in cumulative drug exposure between GALT and other tissues was the result of differences in both the observed peak drug concentration and in clearance rates from the individual tissues.

Conclusion: Results suggest that high and sustained Vicriviroc concentrations in GALT can be achieved by as little as a single oral dose, which may prevent viral replication in these tissues and reduce the depletion of CCR5+ CD4 T lymphocytes.

P116 Characterization of the In Vitro Human Liver Cytochrome P450 (CYP) Mediated Metabolism and Inhibition Potential of Vicriviroc
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Vicriviroc (formerly SCH 417690), a CCR5 receptor antagonist, is currently under investigation for the treatment of HIV infection. Human liver microsomes (HLM) metabolized vicriviroc via N-oxidation (M2/M3), O-demethylation (M15), N, N-dealkylation (M16), N-dealkylation (M41) and carboxylic acid formation (M35b/M37a). The metabolites generated under in vitro conditions were also detected in clinical studies after oral doses of vicriviroc. Incubation with recombinant human CYP3A4 formed all metabolites listed above, while CYP2C9 formed M15 and CYP3A5 formed M2/M3 and M41.
In clinical trials, vicriviroc co-administered with ≥100 mg QD ritonavir (RTV), a potent CYP 3A4 inhibitor, resulted in a Cmax 2 to 3 times higher and an AUC(0–12 hr) 4 to 5 times higher than vicriviroc alone. In vitro pre- or co-incubation inhibition studies with HLM demonstrated that vicriviroc does not significantly inhibit the activities of CYPs 1A2, 2A6, 2D6, 2C9, 3A4, or 2C19 at concentrations up to 26.7 μg/mL (100 X the expected human plasma Cmax following once daily oral doses of 10 mg + RTV), which suggests that vicriviroc is not an inhibitor of major CYP enzymes.

The results suggest that formation of the major vicriviroc metabolites in human liver microsomes is primarily mediated via CYP3A4, and that vicriviroc, at clinically relevant doses, is unlikely to inhibit other co-administered drugs metabolized by the major CYP 450 enzymes.

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P118
Comparing Medical Inpatient and Outpatient HIV-positive Baltimore Populations on Adherence to HIV Medications

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Background: We have previously reported on levels of adherence to HIV medications and psychosocial factors associated with adherence in an outpatient HIV primary care clinic serving disadvantaged, largely African American patients in West Baltimore. In our most recent study of 70 outpatients, missed doses were significantly correlated with depressive symptoms, social instability, and the number of severity of current life stressors, in concert with the literature. This study found uniquely that patients’ trust and confidence in their medical providers were the strongest predictors of better adherence. There are no studies, however, which systematically evaluate adherence and associated factors among hospitalized inpatients, a population that has received insufficient attention.

Methods: Baseline psychosocial and clinical variables were assessed for 90 inpatients and 78 outpatients. Almost none of the inpatients could be classified as optimally adherent (>95%), compared to the outpatients (>50% adherent >95%). Although both populations reported a mean of 3+ depressive symptoms, the inpatients acknowledged more severe psychiatric symptoms. Compared to outpatients, HIV+ inpatients had lower CD4+ cell counts, were more likely to have recently abused drugs, to cope by avoidance and substance abuse, and to be homeless.

Conclusion: HIV+ inpatients suffer from multiple social and psychological co-morbidities that contribute to poor adherence. To improve their prognoses, these problems must be addressed.

P119
HTLV-2 Induces Resistance to CCR5-Dependent HIV-1 Infection Via Selective PBMC Expression of CCL3L1

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Background: In HIV-1/HTLV-2 co-infected IDUs the CCL3/MIP-1alpha induction by HTLV-2 leads to HIV inhibition. CCL3 gene codes for CCL3/LD78alpha and CCL3L1/LD78beta isoforms. CCL3L1 binds more potently to CCR5 than any other chemokine. Possession of a CCL3L1 copy number lower than two (the population average for Europeans) is associated with markedly enhanced HIV/AIDS susceptibility. Here, we analysed the genotype frequency of CCL3L1 and its expression in 8 HTLV-2-infected/HIV-1-exposed-seronegative (HTLV-2/HIV-1ESN) individuals, 7 LTNP-HIV-1/HTLV-2-co-infected and 8 LTNP-HIV-1-mono-infected subjects.

Methods: R5 HIV-1 infection of PBMC from HTLV-2/HIV-1ESN was evaluated for HIV proviral load and for p24 production. CCL3L1 gene copy number and mRNA expression levels were assessed using real-time PCR. CCL3 and CCL3L1 isoforms were identified from spontaneous PBMC cultures by mass spectrometry (MS). Results: R5 infectibility and efficiency of viral replication in primary PBMC from HTLV-2/HIV-1ESN were very low. The median of CCL3L1 copy number was one in HTLV-2/HIV-1ESN three in LTNP-HIV-1 and two in HIV-1/HTLV-2 subjects. CCL3L1 mRNA was more abundant in individuals with HTLV-2 infection than in HIV-1 LTNP. MS analysis evidenced that intact CCL3L1, usually not secreted from healthy subjects, was produced by PBMC of HTLV-2/HIV-1ESN. Noteworthy, CCL3L1 isoform was highly expressed by PBMC of LTNP HIV-1/HTLV-2, but not of HIV-1 LTNP. The high CCL3L1 production, and a persistent IFN-gamma secretion, conferred a CCR5low phenotype to HTLV-2 infected subjects.

Conclusion: HTLV-2 may curtail HIV-1 infection upregulating the CCL3L1/LD78beta chemokine. Apparently, HTLV-2 infection can fully compensate for the functional state conferred by CCL3L1low.

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P121
HIV-HCV Co-infection Among Blood Donors

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Background: The World Health Organisation (WHO) estimate that 170 million individuals worldwide are infected with
Materials and methods: This was a cross-sectional study of HCV testing kits. Supporting epidemiological data and prohibitive costs of the for hepatitis C. This is so due to the non-availability of hepatitis C virus (HCV). However the prevalence of HCV infection varies throughout the world. HCV is transmitted primarily through blood or blood products or contact with infected tissue (blood transfusion intravenous immunoglobulins, intravenous drug abuse and tissue transplant). Since hepatitis C is a preventable disease, accurate description of the prevalence and risk factors for the disease is a pre-requisite for prevention. This study is of public health importance as the National Transfusion Service has no policy to screen donated units of blood for hepatitis C. This is so due to the non-availability of supporting epidemiological data and prohibitive costs of the HCV testing kits.

Methods: This was a cross-sectional study of 1600 regular health blood donors. These donors underwent a routine blood donor selection process. Blood sample of 5–8 ml was collected into a labelled vacutainer. Blood was allowed to clot, after centrifuging 2 mls of serum was stored at -20 degrees celcius for further analysis for confirmation of antibodies to HCV. Serum samples were also analysed for hepatitis B (HBV), Syphilis and HIV 1/2. All antibodies to HCV were determined using ELISA Abbott murex (version 4.0) which has 99% sensitivity and 99.9% specificity. Statistical analysis was performed using EPI Info version 6. Prevalence of HIV-HCV at 95% confidence interval (CI) were calculated. Chi-squared analysis was done to test for 3% level of significance. A p-value of less than 0.05 was considered significant. Fisher’s test-2 tail probability was used to determine for the association between factors when the expected frequency was less than 5. Seropositive samples were not confirmed using molecular methods due to limited resources.

Results: The median age (Q1, Q3) of the blood donors was 31 (22,46) and 56% of them were male. ELISA assay was positive for 28 samples in the studied population yielding an overall prevalence of 1% (95% CI 0.7–1.5%). There was no dual infection for HBV and HCV. 1.2 % Anti-HCV individuals were also positive for HIV1/2. 1.7 % individuals tested positive for syphilis as well as positive to anti-HCV. No association was detected between age and seropositive status (p = 1.000). There was 18% risk of acquiring infection from blood transfusion (RR = 1.18, 0.78<RR<1.79).

Conclusion: Considering the nature of the population studied the prevalence of HIV-HCV co-infection of 1.2% is high. Screening all donated blood for HCV must be mandatory. There is also need to carry out a study of HCV co-infection in patients living with HIV/AIDS since anaemia of chronic illness is a common trigger for blood transfusion in this group.

P124 Probing Cell-to-cell Transfer of Human T-cell Leukaemia Virus Type-1 Using Novel Inhibitors of Viral Entry
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Infection of human cells by human T cell leukaemia virus (HTLV-I) is mediated by the viral envelope glycoproteins. The gp46 surface glycoprotein makes first contact with the target cell through binding to the cell surface receptor Glut-1, thereby allowing the transmembrane glycoprotein to initiate fusion of the viral and cellular membranes. We have now used a soluble recombinant form of gp46 fused to the Fc-region of human IgG (sRgp46-Fc), and a panel of antibodies and inhibitory peptides to probe envelope function during cell-to-cell viral transfer. We have been able to recapitulate the transfer of HTLV-I between cells through sites of tight cell-to-cell contact that have been termed the virological synapse. We now demonstrate that upon contact with HTLV-I infected T-cells, the HTLV-I receptor glucose transporter-1, Glut-1, is redistributed within the membrane of target cells. On the non-infected target cell Glut-1 is re-localized to, and enriched within, the point of synaptic T-cell contact. Importantly, this re-localization of Glut-1 reflects the pattern of envelope accumulation on the HTLV-I infected cell. Moreover, we find that sRgp46-Fc is also able to effect re-localization of Glut-1 on T cells, suggesting that envelope-mediated recruitment of Glut-1 to the site of synaptic transfer may be a crucial event in the cell-to-cell transfer of HTLV-I. Our recent results will be presented, and the implications of our findings for HTLV-I pathogenesis will be discussed.

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P126 Identification and Biological Characterization of Unique B/C Recombinant Strains of HIV-1 In Southern States of India
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Identification and Biological Characterization of Unique B/C Recombinant Strains of HIV-1 In Southern States of India

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Background: HIV-1 subtype-C strains are associated with more than half the infections globally. The molecular nature of the viral subtypes circulating in India is not adequately characterized. In the largest study ever to appear from India, we recently reported a predominance of subtype-C infections in the southern states of the country (Siddappa N.B et al AIDS in press). Unexpectedly, we identified 3 unique B/C recombinant viruses in our cohort that contained envelope of subtype-B origin.

Methods: 608 seropositive volunteers were enrolled during 2000–2004 for this study. Genomic DNA from the blood samples was characterized using a novel PCR that differentially identified subtype-C viruses in the viral LTR. A subset of 115 samples was also analyzed in the env, using DNA sequencing and/or HMA. Results: Out of a total of 608 samples, 602 (99%) were identified to be subtype-C in LTR. Additionally, two subtype-A one subtype-B and 3 B/C recombinants were also identified. Interestingly, env sequences of two of three B/C recombinant viruses phylogenetically clustered with subtype-B strains of the USA and the third one with Thai-B. Subtype-C viruses are hypothesized to have evolved to greater levels of attenuation, hence are less pathogenic to the host. This hypothesis was further supported by the observation that the prominent recombinants of subtype-C (B/C and C/D) invariably retain env sequences derived from subtype-C. In this backdrop, the B/C recombinant viruses we identified in southern India attain importance as these recombinants contain naturally evolved B-env. We are presently isolating molecular clones of these unique B/C recombinants for further characterization of subtype-C viruses.

Conclusion: Globally, the number of circulating recombinant forms of HIV-1 is rapidly increasing. Importantly, the overall incidence of the recombinants is also on the rise. Our identification of two different types of unique B/C recombinants in southern India, at a significant frequency, warrants an extensive nationwide investigation to determine if a new epidemic is emerging in India.

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P129
An Evaluation of the Nutritional Status of Persons Infected by HIV/AIDS: The Case of Ngaoundere Provincial Hospital-Cameroon
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Keywords: Haematology; biochemistry; anthropometry; HIV/AIDS; nutrition

Morphological, haematological and biological alterations in the blood of persons infected by HIV/AIDS have been described by certain studies carried out in and out of Africa in AIDS patients. The objective of our study is to determine the nutritional status of an AIDS infected person in sub-Sahara in order to facilitate a proper care taking by evaluating the anthropometrical, bio-chemical (creatinin, cholesterol, transaminase), biological (red and white blood cells) perturbation parameters. It implies a study of very talking case in which 100 persons (44 men and 56 women) did participate. These persons were given questionnaires in which information on the civil, chemical states, the anthropometrical parameters and the diet of the last two days were asked. Only those of the persons who gave their accord were retained. Blood specimen was taken for HIV-AIDS screening test (Elisa and Western blot) and to dose the transaminases, cholesterol, creatinin by spectrophotometer. Both the weight and skinfold were taken using the Holtain calliper. The results of this study revealed 72 persons infected and 28 negative cases. The transaminases increased enormously (p < 0.000003) in the course of the infection. This increase must either be the consequence of a co-infection VHH-VHB or VHH-VHC responsible of the hepatological cytolysis or the action of opportunized infections. In PLHIV, the increase of transaminase which is two to three times superior to the normal (p < 0.05) translates not only a tissue necrosis but equally reveals a steatosis, a fibrosis and a hepatic cirrhosis. HIV infection and especially the presence of signs of an opportunized infection which provokes a muscular dystrophy revealed a significant drop of the creatinina (p < 0.05). The seropositive persons suffer from hypocholesterolemia which can have a serious effect on the maintenance of their health, ponderal deficit compared to the initial weight (p < 0.05), and present a BMI and degree of skinfold which attests denutrition. The anthropometrical parameters are influenced by the diet and the serological status. Some profound modifications in the composition of surrounding blood cells (leucopenia, anemia) in the infected persons (p < 0.05). The infected women present greater importance (p < 0.05) of energy needs than the non-infected. Certain signs of opportunized illnesses lead to greater energy deficit than others; particularly the case of tuberculosis as a characteristic sign and cough as sign associated with HIV infection. Nutritional support needs to be given to PLHIV and, in a holistic manner, in complement of all others treatments.

P130
Association Between the Presence of CCR5-specific Antibodies and Long Term Non Progression
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Most transmitted HIV-1 strains use CCR5 as coreceptor. Antibodies (Abs) to CCR5 have been detected in highly exposed to HIV-1 but uninfected subjects, thus they could be involved in HIV protection. To assess whether these Abs may also contribute to slow HIV-disease progression, we searched for anti-CCR5 Abs in 499 subjects, including 87 Long Term Non
Progressors (LTNP), 70 Progressors, 135 HIV+ HAART treated, and 207 seronegative donors. We found anti-CCR5 Abs in a fraction of LTNP (22.9%), but not in the other populations studied (p < 0.0001). These Abs efficiently prevent infection of HIV-R5 strains representing subtypes B, C and A by inducing a stable and long last down regulation of CCR5 on surface of T lymphocytes. Follow-up studies showed that the loss of anti-CCR5 Abs, occurred in some subjects, was significantly associated with a progression toward disease. Thus, the anti-CCR5 Abs could be relevant to vaccine design and therapeutics.

**P131**

Clinical Efficacy by Intratumoral Injection of DNA Encoding Human Interleukin-12 in Metastatic Melanoma Patients

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Plasmid DNA encoding human Interleukin-12 (IL-12) was produced under GMP conditions and injected into lesions of nine patients with malignant melanoma (stage IV) previously treated with both, standard and non-standard therapies. The treatment was based on efficacy in preclinical studies with melanoma in mice and gray horses. The DNA was applied in cycles, three injections per cycle, up to seven cycles. Three therapy arms comprised low (2 mg), medium (4 mg) and high (10 to 20 mg) amounts of total DNA. The therapy was well tolerated. Three out of nine patients experienced a clinical response, 2 SD and 1 CR. One patient receiving a low dose of DNA experienced a long-lasting stabilization of the disease for more than three years, while the other two responders received high doses of DNA. All patients but one (P9) experienced a transient response at the intratumoral injection site. Immunohistochemical staining of responders showed local reduction of angiogenesis and lymphocyte infiltrations. All patients, in particular the responders (P3, P7, and P8) exhibited an antigen-specific immune response against MAGE-1 and MART-1. Biopsies of responders showed some increase of IL-12, IP-10 and IFN-γ. (Hu. Gene Ther. 16, 35 (2005)). Combinations of IL-12 DNA with other therapies show significant increase of efficacy in preclinical studies and will be discussed.

**P132**

Abstract withdrawn

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**P133**

More Antiretroviral Drugs Will Reduce Stigma and Discrimination in West Africa

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**Background:** In West Africa, the high level of Stigma and discrimination attached to the victims of HIV/AIDS is due to the lack of many factors including the lack of free and cheaper antiretroviral drugs. This is due to poverty on the part of victims and the lack of good policies by the government to subsidise ARVs.

**Methods:** Discussing sexuality issues publicly in West Africa is like a taboo, therefore several techniques have to be adopted inorder to collect adequate informationfor analysis. The information collected was drawn from four countries, namely; Liberia, Ivory Coast, Ghana and Benin. In the process, schools refugee camps internally displaced campsite night clubs bars and other public places including market grounds and youth centers. Interviews were conducted and questionnaires were given at times and also group discussions- were held inorder to get the individual and general view of the masses.

**Results:** The assessment passed out well with just few minor obstacles relating to some discussions in some areas forbidden by social customs and traditions. In total 300 persons were surveyed, ages 12 to 25. Ninety percent of illiterate the people know that AIDS is a sickness but do not know how it is transmitted, whereas 50% of the students know that AIDS exist and it is real but do not know the actual root causes or what even the acronym stands for. About 20% of them do not know the difference between high risk behavior and low risk ones. About 98% of the students agree that stigma and discrimination affects the rate of growth of infected persons whilst 40% of sexually active youthsaid that they do not like the use of condoms. About 85% of them said that they can never befriended someone with AIDS i.e they cannot eat, talk, shakehands or share clothes. Surprisingly 23% said that if the doctor tells them that they are HIV positive they will either commit suicide or get extraordinarily promiscuous to spread the disease and not to die alone.

**Conclusion:** It is possible to decrease stigma and discrimination by providing information on HIV/AIDS. This should focus on what the virus is how it can be transmitted and prevented. There should be intensive education on how victims of the pandemic should be treated and cared for. Governments shoul subsidise the importation of cheaper and safer antiretroviral drugs and do everything possible to reduce stigma and discrimination as relate to HIV/AIDS. Stigma and discrimination will be reduced because some hope, at least is provided for a longer and healthier life. If drugs are provided to buttress the counseling received, people will feel comfortable when affected.

**P134**

The Immunomodulatory Agent Rapamycin Potentiates the Antiviral Activity of the Fusion Inhibitor T20 Against R5 Strains of HIV-1

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The fusion inhibitor T20 marks the beginning of a new era in the management of HIV-1 disease. By inhibiting viral entry, T20 suppress viral replication in patients carrying strains resistant to reverse transcriptase or protease inhibitors. However, its antiviral activity is compromised by mutations in gp41. Based on our previous work demonstrating that Rapamycin (RAPA) inhibits R5 HIV-1 by down-regulating CCR5 surface expression, we now show that RAPA and
T20 synergize in antiviral activity against R5 strains. Synergy studies using the Median Effect analysis revealed that the IC50 values of RAPA and T20 in the RAPA/T20 combination were reduced 9- and 3-fold, respectively. Three-Dimensional modeling confirmed the observed synergy (synergy volume of 253.85; 95 % CL: 91–147). We also show that the RAPA/T20 combo, but not T20 alone, prevented the emergence of T20 resistance upon continuous passage of R5 HIV-1 ADA on PBMCs for 24 weeks under subinhibitory concentrations of T20. In addition, R5 ADA and YU-2 clones carrying T20 single mutations 36D, 38M or 43K (4–10 fold resistance) or the double mutation 36D/38M (65-fold resistance), were all inhibited in the presence of RAPA. In conclusion, our results demonstrating that the RAPA/T20 combination has synergistic antiviral activity, prevents the emergence of T20 resistance, and inhibits T20 resistant strains, suggest a novel therapeutic approach to enhance the antiviral activity of T20 in patients carrying R5 strains of HIV-1.

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P138
Waking Up to HIV/AIDS in War Ravaged Liberia, The Difficulties in Instituting HIV/AIDS Awareness Programs

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Background: It is extremely difficult to have a clear picture of HIV/AIDS in Liberia. Liberia has been ravaged by arm conflict for the past fourteen years. Due to this it has been very difficult to collect and analyze data in the length and breadth of this tiny West African republic. The bad roads and the constant back and forth movement of refugees and internally displaced persons from one place to another is a contributing factor.

Methods: There were wide ranging places that our assessment covered. We were in the market places, Nightclubs and bars as well as schools and the most important of all we were from door to door. This was a long a hectic process that took more than six months. Questions were being asked in the form of conversation and at some time for the literates questionnaire were issued to be filled in. Gifts were given to people at times for encouragement. Children at various schools were allowed to have time to discuss AIDS and some causes among women and young girls.

Results: There are numerous problems that must be addressed in order to smoothly run programs related to AIDS. Firstly, a vast majority of the people is inaccessible; lack of roads has become a barrier separating advocates from victims and vulnerable people. These people lived in isolated areas and remote areas that can only be reached by foot. Secondly, there is a high level of illiteracy and this is due to poverty. There are very few trained personnel that are available to relate to people in the rural areas and the most vulnerable.

Conclusion: One of the simplest methods in resolving this issue is by providing means by which local trainers can be trained because they know the terrain, language, culture and tradition of the local people. Poverty alleviation programs should be intensified through literacy programs. HIV/AIDS and other STD’s awareness programs should be integrated into schools curricula as a subject and teachers as well as parents should abandoned that age-old legacy of not discussing issues relating to sexuality with adolescents and the public in general.

P139
The Objective of Inducing Broadly Cross-reactive Neutralizing Antibodies Against HIV-1

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Keywords: HIV-1; gP120, gP41; CD4; Chemokine Receptor; Antibody Response

The objective of inducing broadly cross-reactive neutralizing antibodies against HIV-1 is proramic because of the high sequence variability of the viral envelope proteins and the general resistance of primary isolates to neutralizing. The gp120 glycoprotein elicits both virus-neutralizing and non-neutralizing antibodies during natural infection. Non-neutralizing antibodies are often directed against the gp120 regions that are occlude on the assembled trimer which are exposed only upon shedding. Neutralizing antibodies must access the functional envelope glycoprotein complex and typically recognize conserved or variable epitopes near the receptor-binding regions. HIV envelope glycoproteins that are conserved among diverse viral strains are poorly expose to the humoral immune system. The conserved gp120 surfaces involved in binding to its three minimally polymorphic ligands, gP41, CD4 and chemokine receptors, each exhibit particular problems with respect to the elicitation of or sensitive to neutralizing antibodies. The moieties involved in gp120-gP41 association are buried in the interior or of the functional envelope glycoprotein spike. The CD4 binding site is recessed, flasked by variable regions exhibiting considerable glycosylation. The chemokine receptor-binding site is masked by varial loops-V3 and V2. The relatively conserved HIV-1 gp120 core has been structurally analyzed, the outer domain exhibits a variable, heavily glycosylated surface. This concentrated glycosylation may reduce the potential of a large portion of the gp120 surface to serve as an immunogenic target.

P140
Cell-based Assay for Testing Susceptibility of HIV-1 to Protease Inhibitors

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A growing emphasis on using cell-based assays for compound screening and enzyme activity is fueled by the need to decrease
costs, increase success rates, and identify failures during discovery and preclinical development. Moreover, the growth in cell-based screening has led to the development of novel cell lines and technologies to increase the throughput and data output of cell-based assays. Therefore, we have also developed a new reporter system that allows monitoring of HIV-1 protease activity and susceptibility to protease inhibitors in living cells. Our aim was to construct a cell-based assay for assessing the activity of intracellular HIV protease, use it to screen protease inhibitors and test HIV susceptibility to these drugs. Functional bioassay for resistance was constructed without the need to culture infectious HIV. These assays are based on processing of recombinant reporter proteins in mammalian cells using wild-type PR or a pool of patient-derived PR sequences. Assays not involving infectious HIV should be simpler, faster, safer, and more economic and allow implementation in clinical routine labs, which are generally not equipped for virus culture. Moreover, its application for searching inhibitors of this important enzyme will provide faster, high-throughput and reliable results.

The working hypothesis was to test if a transactivator protein conjugated to the cytoplasmic portion of a cellular receptor, via the respective cleavage peptide, could be used as an indicator of HIV protease susceptibility. This assay was constructed in a cell line that expresses an indicator protein under the inducible action of the transactivator. The cleavage of the transactivator protein by the action of the protease resulted in the expression of luciferase or β-galactosidase. In preliminary experiments, the signal obtained correlates with the intracellular activity of the protease. The optimized cleavage assay is currently being used for testing current protease inhibitors to compare the IC50 values to reference virologic assays.

**P141**
**Higher Virus Replication and Rapid Disease Progression Correlate Inversely With SIV tat exon 1 Evolution in Morphine-addicted SIV/SHIV-infected Macaques**

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We analyzed the association between evolution of the 5’ exon of tat and disease progression in a SIV/SHIV macaque model of opiate-dependence and AIDS. We cloned tat sequences using RT-PCR of plasma virus from eight animals at three time points following infection. Six of these monkeys were part of a morphine-dependent cohort, while two served as non-drug using controls. We found a significant inverse correlation between disease progression and tat diversity in plasma by 20 weeks. The morphine cohort segregated into two classifications based on progression: a rapidly progressing group (Group A) and a second set (Group B) that progressed at a rate similar to the two non-morphine controls (Group C). The three animals in Group A exhibited ~40% (p = 0.01) and ~50% (p = 0.028) less diversity than Group B and C animals, respectively. Group A animals showed prominent re-emergence of the wild-type inoculum tat sequence as illness progressed. This suggests that the virus from the original infection represented the most pathogenic form in these cohorts throughout the first 20 weeks of infection. Our results indicate that in vivo morphine dependence can contribute to the pathogenesis of SIV/SHIV infection and that it may do so in conjunction with the evolution of viral proteins, such as Tat. It is unclear if this is a direct effect of morphine on the virus replication/evolution or if it is mediated indirectly through modulation of the immune response, or through the enhanced vulnerability of a protected compartment such as the CNS.

**P142**
**Characteristics of P-glycoprotein (Pgp) Upregulated in Chronic Cocaine Users and HIV Infected Persons**

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**Background:** Chronic cocaine use and HIV independently upregulate cellular expression of a membrane bound efflux protein, Pgp, reducing the bioavailability of HIV-protease inhibitor drugs. Pgp possesses two functionally distinct binding sites that are associated with its efflux function. It is not known if Pgp upregulation by cocaine and/or HIV infection differently modifies the function of the two binding sites of Pgp.

**Materials and methods:** Peripheral blood was obtained from HIV negative chronic cocaine users. Peripheral blood mononuclear cells (PBMCs) were isolated and their Pgp and HIV coreceptor expression was assessed by Flow cytometry. Efflux function of Pgp was assessed also by flow cytometry by measuring the uptakes by Pgp positive CD4 T cells of two different substrates, Rhodamine 123 (R) and Hoechst 33342 (H). Cyclosporine-A and Colchicines were used to induce R- and H-site specific inhibition of the efflux functions.

**Results:** Even though both HIV infection and chronic cocaine use independently upregulated Pgp expression on CD4 T cells, each induced a different level of alteration of R- and H-site specific efflux function.

**Conclusion:** HIV infection and chronic cocaine use similarly increased Pgp expression but presumably induced configurationally different alterations of the efflux pump molecule.

**P143**
**Increased CXCR4-dependent HIV-1 Fusion in Activated T Cells: Role of CD4/CXCR4 Association**

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Activation of peripheral T cells resulted in enhanced fusion with X4 HIV-1 env-expressing cells without increases in the surface CD4 or CXCR4. Biochemical methods and biological assays were used to correlate the increased fusion of activated T cells with changes in CXCR4 isomers and CD4-CXCR4 association. CXCR4 species with molecular weight of 47, 50, 62, and 98 kDa were identified in resting T cells by western blot. Stimulation with PHA/IIL2 induced a reduction in the 47 kDa, and an increase in the amounts of 50 and...
ubiquitinated 62–64 kDa CXCR4, and in the co-precipitation of the 62 kDa CXCR4 with CD4. Stripping of CD4 from the cell surface prior to cell lysis only partially reduced co-precipitation of CD4 with the 62 kDa CXCR4, revealing a pool of intracellular CD4-CXCR4 complexes. Brefeldin A and monensin reduced co-precipitation of CXCR4 with CD4, suggesting that late endosomes play a role in intracellular association of CXCR4 with CD4. Our data demonstrated a correlation between the enhanced susceptibility of activated T cells to HIV-1 fusion and an increase in CXCR4-CD4 complexes that may shutle between late endosomes and the cell surface.

**PI44**  
**A HIV-1 Stimulating Host Factor Induced by HIV-1 Tat Protein**  
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Retrovirology 2005, 2(Suppl 1):P144  
The HIV-1 Tat gene is required for virus replication and disease. Tat was reported to be released from infected cells. Recombinant soluble Tat may be taken up by many cell types and transported to the nucleus as an active transcription factor leading to upregulation of viral replication in bystander cells. However, numerous attempts to use Tat protein or Tat expressing constructs for protective immunization in non human primate model produced controversial results, leading us to ask whether the effects of Tat might be indirect and result from increased expression of secondary mediators like cytokines or growth factors. Immunization with Tat protein produces antibodies to a limited number of linear epitopes in animals and human beings, mainly located in the N-terminus of the molecule. We generated a unique prototypic antibody stimulation alone. Therefore, it seems that TLR2 does play a role in Daudi cell killing and signaling through this receptor works as a ten-fold increase in IFN-g over that produced by anti-TCR antibody stimulation alone. Therefore, it seems that TLR2 does play a role in Daudi cell killing and signaling through this receptor works as a ten-fold increase in IFN-g over that produced by anti-TCR

**PI45**  
**Circulating Human Vγ2/Vδ2 T Cells Express Cytoplasmic RANTES**  
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Retrovirology 2005, 2(Suppl 1):P145  
A likely process of chronic positive selection produces the highly biased peripheral blood γδ T-cell repertoire of adult human beings. The major blood subset expresses the Vγ2/Vδ2 T-cell receptor and responds to phoshoantigen stimulation in the absence of MHC restriction. Chronic expansion of γδ T-cell pool is expected to produce a population of cells with the effector/memory phenotype. A CC chemokine RANTES is produced late after TCR stimulation of αβ T-cells, accumulates into the cytoplasm and represents a marker for non-naive T-cells. We demonstrate here that the vast majority of peripheral human T-cells contain RANTES in the cytoplasmic granules. In vitro expansion after non-peptidic phoshoantigen stimulation mimics the normal γδ T cell response to pathogens, and produces polyclonal Vγ2/Vδ2 T cell population uniformly positive for cytoplasmic RANTES. These cells readily release RANTES from cytoplasmic deplas into the culture medium after TCR stimulation. The presence of stored RANTES suggests a memory phenotype and may mediate effector functions of circulating Vγ2/Vδ2 cells. Phoshoantigen-responsive Vγ2/Vδ2 T cells represent 1 in 40 of circulating D3+ lymphocytes; this is the dominant central memory population in primate peripheral blood that can evolve directly into an effector memory pool.

**PI46**  
**Rapid Activation of an Effector Phenotype in Human Vγ2/Vδ2 T Cells Stimulated With a Toll-Like Receptor 2 (TLR2) Agonist**  
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Retrovirology 2005, 2(Suppl 1):P148  
Approximately 1–10% of circulating CD3+ cells in the blood express the gamma delta (γδ) T-cell receptor (TCR) and, of these γδ T-cells, the majority express the Vγ2/Vδ2 receptor. In HIV-infection, there is an targeted destruction of Vγ2+Vδ2 T cells. This is the only TCR-specific change common to all individuals infected with HIV. Because Vγ2/Vδ2 T cells are potently cytotoxic for tumor cells, loss of these cells may be part of the mechanism that promotes AIDs-related malignancies. Tumor recognition by γδ T cells may require a 60 kDa heat shock protein (HSP60) on Daudi Burkitt’s lymphoma cells. However, HSP60 recognition may not be mediated by the TCR but by another receptor on γδ T cells. Since γδ T cells also respond to microbial infection, this additional activatory receptor may be in the toll-like family of receptors that recognize pathogen-associated molecular patterns. To explore this recognition, we treated isopentenyl pyrophosphate (IPP) expanded Vγ2/Vδ2 with the TLR2 agonist PAM3Cys and analyzed the activation of an effector phenotype by measuring IFN-γ secretion and cell killing. Daudi cell killing appears to be enhanced by the addition of the TLR2 agonist. Intracellular staining of γδ T cells after a two-hour incubation with PAM3Cys and anti-γδ TCR antibody revealed as much as a ten-fold increase in IFN-γ over that produced by anti-γδ TCR antibody stimulation alone. Therefore, it seems that TLR2 does play a role in Daudi cell killing and signaling through this receptor works
synergistically with TCR signaling to induce an early Th1-type immune response by IFN-γ production.

P149 Recognition of Isopentenylpyrophosphate and Daudi Tumor Cells By Distinct Subsets of V_{2/V_{62}} T Cells
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Retrovirology 2005, 2(Suppl 1):P149

Gammadelta (γδ) T cells account for 1–10% of CD3+ lymphocytes in the peripheral blood and mostly express a heterodimeric T cell receptor (TCR) with V_{6}γ and V_{2}δ chains. Although the V_{2}/V_{6} subset is defined by the shared expression of common TCR gene segments, these TCRs are highly diverse due to characteristic N nucleotide insertion and deletion at the complementarity-determining region 3 (CDR3) of both γ- and δ-chains. V_{2}/V_{6} T cells recognize alkylphosphates that are ubiquitous intermediates in isoprenoid biosynthesis and tumor cells derived from hematopoietic malignancies in a non MHC-restricted, TCR-dependent manner. Previous work from our lab demonstrated that a model alkylphosphate, isopentenyl pyrophosphate (IPP), specifically selects J_{1.2} chains and selectively skews the V_{2} repertoire toward longer chain lengths. We assumed that V_{2}/V_{6} recognition of alkylphosphates and tumor cells was common and hypothesized that Daudi B cells, the model tumor target for V_{2}/V_{6} T cells, would similarly promote the outgrowth of V_{2}/V_{6} lymphocytes with longer, J_{1.2} V_{2} TCRs. Peripheral blood mononuclear cells (PBMC) from 6 donors were stimulated in vitro with interleukin-2 (IL2) alone, IL2 and IPP, or IL2 and irradiated (120 Gy) Daudi tumor cells. The frequency of V_{2}/V_{6} lymphocytes increased from 5.8 ± 7.8% on Day 0 to 5.5 ± 5.9%, 34 ± 32%, 47 ± 27% after 2 weeks in culture with IL2, IL2+IPP or IL2+Daudi, respectively. RNA was extracted before and after stimulation, V_{2}C HAINs were amplified from reverse transcribed cDNA, and spectratype analysis clarified the role of V_{2} CDR3 sequence from three donors suggest that recognition of IPP and Daudi is mediated by two distinct V_{2}/V_{6} T cell subsets, thus outgrowing the prevalent model for gd T cell recognition of tumors. Collectively, these experiments help clarify the role of V_{2} CDR3 specificity in alkylphosphate and tumor recognition and demonstrate that discrete subsets of V_{2}/V_{6} T cells mediate alkylphosphate and tumor responsiveness.

P150 Proteomic Analysis of Cervicovaginal Lavage Samples (CVL): Identification of Human Immunodeficiency Virus (HIV) Proteins
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Cervical intraepithelial lesions are morphological characteristics recognized as precursor lesions of cervix cancer, and CVL allows sampling of both cells and mucus of these target cells, by noninvasive means. The objective of this study was to determine if (1) proteins could be recovered from the CVL samples of women with CIN lesions, and (2) if the proteins correlated with the CIN stage. CVL samples were obtained with informed consent from 20 women, histopathologically diagnosed to have CIN I lesions. The protein concentrations of the samples ranged from 0.18–1.34 mg/ml. 1D gel electrophoresis identified marked variations in the protein profiles between the CVL samples, and 2D gel electrophoresis revealed the presence of extensive posttranslational modifications. MALDI TOF mass spectrometry analysis revealed, however, the presence of HIV gag (p24) and envelope glycoprotein (gp41), in 4 out of 20 CVL samples, even though these women were persistently diagnosed to be seronegative for HIV. Western Blot analysis confirmed the presence of these HIV viral proteins and additionally demonstrated phosphorylation of p24 on tyrosine. Furthermore, MS/MS data revealed the presence of sequences which corresponded to HIV gag and envelope glycoprotein.

P151 Characterization of Proviral HIV Latency in Different T Cell Subsets of Patients Undergoing HAART
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Retrovirology 2005, 2(Suppl 1):P151

Background: In an HIV infected person, each body compartment harbors a distinct resident HIV. There is an increasing awareness that each T cell subset harbors a genetically distinct lineage of the virus.

Materials and methods: Peripheral blood was obtained from 5 HIV patients receiving HAART for 2–12 years. Three patients had 400–40,000 HIV RNA copies/ml with between 529 and 1,588 CD4 T cells/µl. Other two had 915 and 453,000 RNA copies/ml with 235 and 266 CD4 T cells/µl. Each T cell subset was sorted by FACSaria, washed and DNA was isolated. Proviral HIV env C2-V3 genes were PCR amplified, cloned and sequenced, and were phylogenetically analyzed by using MEGA (v.2.1).

Results: In each individual, different T cell subsets harbored genetically distinct lines of HIV. In most patients, CD45RO (memory) subset of CD4 T cells were positive for HIV proviral DNA. Only one patient was positive for proviral HIV in naive CD4 T cells. Both naive CD4 and CDB T cells showed highly divergent proviral HIV sequences.

Conclusion: In patients receiving a long-term HAART, proviral HIV DNA in each T cell subset represented a distinct lineage of the virus. Even in patients with less than detectable levels of HIV in the plasma, proviral DNA showed the evidence of drug resistance to antiretrovirals.

P152 Vulnerability of Displaced Women and Children to HIV/AIDS in West Africa
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Retrovirology 2005, 2(Suppl 1):P152

Background: West Africa is considered to be the most unstable region on the Africa continent. Civil wars in Liberia, Sierra Leone
and Ivory Coast couple with political instability in Nigeria, Guinea Bissau and recently Togo have put Women and children at risk to diseases and poverty. These women struggled with their in order to meet the basic necessity of life. We take this as a serious problem because according to Church World service refugees and internally displaced persons are six times more likely to get infected with the virus than their counterparts in normal condition.

**Methods:** There were wide ranging places that our assessment covered. We were in the market places, Night clubs and bars as well as schools and the most important of all we were from door to door. This was a long a hectic process that took more than six months. Questions were being asked in the form of conversation and at some time for the literates questionnaire were issued to be filled in. Gifts were given to people at times for encouragement. Children at various schools were allowed to have time to discuss AIDS and some causes among women and young girls.

**Results:** After visiting the Liberian Refugee Camps in Ghana and the Ivory Coast and also displaced camps in Liberia Guinea and Sierra Leone several data were collected which are clear representation of the vulnerability of women and young girls. About 80% of the women told us that they get involved in risky sexual behaviour in order to keep up their children. Also about 72% of young girls get involved in sexual behaviour because of the pressure from men and also rape. Ten percent told us that whenever they drink alcohol the feel like indulging in sexual activity. The location of wells and market places as well as the closeness of the houses are factors that increase their vulnerability.

**Conclusion:** After this assessment it can be concluded that the main causes of the growing rate of infection in the sub region is poverty and instability. The only way to solve this is to first have political stability in the region where people will go through the normal education process and become soundly education. If they have political stability in the region where people will go through the normal education process and become soundly education. If they are sound they will not be too greedy for power to the detriment of the masses. There are enough resources that can be exploited in order to feed the people of the sub region. The international community should think on stability before fighting the virus.

**P153**

Modulation of Cytokine Production by the Transmembrane Envelope Protein gp41 of HIV-1

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Elevated IL-6, IL-10, IL-8, gro-alpha and TNF-alpha and decreased IL-2 values have been regularly observed in HIV infected individuals. To study the influence of the transmembrane envelope protein gp41 of HIV-1 on the cytokine production by human blood donor PBMCs, additional cytokine arrays (RayBiotech) that measured the release of about 100 cytokines, were used. In parallel a synthetic peptide corresponding to a domain highly conserved amongst all retroviruses, the so-called immunosuppressive (isu-) domain, was studied. The isu-peptide was used as a homopolymer, since unconjugated peptides were inactive. The expression of cytokines such as IL-6, IL-8, IL-10, RANTES, MCP-1, MCP-2, gro-alpha, TNF-alpha, MIP-1alpha, MIP-1beta, MIP-3 increased upon exposure to the transmembrane envelope protein gp41 and the isu-peptide of HIV. In contrast, the expression of IL-2 decreased and the expression of the other cytokines remained unchanged. The extent of changes in the cytokine expression varied from donor to donor. These data confirm and extend previous data obtained with purified HIV-1 and porcine endogenous retrovirus (PERV) particles, the transmembrane envelope proteins gp41 of HIV-1 and p15E of PERV and their isu-peptides. These data indicate that retroviral transmembrane envelope proteins modulate the cytokine production of normal PBMCs and therefore may play an important role in retrovirus-induced immunopathogenesis.

**P154**

Selective Regulation of CD8 T Cell Immune Function by IL-21 in HIV Infected Individuals

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**Background:** HIV infection is associated with skewed matura-

**Methods:** Fresh peripheral blood mononuclear cells of healthy donors (n = 7) and HIV+ patients (n = 10, CD4+200 mm3, VL<200 copies/ml) were cultured for 5 days with IL-21 (50 ng/ml) or IL-15 (50 ng/ml) and analyzed for the expression of intracellular perforin and cellular proliferation (CFSE-dye dilution) in maturation subsets of CD8 T cells based on expression of CD45RA/CD62L.

**Results:** By itself, IL-21 addition significantly increased per-

**Conclusion:** IL-21 selectively augments EM CD8 T cell proliferation and perforin in HIV+ individuals, whereas IL-15 induces pan CD8 T cell activation in both, healthy and HIV+ individuals. The EM CD8 T cells of HIV+ patients are more responsive to IL-21 than healthy control cells.

**P155**

Repopulation of the Vg2Vd2 Repertoire After Prolonged HAART

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HIV infection leads to a rapid and complete loss of cells expressing the Vg2-jg1.2 chain of the gd T cell receptor. This cell subset is
Prevalence of HIV in Kaposi’s Sarcoma (KS) Patients
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Background: A high incidence of HIV/AIDS has been observed in Zambia. Current overall estimates stand at 16%.
Method: This was a prospective study done at a Central African University Teaching Hospital, skin clinic, for five months. 112 KS patients were recruited. Full case histories and clinical examination were taken and done, respectively.
Results: 94.6% of the KS patients were HIV positive whereas 5.4% were HIV negative. The former had epidemic KS while the latter had endemic KS. The mean ages for the two types of KS were 34.9 years (standard deviation = 9.46) for epidemic KS and 34.5 years (standard deviation = 14.4) for endemic KS. The peak incidence of the former type of KS was in the age range 30 to 39 years whereas for the latter type it was more or less uniformly distributed in all the age groups from 10 to 59 years, with a slight peak in the 20 year age range (hence the large standard deviation). The male(M) to female(F) sex distribution for epidemic KS was 1:4.1 while for endemic KS it was 2:1. Also, endemic KS was more common in the low socioeconomic class unlike the epidemic type which cut across all socioeconomic strata.
Conclusion: 1. Most patients with KS have a high prevalence for HIV infection (94.6%).
2. The HIV pandemic has led to an increased incidence of KS in Zambia.
3. Epidemic KS (HIV positive) is roughly equally distributed in both sexes while endemic KS (HIV negative) is twice more common in males than in females.
4. There is no significant difference between the mean age of epidemic and endemic KS patients.
5. Epidemic KS is distributed in all socioeconomic strata whereas the endemic type is common in the low socioeconomic class.

Clonal Selection and Population Dynamics of Vγ2/Vδ2 T Cells in Macaca fascicularis
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HIV infection increases the susceptibility to new M. tuberculosis (Mt) infections, the risk of reactivating latent infections and the risk of rapid TB progression. γδ T cells, in particular the Vγ2Jδ1.2 subset, are thought to be part of the innate immune response to both HIV and Mt. Importantly, both HIV and Mt perturb gd T cell homeostasis, causing a profound and highly specific depletion of the Vγ2Jδ1.2 subset. We used a primate model (M. fascicularis) to investigate the Vγ2 response to mycobacterial infections and we followed Vγ2 population dynamics at the clonal level after infection with attenuated Bacille Calmette-Guerin (BCG). There was a modest increase of circulating Vγ2 T cell and changes in the Vγ2 repertoire following BCG inoculation. The increase of circulating Vγ2 T cell frequency correlated with an increase in Vγ2 responsiveness to secondary stimulation in vitro, both in terms of proliferation capacity and IFNγ production. CDR3 sequence analysis showed the existence of discrete clones that were selected after BCG exposure. Two CDR3 sequences were found frequently in all of the four animals analyzed and both were encoded by multiple nucleotide sequences converging on the same amino-acid sequence. Few other CDR3 sequences were found in more than one animal. A second BCG inoculation caused a dramatic contraction of the Vγ2Jδ1.2 population and specific deletion of the responsive clones, likely as a result of activation induced cell death. These results show that the Vγ2 T cell response to live BCG tends to be clonal in M. fascicularis. The presence of a few preferred CDR3 sequences used frequently in different animals strongly suggests that, if any presenting molecule is involved in Vγ2-2 antigen recognition, it is not highly polymorphic. Our established M fascicularis model provides important information about Vγ2 clonal deletion induced by mycobacterial infection and is a model for the impact of pathogens including HIV and P falciparum on the Vγ2 population.

Vicriviroc, A Novel CCR5 Inhibitor, is NOT a p-glycoprotein Substrate In Vitro
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The CCR5 chemokine receptor is a promising target for antiretroviral therapy because of its role as a coreceptor for HIV entry and propagation of infection. Vicriviroc, a small molecular CCR5 inhibitor being studied in clinical trials, is well absorbed in rats and monkeys; in vitro studies were performed with caco-2 cells to determine its bi-directional permeability and potential as a p-glycoprotein (pGp) efflux substrate.
Caco-2 cells (passage 60 to 61) were grown for 3 weeks to confluency and the integrity of the monolayer was confirmed by TEER measurements in the presence of vicriviroc (50 to 400 mM). For bi-directional permeability studies, vicriviroc was placed on either the apical (A) or basolateral (B) compartment at a concentration of 40 mM and permeability (n = 3) was determined over 2 hrs with an LC/MS/MS assay. Total recovery exceeded 85% in all studies. The passive permeability (A to B) performance of the caco-2 cell monolayers were confirmed with atenolol (Pc = 3 ± 1.7 nm/s) and pindolol (Pc = 200 ± 9 nm/s). Functional expression of pGp was confirmed with the standard pGp substrate digoxin (bi-directional efflux ratios: 4- to 10-fold).

Vicriviroc showed high A to B permeability (Pc = 400 ± 4 nm/s) consistent with its high in vivo oral absorption. The bi-directional efflux ratio of vicriviroc was only 0.6 indicating that it is not a pGp substrate in vitro. These data suggested that pGp is unlikely to affect the oral absorption of vicriviroc and that co-administration of vicriviroc with a pGp inhibitor is unlikely to cause significant drug-drug interactions.