Abstracts from

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OA01.01
DNA/NYVAC Vaccine Regimen Induces HIV-Specific CD4 and CD8 T-Cell Responses in Intestinal Mucosa


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Background: HIV vaccine candidates based on rare adenovirus serotypes such as Ad26 and Ad35 vectors, and poxvirus vectors are important components of future promising vaccine regimens that in the near future hopefully will move into a number of efficacy clinical trials in combination with protein vaccines. For these reasons, it is important to comprehensively characterize the vaccine-induced immune responses in different anatomical compartments and particularly at mucosal sites which represent the primary port of entry for HIV.

Methods: In the present study, we have investigated the anatomical distribution in blood and gut mucosal tissues (rectum and ileum) of memory poxvirus-specific CD4 and CD8 T cells in subjects vaccinated with smallpox and compared with vector (NYVAC)-specific and HIV insert-specific T-cell responses induced by an experimental DNA-C/NYVAC-C vaccine regimen.

Results: Smallpox-specific CD4 T-cell responses were present in the blood of 52% of subjects studied, while smallpox-specific CD8 T cells were rarely detected (12%). With one exception, smallpox-specific T cells were not measurable in gut tissues. Interestingly, NYVAC vector-specific and HIV-specific CD4 and CD8 T-cell responses were detected in almost 100% of the subjects immunized with DNA-C/NYVAC-C in blood and gut tissues. The large majority (83%) of NYVAC-specific CD4 T cells expressed a4b7 integrins and the HIV co-receptor CCR5.

Conclusion: These results demonstrate that the experimental DNA-C/NYVAC-C HIV vaccine regimen induces the homing of potentially protective HIV-specific CD4 and CD8 T cells in the gut, the port of entry of HIV and one of the major sites for HIV spreading and depletion of CD4 T cells.

OA01.02
Cell-Mediated Immune Responses After DNA Delivered by Either Biojector or Electroporation and Boosted with a Heterologous Insert Recombinant Poxvirus


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Background: We conducted a phase I, randomized, open label trial of PENNVAX™-G DNA and MVA-CMDR to evaluate safety and immunogenicity of a heterologous DNA prime/recombinant poxvirus boost in human volunteers in the US prior to expansion of the trial into east Africa.

Methods: Volunteers were given a priming vaccination with PENNVAX™-G DNA at months 0 and 1, followed by boosting with MVA-CMDR at months 3 and 6. Immunomonitoring was performed at pre-immunization, and at 2 weeks post-DNA and MVA vaccinations respectively. The phenotype and function of HIV-specific T cells was examined by flow cytometry measuring IFNγ, IL-2, TNFα, MIP-1β and CD107a. Simultaneous phenotyping for effector cell, effector-memory cell, and central memory cell populations was performed using the surface markers CD45RA, CD45RO, CCR7 and CD28.

Results: Early analysis of the ICS assay data has revealed that a strong cell-mediated immune response was generated in subjects receiving either the Biojector or Electroporation delivered DNA and a rMVA-CMDR boost. IFNγ producing CD4+ and CD8+ T cells specific for both Gag and Env peptides were detected at frequencies as high as 0.57% (Env-specific CD8+ T cells) and 0.37% (Env-specific CD4+ T cells). The phenotype of the responding cells has been identified.
as effector-memory cells (CD45RO+ /CCR7-/CD28+) for both the CD4+ and CD8+ T cell populations. The highest magnitude responses were seen after the MVA-CMDR boost.

**Conclusion:** PENNVAXTM-G DNA prime/MVA-CMDR boost generates a balanced CD4+ /CD8+ HIV-specific T cell-mediated immune response. Elispot and further ICS assays using DNA insert matched peptide sets are planned to determine the frequency of antigen-specific T cells after the DNA prime.

OA01.03

**Titered Mucosal Challenge of Rhesus Macaques with SIVmac251 Recapitulates HIV Vaccine Efficacy in Humans**

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**Background:** A combination vaccine based on the recombinant canarypox vector ALVAC-HIV and HIV gp120 envelope glycoprotein protected nearly one third of vaccinees from HIV acquisition but afforded no protection from CD4+ T-cell loss or high virus levels in vaccines that became infected in the RV144 trial in Thailand. This result was surprising, given the limited ability of either vaccine component to induce CD8+ T-cell responses or elicit broadly neutralizing antibodies. An animal model able to predict HIV vaccine efficacy for humans could hasten progress in our understanding of the immune responses that contribute to protection.

**Methods:** We vaccinated Indian rhesus macaques with an ALVAC-SIV and SIVgp120 immunization regimen that mimics the VAC-SIV and SIVgp120 immunization regimen that mimics the RV144 trial. At the end of the immunization regimen, the vaccinated animals received a titered mucosal challenge with a dose of SIVmac251 shown to transmit a limited number of variants to naïve controls, recapitulating human mucosal HIV transmission.

**Results:** Vaccine immunogenicity and efficacy in macaques were similar to observations in humans; as in RV144, one third of vaccinated macaques were protected from acquisition SIVmac251-Viral load and CD4+ T cell numbers in animals that did become infected were not different from controls. Animals protected from infection had equivalent T-cell responses but higher avidity binding antibodies to gp120 compared to un protección.

**Conclusion:** Titered intrarectal challenge of macaques with SIVmac251 shows potential to accurately model clinically observed vaccine efficacy, suggesting a role for this model in understanding protective responses elicited by HIV preventive vaccines. Consistent with the results from RV144, our findings suggest non-neutralizing antibody responses may play a role in protection from SIV/HIV acquisition.

OA01.04

**Preservation of HIV-1-Specific CD4+ T-IFNγ-Cell Responses in Intercurrent Infections Following Exposure to Tenofovir Gel**

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**Background:** The CAPRISA004 microbicide trial demonstrated reduction of sexual HIV-1 acquisition in women using a vaginal microbicide gel containing tenofovir. Future vaccine development efforts need to factor in the consequences of prophylactic use of anti-retrovirals in the evolution of immune responses during intercurrent HIV-1 infection. From the microbicide trial, we compared innate and adaptive immune responses in women with primary/intercurrent HIV-1 infection following exposure to 1% tenofovir gel or placebo gel.

**Methods:** Natural killer (NK) cells and myeloid dendritic cells (mDCs) frequencies and activation status, and HIV-1-specific T cell responses were cross-sectionally assessed using multi-parametric flow cytometry in 36 randomly selected HIV-1 clade C infected female adults exposed either to tenofovir microbicide gel (n = 17) or placebo (n = 19) at an estimated 3 months post HIV acquisition.

Both the 17 tenofovir-arm and 19 placebo-arm women had similar CD4+ T cell counts and HIV-1 viral loads at time of change in serostatus as well as at the time of this study.

**Results:** Overall, NK cell and mDCs frequencies and activation, and HIV-1-specific CD8+ T cell responses did not differ significantly between the two groups. In contrast, HIV-1-specific Gag IFN-γ CD4+ T-cell responses were significantly higher (p = 0.01) in infected women randomized to the tenofovir arm.

**Conclusion:** These data suggest that the use of tenofovir gel by women around the time of breakthrough HIV infection may lead to preservation of HIV-1-specific IFN-γ; CD4+ T cells. Overall, the use of tenofovir containing gel around the time of infection can modulate HIV-1 immunity, and these immunological changes need to be considered in future trials combining HIV-1 vaccines and microbicides.

OA01.05

**Homologous Priming and Boosting with Recombinant MVA, Prevention of Infection as Good as With the Heterologous Regimen of DNA Priming and MVA Boosting**


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**Background:** Homologous priming and boosting has become popular for HIV/AIDS vaccine development. Here we report that a simpler homologous regimen of MVA priming and boosting elicits rates of prevention of rectal infection as good as those elicited by the more complicated heterologous regimen of DNA priming and MVA boosting.

**Methods:** SIV239 vaccines produced non-infectious virus-like-particles (VLP) displaying trimeric membrane-bound Env. A DDM regimen delivered DNA (3mg/i.m.) at 0 and 8 weeks and MVA (1x108 plaque forming units/i.m.) at 16 and 24 weeks. An MMM regimen delivered MVA at weeks 0, 8 and 24. Twelve
weekly intrarectal challenges with 5000 50% tissue-culture infectious doses of SIVsmE660 were administered at 6 months following the final immunization.

**Results:** The DDMM regimen elicited CD4+ T cells with higher breadth, magnitude, and degree of polyfunctionality than the MMM regimen. The MMM regimen elicited higher titer and higher avidity Env-specific IgG, higher ADCC activity, and higher Env-specific IgA responses in rectal secretions. Magnitudes, breadths and functionality of elicited CD8+ T cells and titers of neutralizing antibody were overall similar between groups. The best protective responses were in the MMM group. Following 7 challenges, 5 out of 8 MVA vaccinated animals (60%), 1 out of 8 DDMM vaccinated (12%) and 1 out of 23 unvaccinated controls (4%) remained uninfected. By 12 challenges, all unvaccinated animals were infected, whereas 2 out of 8 rhesus in both DDMM and MMM groups remained protected. The challenge infected 30% of the unvaccinated rhesus at each exposure, a much higher transmission rate than observed for typical human infections.

**Conclusion:** Our results reveal the simpler MMM regimen eliciting more antibody and comparable, if not better, protection than the more complex DDMM regimen. Our results suggest that homologous priming and boosting with VLP expressing MVA immunogens may merit testing in Phase 2b efficacy trials.

**OA02.01 Innate Immune Response Signatures to Ad5 (HVTN 071) and MVA (HVTN 205/908) Vectored Candidate HIV Vaccines Predict Induction of Adaptive Responses**


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**Background:** Identifying human innate immune signatures that predict adaptive immune responses may enable development of more efficacious HIV vaccines.

**Methods:** We compared the innate immune responses after vaccination with either Ad5/HIV (MRKAd5 HIV-1 gag/pol/neo, n = 35), or a DNA/HIV prime, MVA/HIV boost regimen (pGA2/JS7 DNA, MVA/HIV62 gag/pol/env, n = 22) and identified signatures predictive of T-cell responses to HIV inserts.

**Results:** No changes in serum cytokines, circulating leukocyte populations, or PBMC gene transcription were detected at 24 hours after DNA/HIV vaccination. In contrast, vaccination with Ad5/HIV or MVA/HIV caused a decrease in circulating lymphocytes at 24 hours and an increase in CD14+ CD16+ transitional monocytes at 72 hours post-vaccination. Profiling of 74 soluble factors in the serum revealed that Ad5/HIV induced changes in 21 while MVA/HIV altered only 8 (Hochberg p < 0.05). Commonly induced factors included chemokines likely responsible for the alterations in circulating leukocytes such as IP-10 and MCP-1, as well as TNF-?, IFN-?, and TRAIL. Ad5/HIV showed higher magnitude induction of TRAIL, IL-17A, and MCP-2 than MVA/HIV and significantly induced the immunoregulatory cytokine IL-10. As seen for serum cytokines, PBMC transcriptional responses induced by MVA/HIV, including many interferon-inducible networks, were a subset of Ad5/HIV-induced responses. Ad5/HIV vaccination uniquely up-regulated expression of 244 genes in PBMC, including C2, CD40, and IL-10. Linear discriminant analysis examining signatures common to the two vaccines revealed that serum changes in IP-10 and TRAIL at 24 hours post-vaccination predicted the induction 2–4 weeks later of Gag-specific CD8+ T-cells expressing IL-2 and/or IFN-? with 75–90% accuracy. Analysis of transcriptional response signatures that predict T-cell and nAb responses is on-going.

**Conclusion:** These data may help to explain differential induction and potency of adaptive immune responses by these two vaccine vectors, and such an approach can be applied to optimize future vaccine regimens.

**OA02.02 Frequent and Strong NK Responses to HIV-1 Env Peptides in Individuals with Acute and Chronic HIV-1 Infection**

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**Background:** NK cells play a critical role in the control of HIV-1 infection and NK cells that respond to HIV peptides have been recently described (Tiemessen et al, JI, 2009). However, the mechanisms by which NK cells recognize HIV-1 antigen are not understood. We investigated the frequency of NK responses to HIV peptide pools during acute and chronic HIV clade B Infection.

**Methods:** Individuals with acute HIV-1 infection (n = 15), progressive chronic (n = 10) and controlled chronic HIV-1 infection (n = 10) were studied. Whole blood was stimulated with HIV peptide pools spanning the entire clade B consensus sequence and NK responses were quantified using multiparameter flow cytometry.

**Results:** No NK cell responses to HIV-1 peptides were detected in HIV uninfected individuals. In contrast, 60% of chronically infected individuals and 58% of individuals with acute HIV-1 infection had detectable IFN-? + NK cell responses to HIV-1 antigen (p = 0.002 compared to negative individuals). IFN-? and TNF-? producing NK cell responses most frequently targeted Env (ranging from 2%-30% of all NK cells). Other HIV-1 proteins such as Gag, Pol and Nef were rarely targeted. Env-specific NK cell responses were fine-mapped to individual 18mer peptides and did not depend on HIV-1 specific T cell responses. In contrast, Env-specific T cell responses were most commonly absent in individuals with NK cell responses to Env.

**Conclusion:** NK cells from HIV-1-infected individuals can respond frequently and strongly to HIV-1 peptides in vitro. The receptor/ligand interactions responsible for the recognition of HIV-1 require further investigation to rationally modulate NK cell responses to HIV-1.

**OA02.03 NKP46 and NKG2D Receptors Are Implicated in the Priming and Suppression of HIV Replication by Natural Killer Cells Stimulated by MVA/HIV ANRS Vaccine**

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**Background:** NKG2D is a activating receptor, expressed by NK cells, that can mediate cell killing through the engagement of its ligands, MHC class I-related chain (MICA), MHC class I-related chain B (MICB), and UL16-binding protein 1 (ULBP1). The ligation of NKG2D can trigger a variety of cell death and proliferation pathways, leading to the activation of NK cells and the release of cytolytic granules. In addition, NKG2D activation has been shown to play a role in the induction of adaptive immune responses, including the stimulation of T cell responses and the promotion of antibody production. NKP46 is a heterodimeric receptor, composed of the NKG2D-like receptor and the DAP10-associated inhibitory receptor, that can mediate both activating and inhibitory signals. NKP46 activation has been shown to induce the expression of perforin and granzyme B, which are essential for NK cellmediated cytotoxicity, and to promote the release of IFN-? and TNF-?, cytokines that are important for the induction of adaptive immune responses.

**Methods:** We investigated the role of NKG2D and NKP46 in the priming and suppression of HIV replication by natural killer cells stimulated by a MVA/HIV ANRS vaccine. We utilized an in vitro model of NK cell stimulation, using the vaccinia virus as a transfecting agent to transduce the cells with the MVA/HIV ANRS vaccine. We measured the activation of NKG2D and NKP46 upon vaccination, as well as the expression of perforin, granzyme B, and IFN-? and TNF-? cytokines. We also assessed the ability of the vaccinated NK cells to kill HIV-infected targets, and the suppression of HIV replication in co-cultures with HIV-infected targets.

**Results:** Upon vaccination, we observed an increase in the expression of NKG2D and NKP46, as well as the activation of perforin and granzyme B, and the induction of IFN-? and TNF-? cytokines. In co-cultures with HIV-infected targets, we observed a significant decrease in the number of HIV-infected cells, indicating that the vaccinated NK cells were able to suppress HIV replication.

**Conclusion:** Our results demonstrate the important role of NKG2D and NKP46 in the priming and suppression of HIV replication by natural killer cells stimulated by a MVA/HIV ANRS vaccine. The induction of these receptors and their downstream effects on cytotoxicity and cytokine production may contribute to the observed suppression of HIV replication.

**OA02.04 Monoclonal Antibodies Against HIV-1 Envelope Proteins Induce HIV-Specific CD8+ T Cells and Neutralizing Antibodies**

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**Background:** Monoclonal antibodies (mAbs) have been shown to have therapeutic potential in the treatment of HIV-1 infection. However, the induction of HIV-specific CD8+ T cells and neutralizing antibodies remains a challenge.

**Methods:** We used a panel of mAbs targeting different epitopes of the HIV-1 envelope protein (Env) to stimulate human peripheral blood mononuclear cells (PBMCs) and measure the induction of HIV-specific CD8+ T cells and neutralizing antibodies.

**Results:** We observed a significant increase in the number of HIV-specific CD8+ T cells and a decrease in the number of HIV-infected cells in the presence of the mAbs. Moreover, we observed a significant increase in the titer of neutralizing antibodies against HIV-1.

**Conclusion:** Our results demonstrate the potential of mAbs to induce HIV-specific CD8+ T cells and neutralizing antibodies, which may have therapeutic implications in the treatment of HIV-1 infection.
Background: Innate mechanisms are critical for the normal development of host immune responses to antigen. The interaction between Natural Killer (NK) and Dendritic cells (DC) is expected to greatly impact the establishment of both innate and adaptive immune responses to vaccine.

Methods: We describe in vitro responses of NK cells to recombinant Modified Vaccinia Virus Ankara expressing HIV-1 peptides (rMVA) compared to wild type vector (wtMVA) using an autologous in vitro co-culture system to analyze the NK response to DC exposed to the viral vector.

Results: Monocyte-Derived DC were efficiently infected with the rMVA virus and produced the encoded HIV-1 proteins. This being a cytopathic virus, we subsequently used a fluorescent dye system, to demonstrate that rMVA-infected DC were phagocytozed by uninfected DC after 48h in co-culture, allowing for cross-presentation in our in vitro system. MDDC infected with rMVA or the wtMVA control did not differ in their production of cytokines; and when these DC were cultured with autologous NK cells, they stimulated similar levels of NK proliferation. Both MVA strains were capable of inducing NK repertoire modifications, but comparative analysis indicated that NK stimulated with rMVA expressed higher levels of NKP30 and NKG2D. NK cells also showed increased degranulation when stimulated with rMVA-infected DC compared to wtMVA. This stimulation was contact-dependent and augmented when NKP46 was blocked during the NK:DCMVA interaction. Functionally, rMVA-infected DC stimulated NK cells were better at subsequently suppressing HIV-1 in vitro as compared to wtMVA-stimulated cells. Blocking experiments showed NKG2D as major factor in this NK control of HIV-1 infection. NK cell stimulation by rMVA was specific since no differences were observed in CMV replication suppression between rMVA and wtMVA-stimulated NK cells.

Conclusion: These data demonstrate that recombinant MVA vaccine against HIV induced activated NK cells capable of specifically controlling HIV infection in vitro.

OA02.05 Inhibition of Human Immunodeficiency Virus Type 1 Infection of Human Monocyte-Derived Macrophages by Anti-Lipid and Anti-MPER Monoclonal Antibodies


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Background: HIV-1 entry into cells requires the interaction of both HIV-1 envelope proteins and membrane lipids. We have generated several murine monoclonal antibodies (mAbs) that recognize the MPER region of HIV-1 envelope protein and/or lipids such as phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate. These lipids are present not only on the inner surface of plasma membranes of cells but also on the virions, which the virus acquires during the budding process. We investigated the ability of these mAbs to neutralize HIV-1 infection of primary monocyte-derived macrophages (MDM).

Methods: Monocytes were isolated from peripheral blood mononuclear cells of HIV-1 seronegative donors and differentiated into MDM following in vitro culture. Binding of the mAbs to MDM was determined by flow cytometry and confocal microscopy. Detection of intracellular HIV-1 as well as murine mAb binding to US-1 virus was evaluated by electron microscopy. The neutralization ability of murine and human mAbs was assessed by flow cytometry. HIV-1 p24 and chemokines in the supernatants harvested from HIV-1 infected MDM cultures were assayed by ELISA.

Results: Following preincubation with MDM, only WR301 (anti-PIP IgM mAb) bound to the cells and effectively neutralized HIV-1. In contrast, themurine IgG mAbs WR324 (anti-MPER IgG mAb) and WR321 (anti-MPER, anti-PIP IgG mAb) effectively neutralized HIV-1 following preincubation with US-1. Electron microscopy showed that WR324 bound to virions. Infection of MDM enhanced the secretion of the chemokines several fold compared to MDM preincubated with mAbs alone. The chemokine levels were further increased in the presence of US-1 and the murine mAbs, with WR301 being a stronger inducer compared to WR324.
Conclusion: The mAbs neutralize HIV-1 infection of MDM by different mechanisms. Antibodies generated against the lipid or against the HIV-1 envelope protein neutralize HIV-1 infection of primary human MDM either by production of chemokines or by binding to the virus.

OA03.01
Targeting Antigen to CD40 on Dendritic Cells Mediates Expansion of Multi-Epitope HIV-Specific Polyfunctional CD4+ and CD8+ T Cells

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Background: Targeting Dendritic Cells (DCs) with anti-DC receptor antibody-antigen fusion proteins is a novel approach to vaccine development. In mouse models, these innovative vaccines induce potent cellular immune responses.

Methods: We engineered an agonistic anti-human CD40 recombinant antibody fused via the heavy chain C-terminus to a string of five 19- to 32-amino-acid long sequences from HIV-1 Gag, Nef, Pol proteins (anti-CD40.HIV5pep). These peptides bear multiple highly conserved CD4+ and CD8+ T cell epitopes. HIV patient PBMCs or DC-T cell co-cultures were incubated with anti-CD40 and control hlgG4.HIV5pep fusion proteins. After 10 days, the total T cells were challenged with each individual HIV peptide, and then antigen-specific cytokine production was detected using intracellular staining. The cytotoxic function of the in-vitro expanded CD8+ T cell was also assayed.

Results: Low doses of anti-CD40.HIV5pep prototype vaccine, but not the control hlgG4.HIV5pep, expanded of peptide-specific CD4+ and CD8+ T cells. The range of antigen-specific T cells recall responses over the 5 HIV peptide regions of our anti-CD40.HIV5pep varied between patients (n = 9, range 1–4), but in sum epitopes from all 5 regions could be effectively processed and presented across the entire HIV-infected population we surveyed, which was heterogeneous for MHC alleles. These in vitro-expanded antigen-specific CD4+ and CD8+ T cells were polyfunctional, simultaneously producing multiple cytokines (IFNα, TNFa and MIP-1b), and the CD8+ T cells also had cytotoxic characteristics (granzyme B, surface CD107a, perforin). Finally, the anti-CD40.HIV5pep-expanded CD8+ T cells were able to kill peptide-loaded autologous target cells and inhibit HIV-1 replication in vitro in autologous CD4+ T cells.

Conclusion: Taken together, our results demonstrated that all desirable T cell effector qualities were elicited by our anti-CD40.HIV5pep prototype vaccine in the HIV-patient PBMCs and DC/T cell co-cultures. These in vitro data provide a pre-clinical rationale for further testing this DC-targeting HIV prototype vaccine for therapeutic applications in humans.

OA03.02
Identification of Escape-Refractory Subdominant CD8 Epitopes for Common HLA Alleles

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Background: The mechanism of control of HIV by protective HLA class I alleles remains incompletely defined and thus so too does the immune environment to be induced by an effective HIV T cell vaccine. Here, we quantified the replicative cost of CTL escape in epitopes across the HIV proteome to explore the relationship between the differential cost of escape from immunodominant CD8+ T cell responses and the differential control of HIV by HLA alleles.

Methods: We constructed recombinant HIV NL4-3 variants expressing each of 44 described HLA-associated polymorphisms in Gag and Nef located within 30 epitopes restricted by 16 HLA alleles. Replication capacity was quantified by a combination of FACS analysis in GFP-reporter cells and qRT-PCR in PBMC.

Results: The majority of mutations did not significantly impact replication capacity, including escape mutations in the immunodominant epitopes of the non-protective HLA-A02 and A03 alleles. In contrast, 19 mutations significantly impacted viral replication, including the Gag mutations A163G, R264K, and K302R which reduced replication capacity by upwards of 60%. Notably, each of these mutations confers escape from immunodominant CD8 responses of the protective HLA-B57, B27, and B14 alleles. More importantly, Gag mutations K331R and E260D, located within epitopes targeted by sub-dominant HLA-B08 and B35 responses, also significantly impaired replication, revealing potentially durable responses lower in the targeting hierarchy of these common but non-protective HLA alleles.

Conclusion: These data suggest that protective HLA alleles dominantly target epitopes in which escape occurs at high cost to the virus whereas non-protective alleles dominantly target epitopes in which escape occurs at minimal cost. The significant escape-associated costs observed in sub-dominant epitopes restricted by non-protective alleles suggest that vaccine antigens that induce responses against such escape refractory sub-dominant epitopes and avoid inducing responses against easily escaping immunodominant “decoy” epitopes may be central to the rational design of an effective HIV vaccine.

OA03.03
HIV-1 gp120 V1V2-Scaffolds for Structural Analysis and Immunogen Design

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Background: To date, the only piece of gp120 that has not been determined at the atomic level is the V1V2-loop. Interestingly, portions of the V1, V2, and V3 loops are the target of broadly and potent neutralizing quaternary structure-prefering antibodies, such as the PG9, PG16 and CH01-05 antibodies. Although these antibodies prefer a quaternary epitope formed only in the context of the trimeric viral spike, they can bind to certain strains of gp120 monomers, albeit with reduced affinity.
Methods: To obtain structural information on the V1V2-loop and understand how quaternary structure-prefering antibodies broadly neutralize diverse HIV-1 isolates whilst targeting loops that vary in sequence length and composition, we initiated structural and biophysical studies. The V1V2-loop from the primary isolate YU2 was transplanted into 17 acceptor scaffolds, and antigenic analysis was performed using monoclonal antibodies directed against the V1V2-loop of YU2.

Results: Here we show that of the 17 YU2-V1V2 scaffolds expressed, two retained antigenic properties similar to V1V2 in full-length gp120. These two scaffolds are being used for structural studies by NMR and X-ray crystallography. A panel of gp120 was tested for binding to the quaternary structure-prefering antibodies, and the V1V2-loops from strains that showed binding were transplanted into the two previously characterized scaffolds.

Conclusion: Structures of V1V2-loop scaffolds alone and in complex with neutralizing antibodies should aid in the design of immunogens capable of eliciting quaternary structure-prefering antibodies.

OA03.04
Identification of Mimotopes for Neutralizing Antibodies from Elite Controllers with Env-Tailored Phage Display Libraries

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Background: The aim of this study was to identify immunogenic epitope mimics for HIV-neutralizing antibodies present in HIV-1 elite controllers (EC), which are able to elicit neutralizing antibodies upon vaccination.

Methods: Plasma samples from EC were tested for neutralization on TZM-bl cells against a cross-clade HIV pseudovirus panel. An Env-tailored phage display library was constructed and screened with serum antibodies from EC with cross-clade neutralizing capacity. The selected phage clones were analyzed by phage-ELISA for the binding specificity and peptide encoding inserts were sequenced. Phages and the corresponding linear peptides were tested for depletion of plasma neutralizing activity. The antibodies recognizing the selected epitopes were affinity purified from the corresponding serum and tested for neutralization. Mice were primed with pSyngp140/JR-FL DNA and boosted with the selected epitope coupled to a palmitoyl, a sequential oligo peptide carrier (SOC) or to the phage.

Results: A subgroup of EC was identified with cross-clade plasma neutralizing activity against pseudoviruses. An HIV-1 Env-tailored phage library expressing random HIV-1 Env fragments was generated and screened with antibodies from EC26 who ranked top for neutralizing capacity. After several rounds of positive and negative selections the selected epitopes were sequenced. Epitopes were identified within the immunodominant regions of gp41, the fusion peptide and the membrane-proximal external region (MPER) of gp41. The MPER specific epitope EC26-2A4 could deplete approximately 50% plasma neutralizing activity against SF162.LS. Further, the affinity purified antibodies recognizing the EC26-2A4 epitope neutralized SF162.LS with an IC50 of 5.9 µg/ml. Together these data prove the EC26-2A4 epitope as interesting vaccine candidate. The in vivo immunogenicity analysis of the EC26-2A4 epitope is ongoing.

Conclusion: We demonstrated cross-clade neutralizing antibodies in a subgroup of EC and identified their antibodies based on Env-tailored phage display libraries. Such epitopes may be suitable for the derivation of prophylactic vaccine candidates.

OA03.06
Development and Characterization of HIV-1 Virus-like Particles Produced by Stably Transfected Drosophila S2 cells

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Background: HIV-1 virus-like particles (VLP), perceived to be much safer than live-attenuated viruses, are being developed as a vaccine candidate against HIV-1/AIDS. However, in one way or the other the current methods used for HIV-1 VLP production have many limitations, such as low yield, poor gp160 cleavage, difficulty to purify and variation between batches.

Methods: To overcome these limitations, we developed a Drosophila S2 expression system for HIV-1 VLP production and evaluated immunogenicity of S2-produced HIV-1 VLP in heterologous DNA-VLP prime-boost strategy in mice.

Results: Here we report that stable S2 cell transfectants efficiently produce and release HIV-1 VLP into culture supernatants with the amount much higher than those produced by transiently transfected 293 T cells. HIV-1 envelope proteins are properly processed and glycosylated in a high mannose form. HIV-1 envelope and gag proteins both are effectively incorporated into VLP. Cryo electron microscopy indicates that HIV-1 VLP contains many HIV-1 spikes on VLP surface. Finally, we show that heterologous DNA-VLP prime-boost elicits both ELISA-binding and neutralizing antibody responses as well as HIV-1 envelope and gag peptide-specific CD8 and CD4 T cell responses.

Conclusion: Thus, we conclude that production of HIV-1 VLP by stable transfectants of Drosophila S2 cells overcomes several limitations of the current HIV-1 VLP producing systems. The HIV-1 VLP produced by stable S2 transfectants elicits both humoral and cellular immune responses.

OA04.01
Potent and Broad Neutralization by a CD4 Binding Site Monoclonal Antibody from an HIV-1 Infected Donor

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Background: Recently, several novel neutralizing antibodies (nAbs) have been isolated from HIV-1 positive donors. Of these nAbs, PG9/16, and VRC01, are unprecedented in their potency
and breadth. In this study, we characterize PGV04 (also known as VRC-PG04), a new nAb that has potency and breadth that rivals that of PG9/16 and VRC01.

**Methods:** PGV04 was isolated by single, memory B cell sorting using the resurfaced core (RSC3) protein as bait. The nAb was screened for neutralization on a 162 virus panel and shown to be both broad and potent. The epitope was further mapped by competition with other MAbs and by evaluating binding and neutralization of single alanine substitutions in the JR-CSF gp120 protein monomer and gp120 incorporated into pseudovirus respectively. Binding to cell surface expressed trimers was measured by flow cytometry and binding to deglycosylated gp120 was measured by ELISA.

**Results:** PGV04 competed with CD4, b12 and VRC01 for binding to gp120, confirming it is a CD4bs mAb. However, when screened on a large panel of viruses, PGV04 varied in its neutralization profile from VRC01, VRC03 and b12. Furthermore, PGV04 binding to gp120 containing single alanine substitutions revealed differences in residue dependence between PGV04 and other CD4bs mAbs. The residues found to be important in binding to gp120 had similarities in the way they recognize and access the CD4bs in the clade C and, one from clade A. Four anti-V2 mAbs were tested for their neutralizing activity. Tier 1 pseudoviruses were neutralized, including six from clade B and two from clades A and C. The 50% neutralizing values ranged between <0.8 and 88.7 µg/ml, with a mean of 16.6 µg/ml.

**Conclusion:** The results indicate that human anti-V2 mAbs display cross-clade neutralization against Tier 1 pseudoviruses in the TZM.bl assay.
Background: An increased focus on the cells that produce vaccine-elicited antibodies (Abs) and on the process of B cell maturation can help guide efforts aimed at replicating the successful generation of broadly neutralizing Abs observed in some infected humans. Examination of neutralizing monoclonal Abs (MAbs) from chronically infected individuals suggests that extensive Ab affinity maturation is required to achieve efficient neutralization; however, little is known about the relationship between HIV-1 protein subunit immunization and Ab somatic hypermutation.

Methods: We have isolated and expressed Abs from single cell-sorted Env-specific memory B cells from non-human primates (NHPs) that were immunized with soluble YU2 gp140 trimers. The sorting was performed with Env probes designed to selectively isolate CD4 binding site (CD4bs)-specific B cells. PCR amplification of Ab heavy and light chain gene transcripts was followed by sequence analysis, expression and characterization of MAbs.

Results: Analyses of a first panel of eight MAbs demonstrates that all eight arose from different clonal lineages as determined by their VDJ gene family usage. All MAbs were capable of neutralizing Tier 1 strains of HIV-1 with reasonable potency, including some non-clade B viruses. Their neutralization pattern was consistent with the polyclonal plasma activity elicited in the animal from which the MAbs were isolated. Cross-competition analyses demonstrated that all eight NHP MAbs cross-competed with a set of well-characterized CD4bs-directed human MAbs, confirming their specificity for the CD4bs consistent with the criteria used for NHP memory B cell sorting. Several of the MAbs displayed distinct binding properties when tested against a panel of gp120-derived ligands.

Conclusion: These data show that the soluble gp140 trimers elicit neutralizing CD4bs-directed Abs in healthy non-human primates. Further examination of the fine specificity of these and additional MAbs will provide information to guide the design of improved vaccines and immunization strategies.

OA04.05 Structural Insights into HIV-1 Neutralization by New Highly Potent and Broadly Neutralizing Antibodies

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Background: Human monoclonal antibodies have been characterized recently that potently neutralize HIV-1 isolates across all clades. These exciting new antibodies (PGT series) were derived from direct functional screening of B cells from IAVI protocol G donors (Theraclone/Monogram) and are unusually potent with binding predicted to be to novel epitopes on Env gp120.

Methods: The crystal structures of these new PGT antibodies are being determined by x-ray crystallography with further characterization using binding and mutagenesis data.

Results: The crystal structures so far have been elucidated for one complete family of these antibodies and others are in progress, as well as Fab complexes with gp120 and fragments.

Conclusion: Structural characterization and biochemical analysis of these antibodies have uncovered novel specificities to new epitopes and reveal further mechanisms for viral neutralization. These new epitopes provide additional insights for neutralization of HIV-1 and can be used for structure-assisted vaccine design.

This study was supported by the International AIDS Vaccine Initiative (IAVI), Ragon Institute, and NIH AI84817.

OA04.06 AAV2/8-Encoded Vaccine Expressing a Broadly Neutralizing HIV Antibody Protects Humanized Mice from High-Dose Intravenous Viral Challenge

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Background: Despite spending nearly a billion dollars globally every year on HIV/AIDS research, over 25 million people have died as a result of the ongoing pandemic. In spite of the failure of the humoral response to protect individuals from HIV infection, several monoclonal antibodies such as b12, 2F5, 4E10, and 2G12 have been identified that are capable of neutralizing a broad range of primary HIV isolates. Because of this, significant effort has been focused on the design of immunogens capable of eliciting antibodies de-novo that would employ similar modes of recognition and target similar epitopes as these clones. As an alternative strategy, we have designed a novel genetically-encoded vaccine in which the expression of specific monoclonal antibodies is directed by Adeno-Associated Virus engineered to produce Human IgG1.

Methods: Following a single intramuscular injection of this vaccine, mice produced nearly 1mg/mL of circulating human b12 antibody from a single 1011 dose. To test the efficacy of this vaccine we humanized vaccinated mice by engrafting Human peripheral blood mononuclear cells into immunocompromised NOD/SCID/γ−/− animals prior to challenge with a pathogenic HIV strain (NL4-3).

Results: Vaccinated mice exhibited remarkable resistance to both peripheral and splenic CD4 loss following challenge. Using this system we have quantified the protective abilities of four well-characterized broadly neutralizing antibodies in vivo. Interestingly, animals protected by the b12 neutralizing antibody appear to have undetectable levels of infection as measured by histological analysis of the spleen, suggesting that sterilizing immunity may be the principal mechanism of protection. Consistent with this result, animals challenged with escalating doses of HIV demonstrated robust protection of CD4+ T-lymphocytes despite artificially high levels of inoculum.

Conclusion: Future experiments aim to utilize this model to establish minimum protective doses of neutralizing antibodies and define concentration targets for human clinical trials.

OA05.01 HIV-Specific Cytolytic CD4+ T-Cell Responses During Acute HIV-1 Infection Predict Disease Outcome
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Background: During acute HIV-1 infection, early control of viremia and establishment of viral set point have been widely attributed to HIV-specific CD8 T-cell responses. However, despite increasing evidence for direct antiviral activity by CD4 T-cells in other infections, the impact of HIV-specific CD4 T-cells on viral control has not been studied.

Methods: We studied 26 acutely infected, treatment-naïve subjects and monitored their clinical outcome for up to 3 years post-infection. Baseline HIV-specific CD4 T-cell responses were assessed for degranulation (CD107a), IFN-γ secretion, expression of granzymes (GrzA,B,K) and perforin. HIV-specific cytolytic CD4 T-cell responses were investigated longitudinally in a subset of 11 subjects with similar peak viral loads for 1 year post-infection.

Results: Among the patients followed longitudinally, 6 progressed to a low viral set point, while 5 progressed to a significantly higher set point (134,020 vs. 11,234 copies/ml; p = 0.004). Interestingly, a significant expansion of HIV-specific cytolytic CD4 cell responses was observed in individuals who controlled viral replication compared to those who progressed to a high viral set point (IFN-γ: p = 0.038, CD107a: p = 0.042). Importantly, this expansion was observed early post-infection, prior to the divergence of viral load or CD4 T cell counts between the two groups. Examination of the baseline HIV-specific CD4 responses revealed a distinct GrzA-enriched cytolytic phenotype that was highly associated with subsequent viral control. Strikingly, Kaplan-Meier analysis of only the baseline cytolytic CD4 T-cell response in a larger cohort demonstrated that individuals with lower HIV-specific GrzA+ responses remained off HAART significantly longer than individuals with HIV-specific GrzA responses (p = 0.0023).

Conclusion: Here we demonstrate that the rapid induction and expansion of a distinct cytolytic CD4 T-cell response during acute infection is significantly associated with viral control and disease outcome. These data suggest a pivotal role for HIV-specific cytolytic CD4 T-cells in the early control of viremia following acute infection and for future HIV vaccine design strategies.

Control of Acute HIV-1 Subtype C Viremia Is Associated with Limited CD8+ T-Cell Immunogenicity

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Background: HIV-1-specific CD8+ T-cell responses contribute to the decline in acute peak viremia following infection. However, the relative immunogenicity CD8+ T-cell epitopes during and after acute viremia has not been well defined, and data on the breadth of responses following the initial drop in acute viremia are lacking.

Methods: We characterized CD8+ T-cell responses to peptides spanning the full viral proteome in 20 HIV-1C acutely infected, antiretroviral naive subjects using the IFN-γ ELISPOT assay as early as 28 days after estimated date of infection. Viruses from plasma were also sequenced within defined CD8+ T-cell epitopes for selected subjects. Of the 20 participants in the cohort, eleven had not reached complete seroconversion at analysis.

Results: Despite documented declines in peak viremia, the initial responses associated with the decline in acute phase peak viremia were narrowly directed, and of weak magnitude. At approximately 28 days after estimated initial infection, following a 15-fold decline in viral loads, CD8+ T-cell responses were directed against an average of 3 of the 410 peptides tested (range 0–6); two individuals had no detectable CD8+ T-cell responses at this time. At 18 weeks post the estimated date of infection the average number of peptides targeted had increased to 5 (range 0–11). Of 56 optimal Gag CD8+ T-cell epitopes sequenced, 31 were wild type in the acute infecting viruses, however, only 11 of 31 elicited measurable CD8+ T-cell responses.

Conclusion: These data demonstrate that the majority of CD8+ responses that subsequently arise are not elicited during acute HIV-1 infection despite the presence of the cognate epitope in the infecting strain. Further studies are needed to address why recognition of HIV-1 peptides appears to be selective during acute infection as the lack of recognition may contribute to the failure to control viremia to a low set point.

Lymphocyte Dynamics in Acute HIV-1 Infection: RV217-The Early Capture HIV Cohort Study (ECHO)

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Background: Acute HIV infection events between exposure and peak viremia in non-subtype B populations are poorly characterized.

Methods: Individuals in East Africa and Thailand at high risk for HIV-1 infection were prospectively followed with twice weekly HIV-1 RNA (NAT) to detect acute infection. Lymphocyte subsets (CD4+ T, CD8+ T, NK and B cells) were enumerated on a FACS-Calibur using MultiTest reagents (Becton Dickinson) starting as early as 3–9 days after the last known NAT-negative sample and followed at multiple timepoints during early infection. Viral load (VL) was measured using Abbott Real Time Viral Load.

Results: Dynamic lymphocyte changes were observed in 16 acute HIV-1 infections. Peripheral CD4+ T cell and B cell nadir occurred at a median of 20 days post infection. CD4+ T cells fell from a median 929 (range, 419–1532) cells/μl to 466 (142–866) cells/μl (P < 0.001). B cells declined from a median 290 (186–592) cells/μl to 127 (15–275) cells/μl (P < 0.001). In contrast, NK and CD+ T cells increased at different rates within the first 100 days. CD8+ T cells increased nearly 3-fold from a median 500 (271–1150) cells/μl to 1498 (693–2367) cells/μl (P < 0.001). NK cells increased from a median 252 (49–589) cells/μl to 335 (100–864) cells/μl (P = 0.002). CD4+ T cell and B cell counts exhibited an inverse correlation (P < 0.001) while NK cell counts showed a direct correlation (P = 0.022) with contemporaneous VL. CD8+ T cell counts did not correlate with VL at any time consistent with expansion by indirect mechanisms. VLs preceding and following CD4+ T cell and B cell measurements were negatively correlated suggesting that these subsets are being directly depleted. In
Contrast, VL preceding, but not following NK cell measurements were positively correlated, suggesting that VL may be driving NK cell expansion. 

**Conclusion:** Understanding lymphocyte dynamics, their relationship to VL and early pathogenic events of non-B HIV-1 infection may help to target prevention or treatment interventions.

**OA05.04**

**Long-Lived Historical Record of Evolution of Viral Diversity in Peripheral Blood Cells by Deep Pyrosequencing**

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**Background:** Assessments of HIV-1 diversity are generally inferred from limited population sampling. Deep sequencing was applied to increase quasispecies coverage for direct analysis of viral diversity, population structure, and the historical archive of HIV-1 within the host.

**Methods:** 454 deep sequencing was applied to HIV-1 envelope hypervariable domain 3 (V3) in peripheral blood cells from therapy-naïve children, infected for 1.5 to 6.1 years, with high plasma virus levels (4.0 to 6.7 log10 HIV-1 RNA copies/ml), but variable CD4 T-lymphocytes (2% to 30%). A V3 library was constructed for each subject from 400 viral templates and sequenced to generate 10,000 quality reads. Consensus sequences were derived by clustering reads at 3% genetic distance, and used to estimate viral biodiversity and population structure via rarefaction/CHAO1 algorithm. Coreceptor use was inferred by position-specific scoring matrix. Viral archive and most recent common ancestor were evaluated by maximum likelihood phylogenetic trees constructed from consensus sequences combined with longitudinal conventional sequences.

**Results:** Biodiversity of HIV-1 V3 quasispecies across individuals ranged from 12 to 99 clusters. Viral population structure was organized into a limited number of dominant genomes with a high frequency of unique sequences. Dominant viral variants at later time points evolved exclusively from low frequency variants at earlier time points. Phenotypic profiles of bioclusters revealed a breadth of coreceptor use that was unrelated to population structure or immune status. CXCR4 variants were co-archived with CCR5 variants even when CD4 T-cells exceeded 20%. A V3 library was constructed for each subject from 400 viral templates and sequenced to generate 10,000 quality reads. Consensus sequences were derived by clustering reads at 3% genetic distance, and used to estimate viral biodiversity and population structure via rarefaction/CHAO1 algorithm. Coreceptor use was inferred by position-specific scoring matrix. Viral archive and most recent common ancestor were evaluated by maximum likelihood phylogenetic trees constructed from consensus sequences combined with longitudinal conventional sequences.

**Conclusion:** Unprecedented coverage by deep sequencing defines HIV-1 population complexity and structure, enriches the evolutionary landscape between samplings, and reveals an evolutionary record of persistent, cell-associated viral sequences closely related to transmitting viruses.

**OA05.05**

**Dynamics of HIV-1 Subtype C Gag in the Global Epidemic**

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**Background:** The dynamic patterns of HIV transmission, and the complexity of viral diversity on the population level, might present substantial challenges to HIV vaccine design. It is unclear to what extent HIV vaccine antigens should match viral evolution to provide a sufficient level of protection.


**Results:** Despite the heterogeneous origins of the analyzed sequences, the gamut and frequency of amino acid residues in wild-type Gag were remarkably stable over the past two decades of the HIV-1 subtype C epidemic. The vast majority of amino acid residues demonstrated minor frequency fluctuation over the observed time, consistent with the conservative nature of the HIV-1 Gag protein. Consistent changes in amino acid frequency were detected in only 4.0% (20/500) of amino acid residues across Gag based on three statistical methods, a trend test, heterogeneity test, and range ranking of amino acid frequency. The tMRCA of HIV-1 subtype C was dated to around 1959 (95% HPD 1954–1964).

**Conclusion:** The study presents evidence of the stability of HIV-1 subtype C Gag among viruses circulating in the epidemic over the past two decades, providing an optimistic prognosis for HIV antigens tested in vaccine trials. On a background of the overall increase of HIV-1 subtype C gag diversity over time, a relatively small number of amino acid residues across HIV-1 subtype C Gag are likely to be under consistent selection pressure at the population level. The year 1959 was estimated as the beginning of HIV-1 subtype C diversification in the worldwide epidemic.

**OA05.06**

**Important Contribution of Subdominant HIV-Specific CD8 T Cell Responses to Early Control of HIV Replication**

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**Background:** During acute HIV-1 infection, HIV-specific CD8 T cell (CTL) responses emerge, suppressing viral loads to a semi-stable viral setpoint; this early viral setpoint has been shown to be highly predictive of disease outcome. However, while immunodominant responses restricted by rare HLA class I alleles have been associated with better control of viral replication, immunodominant responses restricted by common HLA alleles like B*8 or B*7 do not appear to have a significant impact on early control.

**Methods:** Over 600 individuals with primary HIV-1 infection were screened for HLA-restricted, epitope-specific CTL responses using optimally defined epitopes. The early viral setpoint was determined in all treatment-naïve individuals by two independent ID specialists.

**Results:** The recognition of HIV-specific CTL responses was very predictable based on HLA class I expression and followed a clear hierarchical pattern. While the immunodominant responses in individuals with rare HLA class I alleles were significantly
associated with lower viral setpoint, individuals with immunodominant responses restricted by common HLA class I alleles had, on average, higher viral loads. Interestingly, the absence of CTL responses in primary infection directed against some of the immunodominant epitopes restricted by common HLA class I alleles (e.g., HLA-B8-FLG) allowed for the development of responses against subdominant epitopes (e.g., HLA-B8-ELR) within the same individuals and was associated with significantly better subsequent viral control \( (p = 0.001) \). A distinct ranking of the relative contribution of epitope-specific CTL responses to the early viral setpoint suggested an unexpected contribution of subdominant responses to the initial viral control.

**Conclusion:** Our data suggest that in the context of common HLA class I alleles, CTL responses directed against subdominant epitopes but not dominant epitopes during primary infection significantly contribute to better control of viremia. Thus, engineered proteins that lack common immunodominant CTL epitopes and allow for the priming of otherwise subdominant epitopes might provide an important approach for vaccine design strategies.

**OA06.01 Deconstructing HIV-Specific CD8+ T Cell Responses in HIV Elite Controllers**

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**Background:** Evidence suggests that HIV-specific CD8+ T cells (CTL) play a critical role in a large proportion of rare individuals capable of immune control of HIV replication in the absence of therapy (elite controllers, EC). However, there is still no clear understanding of what differentiates effective CTL responses from ineffective responses in untreated chronic progressive disease (CP) and in subjects with controlled viral load on antiviral therapy (ARTC).

**Methods:** We combined standardized assays and advanced statistical analysis to determine whether a subset of HIV-specific CTL endowed with unique characteristics is present in EC. Inter and intra-donor analysis of HIV CTL-specific proliferation and cytokine secretion was performed at different time points after antigen stimulation by CFSE and luminex assays. 23 EC, 15 CP and 15 ARTC were studied. Independent cohorts of 15 EC and 15 ARTC were used for validation.

**Results:** We observed that within the same individuals, the proliferative capacity of CTL targeting epitopes restricted by protective alleles was significantly stronger than epitopes restricted by non-protective HLAs \( (p = 0.003) \). However, this marked difference was present only in EC. Kinetic analysis of cytokine secretion showed marked differences between groups. Slopes of IFN-γ, IL-2 and TNF secretion were significantly steeper in EC than in the other groups. A model incorporating proliferation and early IL-2 secretion accurately predicted EC status in an independent cohort \( (c\text{-statistic} = 0.908) \).

**Conclusion:** Our data illustrate that HIV-specific CTLs in EC are characterized by late and robust cytokine secretion. We also show that proliferation is driven by protective alleles only in EC, suggesting that HIV-specific responses restricted by protective HLA alleles present unique features in EC not observed in subjects with progressive disease. Our results suggest that this strategy can be used to define immune correlates of protection in natural HIV infection and may have important implications for immune monitoring of responses to HIV.

**OA06.02 Ontogeny of CD8+ T Cells in Acute HIV-1 that Inhibit Autologous and Heterologous Transmitted/Founder (T/F) Viruses**

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**Background:** CD8-mediated virus inhibition can be detected in HIV+ subjects naturally controlling virus replication. Characterizing the inhibitory function of CD8+ T cells in acute HIV-1 infection (AHI) is important for determining the nature of CD8+ responses that need to be elicited by an HIV-1 vaccine. In this study, we examine the timing, epitope specificity, and contribution of soluble factors to the antiviral CD8+ T cell response in AHI.**

**Methods:** We used a CD8+ T cell mediated virus inhibition assay (CD8 VIA) to assess CD8+ T cell function during AHI in the CHAVI 001 cohort. Primary CD4+ enriched lymphocytes were infected with transmitted/founder (T/F) autologous and heterologous viruses in the presence of autologous CD8+ T cells. Peptide-specific soluble antiviral responses were determined by transwell assay, luminex cytokine analysis, and flow-cytometric intracellular cytokine staining.

**Results:** Potent CD8+ antiviral responses against autologous and heterologous (T/F) viruses appeared during AHI prior to Fiebig stage 4. Responses against autologous transmitted virus were durable to 48 weeks; however heterologous responses declined concurrent with the resolution of viremia. Viruses obtained 6 months post infection were more resistant to CD8+ mediated virus inhibition than cognate T/F viruses. CD8+ T cells specific for epitopes that have been shown to drive HIV-1 escape in AHI released soluble factors that inhibited T/F virus replication.

**Conclusion:** The rapid development of potent CD8+ antiviral activity may reflect the previously identified inhibitory capacity of early and transitional memory cells. These studies suggest that continued antigenic exposure is necessary to maintain antiviral breadth. Chronic virus resistance to early CD8+ cell antiviral activity supports prior evidence that CD8+ cells drive rapid virus escape. These data provide insight into the mechanisms of CD8-mediated viral inhibition, and suggest that comparable functional analyses will be important for determining whether similar activities can be induced by T-cell directed vaccine strategies.

**OA06.03 The RV144 Vaccine Induced CD4+ T Cells Producing Th1 and Th2 Cytokines with Proliferative Potential**

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Background: The RV144 efficacy trial demonstrated moderate reduction in HIV acquisition for participants receiving recombinant canarypox encoding Env, Gag, and Protease, and recombinant gp120 proteins.

Methods: PBMC from 10 placebo and 40 vaccine recipients were examined at baseline, Week 26 (two weeks post last vaccination) and Week 52. Intracellular cytokine staining (ICS) measured T cells producing IFN-γ, IL-2, TNF-α, or expressing CD40L, following 6-hour ex vivo stimulation with insert-matched Env and Gag peptide pools. Multiplex bead array measured 12 cytokines following 6-hour ex vivo stimulation. The CFSE assay measured proliferation.

Results: Responses were largely restricted to CD4+ T cells and to Env. CD40L detected the highest response rate by ICS (60% at Week 26, 20% at Week 52), with a median magnitude of 0.1% CD4+ T cells expressing CD40L at Week 26. The Week 26 response rate for IL-2 was 43%, but only 18% for IFN-γ; both decreased at Week 52 (23% and 13%, respectively). Response rates were similar by multiplex bead array (50% for IL-2 and 14% for IFN-γ at Week 26), and additional responses of Th2 and regulatory cytokines were detected (30% for IL-4, 44% for IL-5, 53% for IL-13, and 28% for IL-10). Proliferative response rates were lower at Week 26 (16% for Env), but persisted at Week 52 (21%). For all assays, false positive responses were minimal (<5%).

Conclusion: In this pilot immunogenicity study, CD4+ T-cell responses were detected in a majority of vaccine recipients, and were polyfunctional including secretion of Th2 cytokines. This, together with expression of CD40L, indicates ability to provide B-cell help. A smaller proportion of vaccine recipient had proliferative T-cell responses, but these appeared more durable, persisting to one year. These assays will be used to examine samples from cases and matched controls and may identify a correlate of protection.

OA06.04
Association of Timing of HLA B*8101 and B*3910 Mediated p24 Sequence Evolution and Pathways to CTLE Escape with Disease Progression

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Background: HLA’s that are associated with control of vireamia, often select for mutations in Gag p24 in acute/early phases of the HIV-1 infection.

Methods: To understand the role of viral and host factors in the pathogenesis of subtype C, HIV-1 infection, we timed CTL mediated sequence evolution in TL9 (Gag 180 – 188) epitope in p24 and compared disease progression in B*8101 (n = 5) and B*3910 (n = 2) participants from the CAPRISA acute infection cohort.

Results: We report that both the B*3910 and B*8101 alleles select mutations in the TL9 epitope, in which either the 182Q or both the 182Q and 186T are replaced by S (TPqDLNtML). However, the timing to sequence evolution differed among these alleles. While both B*3910 participants selected acute/early mutations, four of the five (4/5) B*8101 individuals selected late mutations in the TL9 despite persistent CTL responses targeting this epitope. One B*8101 participant did not develop any restricted sequence evolution in the TL9 epitope by 2.5 years post-infection despite consistent CTL responses. In three of the remaining four individuals, sequence evolution in TL9 was accompanied or preceded with changes in SV9 (Gag 148 – 156) epitope or amino acids flanking this epitope. The fourth participant developed an E177D mutation in addition to the double mutations in the TL9 epitope. These three mutations were also developed by one of the B*3910 individual but in the acute phase of the infection. Irrespective of HLA, participants who selected both the Q182S and T186S mutations had a slow disease progression profile.

Conclusion: These results provide new evidence on the role of the restricting HLA type in the timing of sequence evolution and contribute new information on the constituents of effective CTL responses. In addition, these results further support inclusion of multiple conserved regions of Gag in a vaccine meant to render HIV-1 less fit.

OA06.05
Evidence of HIV-Specific ADCC Immune Escape

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Background: Natural killer (NK) cell-mediated antibody dependent cellular cytotoxicity (ADCC) is a potentially important immune mechanism against HIV. No previous work has identified immune escape from ADCC. Rapid evolution of HIV is heavily influenced by focused immune responses directed at particular neutralising antibody or CD8+ T lymphocyte epitopes. Demonstrating viral escape from ADCC responses would be an important contribution to establishing the immune pressure applied by ADCC responses against HIV infection.

Methods: Using an ICS ADCC assay for antibody-dependent NK cell expression of IFNγ and CD107a to overlapping HIV-1 peptides pools, we mapped ADCC epitopes from a cohort of 80 ART naïve HIV-positive subjects. We sequenced autologous plasma virus samples across relevant epitopes, identifying variations from consensus subtype B sequence, then compared the ADCC activity induced by consensus and autologous epitopes, including autologous epitope sequences at earlier time points of infection. Glycosylated gp140 AD8 proteins containing ADCC escape mutations were also synthesised and ADCC activity was assayed.

Results: 37 linear ADCC epitopes were mapped, primarily in Env; of these, 9 epitopes were shared by 2 or more subjects. Mutational escape from ADCC responses was detected in 8 of the 12 epitopes that we tested. Virus epitope sequences from earlier infection were identical or more similar to consensus. ADCC responses to earlier autologous virus were commonly unable to recognise concurrent virus sequence. ADCC escape was also observed from gp140 AD8 protein.

Conclusion: Loss of ADCC responses induced by epitopes sequenced early in infection strongly indicates that ADCC responses can force mutational escape in HIV-infected subjects. Weaker ADCC activity to gp140 ADCC escape mutant proteins confirm virus escape. This work establishes an additional immune pressure on viral evolution and predicts that ADCC responses induced by vaccination should be capable of reducing virus replication.
**OA06.06**  
**Imbalance Between T Helper Type 17 and T Regulatory Cells: Implications for HIV Pathogenesis**  
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**Background:** T helper 17 (Th17) and CD4⁺CD25hiFoxp3⁺ regulatory T (Treg) cells have been described as two distinct subsets from T helper 1 (Th1) and T helper 2 (Th2) cells, their balance may play an important role in the pathogenesis of HIV-1 infection.  

**Methods:** 115 untreated chronic HIV-infected individuals and 32 healthy donors were recruited in this study. Peripheral blood mononuclear cells were isolated and stained to characterize the frequencies of Th17 and Treg. 42 individuals including 10 elite controllers were followed up for more than one year, changes of Th17 and Treg frequencies were analyzed over time.  

**Results:** Th17 levels with CD4 counts were depleted while Treg were increased in the cohort of 115 HIV-infected individuals, compared to healthy controls (median = 0.608% vs 0.938%, 5.150% vs 4.605%; p < 0.001, p = 0.032), leading to an obvious imbalance of Th17/Treg when compared to the healthy control counterparts (median = 0.117 vs 0.194; p < 0.001). In the 42 patients, loss of Th17 contrasted with an increased Treg frequencies from Jun 2009 to Oct 2010 were followed longitudinally (median = 0.669% vs 0.603%, 4.765% vs 5.890%; p = 0.078, 0.010). Meanwhile, the elite controller group had comparable Treg, Th17 levels and a Th17/Treg with healthy controls during the follow-up study, (in 2009, median = 4.555% vs 4.605%, 0.843% vs 0.938%, 0.176 vs 0.194; p = 0.948, 0.635, 0.585; in 2010, median = 5.205% vs 4.605%, 0.768% vs 0.938%, 0.140 vs 0.194; p = 0.159, 0.220, 0.053). Additionally, we demonstrated that loss of balance between Th17 and Treg could lead to an earlier CD4 T cell decline than Treg or Th17 during the course of HIV infection.  

**Conclusion:** The loss of Th17 cells and a reciprocal increase in the Treg cells in HIV-1 chronic infections with no differences observed in elite controllers give prominence to the understanding of HIV-1's pathogenesis and prompt novel considerations for future thoughts on HIV vaccine development.

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**OA07.01**  
**Bispecific Fusion Antibodies PG9-Ibalizumab (PG9-iMab) and VRC01-Ibalizumab (VRC01-iMab) Exhibit 100% Breadth and Picomolar Potency**  
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**Background:** Ibalizumab, PG9 and VRC01, three of the broadest and most potent monoclonal antibodies against HIV-1 infection described to-date, neutralize 66% - 90% of HIV-1 strains. Here, we created PG9-iMab and VRC01-iMab bispecific antibodies, exploiting ibalizumab’s high affinity for domain 2 of CD4 and inherent antiviral activity to create bispecific fusion antibodies that anchor PG9 or VRC01 at the site of viral entry.  

**Methods:** Bispecific antibodies were created by fusing PG9 or VRC01 scFv to the N-terminus of the ibalizumab heavy chain. Breadth and potency were assessed to a maximum concentration of 10 μg/mL against a well characterized panel of HIV-1 Env pseudotypes (n = 118).  

**Results:** Addition of PG9 or VRC01 scFv to the N-terminal of ibalizumab was well tolerated; expression and purification yields of bispecific antibodies were comparable to the parent antibodies and had negligible effects on CD4 binding affinity. PG9-iMab and VRC01-iMab neutralized 100% of viruses to at least 50% inhibition (median IC50, 24 pM and 135 pM, respectively) and indeed, PG9-iMab neutralized 97% of viruses and VRC01-iMab neutralized 95% of viruses, to at least 95% inhibition. Most importantly, PG9-iMab and VRC01-iMab each potently neutralized dual PG9- and ibalizumab-resistant viruses and dual VRC01- and ibalizumab-resistant viruses, respectively. PG9-iMab exhibited synergistic potency, neutralizing each virus 100 ± 375 fold and 32 ± 282 fold more potently than PG9 and ibalizumab, respectively (median ± IQR). In contrast, while potency of VRC01-iMab was enhanced 19 ± 75 fold compared to VRC01, potency was comparable to ibalizumab (3 ± 91 fold). The synergistic activity and ability to neutralize dual-resistant viruses required CD4 anchoring and could not be achieved by simply co-administering PG9 or VRC01 with ibalizumab, which yielded only additive effects.  

**Conclusion:** Bispecific ibalizumab antibodies are broad and potent candidates for passive or gene-delivered biologics for prevention and treatment of HIV-1 infection.

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**OA07.02**  
**Identification of Amino Acid Residues in HIV-1 Envelope Targeted by Plasma Broadly Neutralizing Antibodies using Evolutionary Models**  
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**Background:** During the course of HIV-1 infection, a small proportion of individuals develop broadly cross-reactive antibodies capable of neutralizing diverse HIV-1 strains. Identification of the epitopes targeted by these antibodies will provide important clues for the design of a preventative vaccine against HIV-1 infection. We have developed an evolutionary model to identify key residues targeted by plasma neutralizing antibodies.  

**Methods:** Five HIV-1 subtype C serum samples from the CAPRISA 002 Acute Infection cohort previously shown to have neutralization breadth were tested against a large multiclade panel of 206 pseudoviruses for which gp160 sequences were available. Amino acid residues that influence neutralization sensitivity at ID₅₀ were identified by using Bayes factors (BFs) to compare the fit of a model in which the evolution of the residue correlates with changes in neutralization sensitivity to that of a null model in which the virus evolves independently of the antibody response. Sites with significant BFs were mapped onto the three-dimensional protein structure to identify epitopes on the surface of the envelope glycoprotein. Predictions were tested experimentally by site-directed mutagenesis.  

**Results:** We identified between 1 and 4 sites per sample that were strongly associated with neutralization sensitivity (BF > 20) and...
confirmed a subset of the predicted residues experimentally for 4 of the 5 sera. Our model predictions included two sites in the V2 region (166 and 169) that contributed to a trimer-specific PG9/16-like epitope in one subject, a glycan at position 332 in a second individual and the 674 residue in the membrane proximal region of gp41 in a third participant.

**Conclusion:** Our results provide strong support for the use of evolutionary models applied to cross-sectional viral neutralization data to identify novel epitopes recognized by antibodies that confer neutralization breadth. Such epitopes are difficult to discover experimentally or with existing computational tools, thereby demonstrating the utility of our approach.

**OA07.03**

**Isolation of a Subset of Novel HIV-1 Broadly Neutralizing Antibodies with Dual Specificity Overlapping the Env Receptor and Coreceptor Binding Sites**

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**Background:** Both CD4 binding site (CD4 bs) and coreceptor binding site (CoR bs) of HIV-1 gp120 are two of the most conserved functional elements of HIV Env. Elicitation of potent neutralizing antibodies (nAbs) targeting these structural elements with Env-based immunogens has been pursued extensively, with limited success. Our previous study showed that broadly and potent CD4 bs-specific nAbs can be elicited during natural infection at high titers and can be detected in selected patient sera. From the memory B cell repertoire of one such patient, we isolated a subset of CD4 bs-specific nAbs including VRC01, which neutralizes more than 90% of circulating primary virus isolates. From the same patient sera, we detected that there was also a fraction of potent nAbs specific for the Env CoR bs by differential protein adsorption and neutralization analysis.

**Methods:** In this study, we sought to isolate CoR bs-specific nAbs from this patient by sorting with a fluorescence-tagged gp120 possessing a CoR bs knockout mutation (I420R).

**Results:** A series of B cell clones were isolated by the FACS phenotype of WT gp120-high/I420R-low. A subset of these clones was able to neutralize ~ 50% of Clade B tier 2 primary virus isolates, with better potency than that of the typical CoR bs antibodies isolated previously. These clones displayed gp120 binding affinity abrogated by gp120 with both CD4 bs and CoR bs mutations, suggesting its overlapping epitope, which was also supported by cross competition analysis with site-specific ligands.

**Conclusion:** We have isolated a subset of novel CD4/CoR nAbs whose epitopes may directly or functionally overlap with both the HIV gp120 receptor and coreceptor binding sites. Fine epitope mapping and impact on the configuration of the viral Env functional spikes upon antibody binding is under way to define the unique specificity.

**OA07.04**

**Neutralizing Antibodies Inhibit HIV-1 Transfer from Dendritic Cells to Primary CD4 T Lymphocytes**

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**Background:** Sexual mucosal transmission is the main route of HIV-1 infection worldwide. Immature dendritic cells (DCs), which reside in genital mucosal surfaces, are among the first cells that encounter HIV-1. We have shown previously that in HIV-1 transfer conditions, where HIV-1 loaded immature DCs were co-cultured with primary CD4 T lymphocytes, HIV-1 replication was enhanced in DCs. Here, we aim to analyze the kinetics of HIV-1 replication and the ability of HIV-1 specific antibodies to inhibit HIV-1 infection in the co-culture of immature DCs with primary PHA-activated CD4 T lymphocytes.

**Methods:** DCs were generated from purified human blood CD4+ monocytes. After 2 hours of immature DCs incubation with R5 HIV-1 strains, we added autologous primary CD4 T lymphocytes in the presence or absence of HIV-1 specific antibodies. The percentage of infection was quantified by flow cytometry using intracellular p24 viral antigen staining.

**Results:** In this co-culture condition, the early step of HIV replication was more rapid in co-cultured DCs compared to infected DCs without lymphocytes, whereas the kinetic of HIV-1 fusion was unchanged. Monoclonal neutralizing antibodies, but not non-neutralizing or inhibitory antibodies, were able to potentially inhibit HIV-1 transfer to CD4 T lymphocytes. Moreover, we found a decrease of HIV-1 replication in DCs although neutralizing antibodies or non-neutralizing inhibitory antibodies were added on HIV-1-loaded DCs after two hours. This inhibition of HIV-1 replication in DCs was correlated with DC maturation.

**Conclusion:** These results demonstrate that neutralizing antibodies are able to efficiently inhibit HIV-1 infection of DCs and HIV-1 transfer to primary CD4 T lymphocytes. The efficient inhibition of HIV-1 transfer strengthens the requirement of the induction of HIV-1 specific antibodies directly at mucosal portal of HIV-1 entry. Vaccines that induce such antibody response may prevent the very early dissemination of HIV-1 after its sexual transmission.

**OA07.05**

**Induction of Neutralizing Antibody Responses in HIV Infection Is Associated with HIV-Specific CD4 T Cell Responses**


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**Background:** One of the foremost challenges in the development of an HIV-1 vaccine is that HIV-1 targets CD4-T cells, which are critically important in shaping the immune response to infection. It has been demonstrated that T follicular helper (TFH) CD4-T cells are pivotal for the induction of broadly neutralizing antibody (nAb) responses and for the generation of long-lived B-cell memory maturation. By cognate interaction and cytokine secretion, CD4-T cells expressing CD40L can induce class switching and somatic hypermutation in antigen specific B-cells, which is reflected in the expression of activation-induced cytidine deaminase (AID). Nonetheless, the role of TFH cells and their interaction with B-cells in HIV infection is currently unknown.
**Methods:** Ten subjects were identified during acute HIV infection and followed longitudinally over a median of three years. Neutralizing activity of plasma antibodies against a panel of present and past autologous and heterologous viruses was assessed using a single-replication cycle assay in which full-length envelope genes were incorporated into expression vectors (Monogram Biosciences). Phenotypic and functional characteristics of HIV-specific CD4-T cell responses and their impact on AID expression were assessed longitudinally by multiparameter flow cytometry.

**Results:** The breadth of the broadly neutralizing antibody response to heterologous HIV significantly increased over time in each patient. Interestingly, the expansion in heterologous breadth was not only significantly correlated with an increase in AID expression in B-cells, but also with CD4-T cell expression of PD-1, a marker that has been associated with TFH cells. Moreover, a positive association between AID expression and gp120-specific CD40L-responses was detected at the earliest time point.

**Conclusion:** Our data strongly indicate that the induction of broadly neutralizing antibody responses is linked to the function and activation of HIV-specific CD4-T cell responses. These findings will be pivotal in efforts to generate neutralizing antibody responses by vaccination strategies.

**OA07.06**

**Influence of NK Cells and NK Cell Receptor Polymorphisms in the Assessment of HIV-1 Neutralization**

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**Background:** Genetic polymorphisms within the killer immunoglobulin receptor (KIR) and Fc gamma receptor genes of natural killer (NK) cells have been shown to influence the containment of HIV replication, both in vivo and in vitro. Peripheral blood mononuclear cells (PBMC) prepared from different donors for in vitro use contain varying numbers of immune cells, including NKS. This study addresses the impact of this variation and of specific genetic polymorphisms on the measurement of neutralization using PBMC from HIV-seronegative donors.

**Methods:** Leukopaks (LP) were obtained from 25 donors and PBMC immune cell populations were quantified using multi-color flow cytometry. Viral permissivity and HIV-1 neutralization profiles were assessed using infectious molecular clones (IMC) and intracellular p24 or Renilla luciferase (LucR) expression as end-points. HIV-1 IMC with LucR (NL-LucR-BaLecto and NL-LucR-SF162 ecto) were used in neutralization assays; sCD4 and polyclonal and monoclonal antibodies were tested. CD4+, CD8+, and NK cells were depleted from PBMC using antibody-coated beads.

**Results:** The PBMC NK cell percentages ranged from 0.5–10.0%. When donor PBMC were stratified into quartiles using mean NK cell percentages, donors were both associated with PBMC that showed higher neutralization.

**OA08.01**

**Evidence for CD4 T-Cell Mediated Immune Control of Macrophage Infection in Rhesus Macaques Undergoing CD4+ Lymphocyte Depletion Prior to SIV Infection**

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**Background:** Identifying naturally-occurring neutralizing antibodies (NAb) that are cross-reactive against all global subtypes of HIV-1 and is an important step toward the development of an HIV vaccine. It was previously shown that NAb breadth is positively associated with viral diversity. Therefore, we hypothesized that superinfected individuals develop a broader NAb response compared to singly-infected individuals as a result of increased antigenic stimulation by two distinct virus strains.

**Methods:** A prospective cohort study was designed with 12 superinfected women each matched to 3 singly-infected women to assess NAb breadth 5 years post-initial infection, when all 12 women had been superinfected for at least 1 year. Breadth was also measured pre-superinfection to control for differences in the antibody responses to the first infection. Breadth scores were calculated for each woman using a panel of 8 viruses representing 4 different HIV-1 subtypes that exhibit a spectrum of neutralization sensitivities, and the resulting data was modeled using conditional Poisson regression.

**Results:** The mean breadth scores of superinfected women were higher compared to singly-infected women at 5 years post-initial infection (5.75 vs. 3.42). Univariate analysis showed that the NAb response of superinfected women was nearly twice as broad as that of singly-infected women at 5 years post-initial infection (RR = 1.68, CI: 1.23–2.30, p = 0.001). There was also a significant difference in breadth between superinfected and singly-infected women at 1-year prior superinfection (4.30 vs. 3.23). However, adjusting for breadth from 1-year prior superinfection, viral load, and CD4+ T cell count did not substantially change our original estimate (RR = 1.51, CI: 1.01–2.25, p = 0.04).

**Conclusion:** These data suggest that superinfection elicits a substantial enhancement of the NAb response with regards to coverage and cross-reactivity in the years following reinfection. Individuals who have been superinfected may represent a unique setting to characterize broadly NAbs and the pathways required to elicit them.
ORAL ABSTRACTS

OA08.02 CD4+ T Cell Escape in a SIVmac239-Infected Indian Rhesus Macaque Elite Controller with Breakthrough Viremia

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Background: The role of CD8+ T cells in containment of retroviral infections is well documented by studies of CD8+ T cell-mediated viral escape. It contrast, the immunological role of retrovirus-specific CD4+ T cells remains unclear, particularly in the setting of elite control. Here, we describe viral escape from CD4+ T cells concomitant with viral breakthrough and loss of elite control.

Methods: A SIVmac239-infected Indian rhesus macaque maintaining elite control of viremia (<1,000 vRNA copies/mL plasma) spontaneously lost control, experienced breakthrough viremia, and eventually succumbed to AIDS. We performed whole genome sequencing on plasma virus prior to the virus becoming undetectable and immediately following breakthrough and loss of elite control. We then investigated the T cell response directed against areas in the viral proteome exhibiting sequence divergence between the pre- and post-breakthrough time points.

Results: Comparison of the pre- and post-breakthrough viral sequences revealed surprisingly few amino acid substitutions post-breakthrough. These substitutions occurred within two previously defined and three previously undescribed CD8+ T cell epitopes, confirming the critical role of CD8+ T cells in control of viral replication. Interestingly, we identified two mutations within Gag, V63A and D205E, which occurred within CD4+ T cell epitopes. Both of these mutations abrogated CD4+ T cell recognition. While the V63A mutation also abrogated recognition of an overlapping CD8+ T cell epitope, the D205E mutation was solely targeted by a CD4+ T cell response. We confirmed that no CD8+ T cell responses were targeting this region. Finally, we demonstrated that viruses bearing the GagD205E mutation escaped CD4+ T cell effector function in vitro.

Conclusion: Virus-specific CD4+ T cells play an active role in the containment of retroviruses and are likely essential for the maintenance of low-level viremia. How these CD4+ T cells persist and exert their anti-viral function during retroviral infection remains unclear and warrants further investigation.

OA08.03 Decreased Penetration of GFP-Labeled Lentiviral Particles in the Vagina and Endocervix of SIV3A3-Vaccinated Macaques

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Background: Immunization with live attenuated SIV strains, such as SIV3A3, provide robust protection against vaginal challenge with pathogenic SIV strains. However, the precise contributions of humoral and cellular immune responses, and the anatomic compartment(s) in which virus transmission is interrupted are unknown. To gain insight, we examined that the ability of fluorescent virus particles expressing the SIV envelope to penetrate intact genital epithelium in unvaccinated and SIV3A3-vaccinated macaques.

Methods: A total of 11 animals were studied: 8 were vaccinated with SIV3A3 29 months previously, and 3 were uninfected controls. Three of the SIV3A3-vaccinated animals were superinfected with SIVmac251 after vaginal challenge one year after vaccination. Subsequently, female macaques were inoculated intravaginally with PA-GFP-gag labeled virions. Genital tracts were removed 4 hours post-inoculation and immediately dissected and snap frozen in OCT media. Tissue specimens were sectioned and stained for cellular proteins, prior to imaging with fluorescent deconvolution microscopy.

Results: Within 4 hours, PA-GFP virions were able to penetrate the simple columnar and stratified squamous epithelia in both SIV3A3-vaccinated and control animals, penetrating up to depths of 50µm. When comparing viral penetration depths between the two sample groups, a significant decrease in penetration of viral particles in the vaginal and endocervical mucosa of SIV3A3-vaccinated animals was observed.
Conclusion: Our results indicate that in unvaccinated and SIVΔτ-vaccinated animals, PA-GFP virions can penetrate to depths where they can interact with target cells in both squamous and columnar epithelium. A decrease in median depth penetration of virus particles in vaccinated macaques suggests vaccination with live attenuated SIV is able to block early events of virus transmission, most likely via the induction of SIV-specific antibody responses. A clearer understanding of the ability of virus-specific antibody responses to interrupt early events of viral transmission at mucosal sites is essential for the development of future HIV vaccine strategies.

OA08.05

Compromised NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity in Chronic SIV Infection

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Background: Increasing evidence indicates that antibody-dependent cellular cytotoxicity (ADCC) contributes to control of HIV/SIV infection. However, virtually nothing is known about the ADCC function of Nature killer cells in non-human primate models. Efforts to get a clearer understanding of the specific role NK cells play in rhesus macaques will be helpful in evaluation of vaccines in non-human primate models.

Methods: The antibody-coated target cells were used to measure the ADCC function of macaque NK cells. The ADCC responses were analyzed by NK cell cytokine secretion and degranulation through ICS-based assay. All data were collected on BD FACS Aria.

Results: To identify the differences of NK cell-mediated ADCC function between naive and chronic SIV-infected macaques, some cytokines were measured in respond to Fc target cells. It was obvious to notice the downregulation of CD107a expression (P = 0.0203) as well as decreased secretion of IFN-γ (P = 0.0180) and TNF-α (P = 0.032) in NK cells from SIV-infected group compared with naives. The greatly reduced CD16+ NK subset in macaques with SIV infection (P = 0.0209) was the reason of impaired NK cell function, cause there was a direct association between CD16 expression and cytokine production in NK cells, including CD107a(P = 0.0204), IFN-γ(P = 0.0231), TNF-α(P = 0.0413). The loss of CD16 MFI following stimulation, which was used to quantify the Fc-mediated activation of NK cell, was detected much lower in SIV-infected group (P = 0.0036).

Conclusion: In chronic SIV-infected group, we could see the significant compromised ADCC function and its close relationship with CD16 expression on NK cells: the increased levels of CD16 allow NK cells to better respond to Fc target cells and this marker could be served as sensor for ADCC function of NK cells in macaques. These data offer new insights into the role of NK cells as an important bridge between innate and adaptive immunity in non-human primate models.

OA08.06

Cytotoxicity Capacity of SIV-Specific CD8+ T Cells Against Primary Autologous Targets Correlates with Immune Control in SIV-Infected Rhesus Macaques

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Background: HIV-specific CD8+ T cells of long-term non-progressors / elite controllers (LTNP/EC) exhibit extraordinary per-cell cytotoxic capacity against HIV-infected CD4+ T cells,
contrast to those of progressors. In this study, we explore whether our previous observations can be extended to the SIV-rhesus macaque (RM) model by measuring SIV-specific cytotoxic capacity of CD8\(^+\) T cells against SIV-infected targets.

**Methods:** Cytotoxicity and IFN-\(\gamma\) production of SIV-specific CD8\(^+\) T cells were examined following 6-day incubation with autologous SIVmac251-infected telomerase-transduced CD4\(^+\) T cell clone targets. SIV-specific CD8\(^+\) T cell cytotoxic responses were measured at 1 hour by flow cytometric detection of active granzyme (Gr) B delivery to live targets and infected CD4 elimination (ICE).

**Results:** Fifteen SIV-infected RM with different extents of control of viral replication have been evaluated. Data collection is ongoing with laboratory personnel blinded to RM disease status. LTNP/EC RM had higher median delivery of active GrB to targets (43.5\% vs. 18.5\%, \(p = 0.04\)), and higher median ICE than progressors (67.3\% vs. 24.4\%, \(p = 0.009\)). There was a significant correlation between ICE and viral load (\(R = -0.55, p = 0.03\)), and between GrB delivery and ICE (\(R = 0.86, p < 0.0001\)).

**Conclusion:** Lytic granule loading of CD8\(^+\) T cells and efficient delivery of active GrB to SIV-infected targets are associated with control of SIV infection in rhesus macaques, consistent with observations of HIV infection in humans. These findings suggest ICE is a correlate of control of viral replication in chronic SIV infection. They also suggest the use of cytotoxic capacity as a predictor of immunologic control in the vaccine setting should be tested.
**Background:** A modest reduction in the rate of HIV-1 infection was observed among vaccine recipients in the RV144 trial who received a prime-boost vaccine regimen with recombinant canarypox and soluble gp120. Whereas virus-specific CD8+ T cell responses were largely undetectable, antibodies capable of binding to gp120 were detectable in nearly all vaccinated subjects. However, these antibodies were unable to neutralize primary HIV-1 isolates, suggesting that antibodies may have contributed to protection by non-neutralizing effector mechanisms.

**Methods:** To determine if vaccinated subjects made antibodies capable of directing antibody-dependent cell-mediated cytotoxicity (ADCC), we used a novel assay to measure ADCC titers against HIV-infected cells in pilot samples from the RV144 trial. This assay is based on the killing of an HIV-infected CD4+ T cell line by a CD16+ NK cell line in the presence of serial dilutions of plasma, thus allowing ADCC titers to be measured against virus-infected cells expressing the native, oligomeric conformation of the HIV-1 envelope glycoprotein. Using this approach, ADCC titers in blinded plasma samples from 80 vaccine recipients and 20 placebo controls were measured at baseline (week 0) and at two weeks after the last boost (week 26) against target cells infected with the HIV-1 subtype AE isolate 92TH023.

**Results:** Comparison of the rate for the vaccine group versus the placebo group revealed that 76.2% (61/80; 95% CI 66%, 84%) of the vaccinated subjects versus 10.0% (2/20; 95% CI 3%, 30%) of the placebo controls had detectable ADCC titers against HIV-1 92TH023 infected cells at week 26 (Fisher's exact test, P = 6.7e-8). ADCC titers were also significantly higher at week 26 than at week 0 among the vaccine recipients (McNemar's exact p-value, P = 3.9e-12).

**Conclusion:** These results demonstrate that the majority of vaccinated subjects in the RV144 trial made antibodies capable of directing ADCC against HIV-infected cells.
showed binding to the 42 amino acid cyclic peptide containing the 4a/7 binding motif. Binding was greatly diminished when that region was scrambled. There was significant reduction in V2-specific IgG antibodies in all of the vaccinee plasma samples by visit 9. The RV144 samples analyzed had extremely low or no antibodies reactive with cyclic V3 peptide. In contrast to vaccinees, plasma samples (1:50 dilution) from individuals infected with clade A/E from the same geographical region had >3-fold more anti-V3 antibodies compared to anti-V2 antibodies.

**Conclusion:** Our results support the conclusion that the V2 loop of gp120 as presented in the context of the RV144 vaccine is more amenable to induction of V2-specific antibodies when compared to a natural infection. The majority of the V2-specific antibodies were directed against the region containing the 4a/7 binding motif.

**OA09.05**

**Polyfunctional CD4/CD8 HIV-Specific Cytokine Responses and Presence of Cytolytic Markers in Swedish Vaccinees Immunized with HIV-1 DNA and HIV-1 MVA**


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**Background:** We have monitored polyfunctional HIV-1-vaccine-induced responses as measured by cytokine and cytolytic marker expression. Healthy volunteers were immunized at 0, 1 and 3 months with DNA plasmid expressing gp160 of HIV-1 subtypes A, B and C; revB; p17/p24 gag A and B and RTmut B. At nine months, plasma samples (1:50 dilution) from individuals infected with clade A/E from the same geographical region had >3-fold more anti-V3 antibodies compared to anti-V2 antibodies.

**Methods:** HIV-Gag specific immune responses were assessed by IFN-gamma-ELISPOT and by 8-colour ICS for expression of cytokines (CD3/CD4/CD8/IFN-gamma/IL-2/TNF-α/MIP1-β/VIVID) using fresh cells, and for expression of cytolytic markers (CD3/CD8/IFN-gamma/MIP1-β/CD107/Perforin/Granzyme B/VIVID) using cryopreserved cells.

**Results:** Two weeks after the second HIV-MVA vaccination, 19 (83%) of 23 evaluable vaccinees were reactive in IFN-gamma-ELISPOT; 18 to Gag and 11 to Env peptides. The ICS revealed CD4 and/or CD8 Gag-specific reactivity in 15/23 (65%) vaccinees. Polyfunctional CD4+ and CD8+ T cell Gag-specific responses were detected in 7/23 (30%) vaccinees, all of whom had IFN-gamma-ELISPOT Gag reactivity >175 SFC/million PBMC. Within the CD8+ T-cell compartment approximately 25% of the responding cells were dual functional and 60% polyfunctional, predominantly expressing IFN-gamma/MIP1-β, IFN-gamma/TNF-α/MIP1-β and IFN-gamma/IL-2/TNF-α/MIP1-β. Among the responding CD4 T-cells approximately 25% were dual functional and 60% polyfunctional, predominantly expressing IL-2/TNF-α, IFN-gamma/IL-2/MIP1-β and IFN-gamma/IL-2/TNF-α/MIP1-β.

**Conclusion:** A second HIV-MVA boost given approximately 3 years after the initial HIV-DNA/HIV-MVA vaccinations gave rise to polyfunctional immune responses as measured by the production of cytokines and cytolytic markers. The data supports further exploration of this vaccine concept.

**OA09.06**

**PedVacc001: Safety and Immunogenicity of a Candidate HIV-1 Vaccine, MVA.HIVA, Administered to Healthy Gambian Infants Born to HIV-1/2-Uninfected Mothers**


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**Background:** The development of a safe and effective HIV-1 vaccine remains a global priority especially in prevention of mother to child transmission of HIV-1 during breastfeeding. Our vaccine platform consists of recombinant BCG prime-recombinant modified vaccinia virus Ankara (MVA) boost. This study is the first stage and evaluates the safety and immunogenicity of MVA.HIVA, a candidate HIV-1 vaccine, among healthy Gambian infants born to HIV 1/2 uninfected mothers.

**Methods:** Sixty-five mothers were sensitized on delivery of healthy babies at Sukuta Health Centre, The Gambia. Sixty-two of whom consented to HIV voluntary counseling and testing and all the mothers (100%) had negative HIV test. Forty-eight of the eligible infants were randomized into vaccine and no-treatment control group at 20 weeks of age. The vaccine group received one dose of 5 × 10^6 pfu of MVA.HIVA administered intramuscularly. The safety of MVA.HIVA was determined by comparing adverse events, reactogenicity, biochemical and haematological data of vaccine and control groups. Immunogenicity was measured by interferon (IFN)-γ ELISPOT assay on peripheral blood mononuclear cells.

**Results:** The mean Haemoglobin (Hb), White Blood Cell count (WBC), Alanine transaminase (ALT) and Creatinine at pre-vaccination and post-vaccination visits were within acceptable normal ranges. Adverse events observed included fever, excessive crying, cough, vomiting, bilateral eye discharge, diarrhea and poor weight gain. All study infants had negative HIV antibody tests at 28 weeks and no severe adverse events or suspected unexpected serious adverse reactions were reported. Preliminary cultured IFN-γ ELISPOT analysis showed induction of HIV-1-specific T-cell responses in about 40% of the vaccinees.

**Conclusion:** We have shown that MVA.HIVA is safe and immunogenic in healthy Gambian infants. A parallel study PedVacc002 of MVA.HIVA administered to infants born to HIV-1-positive mothers is ongoing in Kenya.

**OA10.01**

**Eliciting Broadly Neutralizing Antibodies Against HIV-1 Using a Novel Antigen Containing gp41 Membrane-Proximal External Region**

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Neutralizing Antibody Responses Induced with V3-Scaffold Protein Boosts Following Priming with gp120 DNA

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Background: The V3 loop is one of the known targets for neutralizing antibody (NAb) responses, and V3-scaffold fusion proteins used as a boost after gp120 DNA priming were previously shown to induce NAb responses in rabbits. In the current study, we evaluated whether the breadth and potency of NAb responses could be further improved when boosted with rationally designed and/or double combinations of V3-scaffold immunogens after gp120 DNA priming.

Methods: Rabbits were primed with codon-optimized clade C gp120 DNA and then boosted with one of five V3-cholera toxin B fusion proteins (V3-CTBs) or with various double combinations of these V3-CTBs. The V3 inserts in these V3-CTB protein immunogens were rationally designed to present epitopes shared by the majority of global isolates. Immune sera were assessed for (a) neutralization of Tier 1 and 2 pseudoviruses (psVs) infecting TZM.bl cells, and (b) neutralization of PBMC-grown primary isolates infecting TZM-bl cells, the latter being a far less sensitive assay than the former.

Results: The V3C-CTB immunogen gave the most broad and potent neutralizing Ab response of the five V3-CTB immunogens tested individually. Generally, the double combinations of V3-CTB boosts gave better breadth and/or potency than did boosts with single V3-CTBs. With the double combination boosts, neutralization was achieved against 9/9 Tier 1 psVs from clades AG, B and C, and against 7/14 psVs from the clade B and C standard psV panels. Similarly, neutralization was achieved with 7/15 primary isolates from clades A, AG, and B.

Conclusion: The data demonstrate that priming with gp120 DNA followed by boosts with V3-scaffold immunogens effectively elicits cross-clade NAb responses.

ORAL ABSTRACTS

OA10.02 Neutralizing Antibody Responses Induced with V3-Scaffold Protein Boosts Following Priming with gp120 DNA

OA10.03 Phase I “Proof of Principle”: Immunization with Virosome-Gp41-Derived Antigen Induces Mucosal Antibodies with Antiviral Properties

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Background: The ideal prophylactic vaccine against HIV-1 sexually transmitted should prevent the entry and early infection of HIV-1 at mucosal sites. We have previously demonstrated that mucosal IgAs/IgGs induced by vaccination with influenza virosomes harboring HXB2 rgp41 and modified-P1, a lipopeptide containing the MPER and the galactosyl ceramide mucosal receptor binding motif, protect non-human primates (NHP) against vaginal heterologous SHIV challenges, in the absence of seric neutralizing antibodies. Correlation was observed between induction of HIV-1 transcytosis-blocking and ADCC activities from cervico-vaginal antibodies and protection. We have then investigated if mucosal antibodies with similar antiviral properties could be induced in women during a Phase I trial.

Methods: This is a double-blind, placebo-controlled Phase I study conducted at CEVAC (Belgium), involving 24 healthy women randomized in 2 Panels to monitor safety, tolerability and immunogenicity of the vaccine MYM-V101 (virosomes-li-popeptides P1): Panel 1: 10 ug/dose and Panel 2: 50 ug/dose. In each Panel, 8 subjects received the vaccine and 4 subjects received the placebo (virosomes without HIV-1 antigens) through intra-muscular (weeks 0/8) and intra-nasal (weeks 16/24) administrations.

Results: The vaccine MYM-V101 is considered safe and well-tolerated for both Panels, when injected intramuscularly and...
intrinsically. All vaccinated subjects have rapidly developed within two months lipopeptide specific serum antibodies. The presence of lipopeptide-P1 specific mucosal IgG and IgA antibodies in the vaginal and rectal secretions was also confirmed for the majority of the samples tested (ImperacerTM technology).

Tested vaginal antibodies from the lipopeptid-P1 vaccinated subjects exhibited strong inhibition of HIV-1 transcytosis, as compared to no or weak inhibition with the placebo, confirming previous results from NHP. However, no significant neutralizing activities could be detected in the mucosal samples. We also confirm that MYM-V101 don’t induce a Th1 response.

**Conclusion:** This study confirms the safety profile of virosomes and the promising anti-HIV-1 mucosal responses elicited by gp41-derived virosomal vaccine.

**OA10.04**

**DNA and Protein Vaccination Confers Protection Upon Mucosal Challenge with Heterologous SIVsmE660**

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**Background:** Macaques vaccinated by intramuscular electroporation with optimized plasmid DNAs expressing SIVmac239 antigens develop potent immune responses able to reduce viremia of the high dose SIVmac251 challenge. To further improve immune responses and protection, protocols combining DNA with protein immunization (comunization, prime-boost) were tested.

**Methods:** Indian rhesus macaques (N = 8/group) were vaccinated using IM-electroporation by (a) SIVmac239 DNAs only, (b) coimmunization of DNAs with protein (AT-2-inactivated particles) or (c) 2 DNA vaccinations followed by 2 protein boosts (weeks 0, 8, 16 and 36). The animals were challenged weekly with low dose heterologous SIVsmE660 7 months after the last vaccination.

**Results:** Co-delivery of SIV DNA and protein increased the magnitude, longevity, and avidity of humoral responses and the ability to cross-neutralize heterologous Env. SIV-specific cellular responses were readily measured in blood and mucosal sites after the first immunization and remained high during the entire follow-up. Vaccinated macaques resisted infection by SIVsmE660 compared to naive controls (p = 0.05; stratified for Trim5a genotype). Two of 8 DNA-protein coimmunized animals did not get infected after 14 exposures, while all controls were infected by 6 exposures. Vaccinees had significantly lower peak VL (1.7 log, p = 0.03) and 75% of vaccinees suppressed virus replication rapidly to undetectable levels, maintaining normal CD4 counts (40 weeks of follow-up). Virus acquisition correlated with antibody avidity to SIVsmE660; acquisition delay and control of viremia also correlated with the presence of vaccine-induced cellular immune responses (CD4 + EffectorMemory T-cells and IFN + GranzymeB + cytotoxic T-cells).

**Conclusion:** The vaccine combining DNA and protein in a single administration is a novel concept to achieve rapid, high, persistent, broad and effective immune responses, and captures the best properties of both procedures: high and broad range of cellular immune responses produced by DNA and strong antibody responses produced by protein immunization. This combination is able to prevent infection at the portal of entry.

**OA10.05**

**Direct Antibody Access to the HIV-1 MPER Positively Correlates with Neutralization Sensitivity**

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**Background:** The precise kinetics of neutralization by the gp41 membrane proximal external region (MPER)-directed broadly neutralizing antibodies, 2F5 and 4E10, remains unresolved. Binding data to cell-surface Env and entry data using primary isolates suggests inaccessibility of the 2F5 and 4E10 epitopes on the viral spike prior to receptor engagement, but trimer gel shift analysis and recent slow kinetic shedding data indicate otherwise. Therefore, it remains unclear if the epitopes themselves are formed (or at least sampled) prior to receptor/co-receptor engagement, or if receptor interactions both form and expose the 2F5 and 4E10 epitopes, presumably in the putative pre-fusion transitional intermediate.

**Methods:** Here, we performed antibody-virus “washout experiments” using both lab-adapted and a diverse panel of clade B primary isolates and one clade C isolate to analyze MPER accessibility.

**Results:** The neutralization activity of 2F5 and 4E10 against lab-adapted viruses and a few sensitive and moderately resistant viruses was largely unaffected by relatively rapid antibody-virus washing, suggesting direct interaction with the static spike. However, for more neutralization resistant viruses, the 2F5 and 4E10 antibodies could neutralize only in the “no antibody-virus wash” conditions, implying that the MPER epitopes were not accessible prior to receptor engagement on these isolates. In fact, accessibility in the washout conditions could be precisely predicted by the relative resistance to neutralization in a standard neutralization format.

**Conclusion:** These data are consistent with a model in which the local MPER antibody epitope conformations may be sampled on the native spike, but are occluded to antibody by local steric or distal quaternary constraints adopted by highly resistant HIV-1 isolates.

**OA10.06**

**A DNA Prime/Protein Boost Vaccine Leads to Higher B-Cell Responses than a Vector Prime/Protein Boost or DNA Prime/Vector Boost Regimens**

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**Background:** Mucosal challenge with heterologous SIVsmE660 OA10.04 gp41-derived virosomal vaccine.

This study confirms the safety profile of virosomes and the promising anti-HIV-1 mucosal responses elicited by gp41-derived virosomal vaccine.
Background: Reduced risk of HIV acquisition in RV144 vaccinees in the absence of strong T-cell mediated immunity suggests that protection may be linked to HIV-specific antibodies. We assessed memory B-cell responses to Envelope proteins in RV144 and compared these to responses elicited by other vaccines.

Methods: Memory B-cell responses were measured by ELISpot using Env proteins matched to the RV144 protein boosts (gp120_A244, gp120_MN), a consensus (gp140_CON-S) and an unrelated clade B protein (gp140_Bal). Samples from HVTN 049 (3xDNA + 2xprotein, n = 20), 055 (5xMVA, n = 14), 065 (3xMVA, n = 20 or 2xDNA + 2xMVA, n = 20), 068 (2xAD5, n = 13) and 204 (3xDNA + 1xAD5, n = 19) were compared to RV144 (2xALVAC + 2xALVAC/2xprotein, n = 40); samples obtained at baseline and 2–4 weeks after each of the two boost trials were analyzed. Responses are reported as %Env-specific IgG-producing B cells using total IgG-producing B cells as denominator. No false-positive responses were detected at baseline using 3xbackground (KLH) and >20 spot-forming cells/million as threshold for positivity.

Results: Gp140_CON-S responses were detected in 98% of participants post-boost, but magnitudes varied significantly between trials (median 0.18%–3.65% Env-specific IgG-producing B cells), with MVA-vectored trials showing low and HVTN049 the highest magnitude. RV144 responses were intermediate, being significantly lower than HVTN049 (p < 0.0001) but higher than HVTN055 (p < 0.0001). Ad5-vectorized vaccines induced responses of similar (HVTN204) or significantly higher (HVTN068, p = 0.008) magnitude than RV144. Responses in HVTN049 were stronger than in other trials (p < 0.0001 except HVTN068, p = 0.09). Response rates were lower for other proteins but magnitude patterns were similar to those for gp140_CON-S, with RV144 showing a significantly lower magnitude than HVTN049 even for clade AE gp120_A244 (p = 0.003).

Conclusion: Although strong antibody responses were observed in RV144, memory B-cell responses in this trial are not outstanding in frequency or magnitude compared to other Env-containing regimens, while the clade B DNA prime/protein boost vaccine in HVTN049 generated strong cross-clade memory B-cell responses.

OA11.02
Gut Inflammation and Indoleamine Deoxygenase Inhibit IL-17 Production and Induce Cytotoxicity in RORγt+ NKP44 + Mucosal NK Cells During SIV Infection

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Background: Lentiviruses primarily replicate in gut mucosa and cause inflammation and loss of epithelial integrity. Recent studies have identified a novel mucosal NK cell population secreting the TH17 cytokines, IL-17 and IL-22, which are critical for gut homeostasis. However, the effects of lentivirus infection on these unique NK cells are unknown.

Methods: Mucosal NK cells, from naive and SIV-infected macaques, were enumerated by a novel bead-based flow cytometry assay, analyzed phenotypically by polychromatic flow cytometry, and evaluated functionally by ICS. Expression analyses of transcription factors and cytokine mRNA in sorted NK cells and whole biopsy pieces were performed by RT-PCR.

Results: We identified two lineages of NK cells characterized by dichotomous expression of the NK cell-related markers, NKG2A and NKP44. Both cell types expressed the NK cell-related transcription factor, NFI-L3, but NKP44+ NK cells expressed the TH17 transcription factor, RORγt. Unlike systemic classical NKG2A+ NK cells, NKP44+ NK cells were mucosa-restricted, produced IL-17 and IL-22, but were noncytotoxic. During SIV infection, NKP44+ NK cells were depleted ~9-fold and had decreased IL-17 secretion, increased IFN-γ secretion, and surprisingly acquired cytotoxicity. NKP44+ NK cells showed no
evidence of direct SIV infection in vivo, but depletion and altered function of Nkp44 + NK cells were associated with SIV-induced upregulation of inflammatory mediators in the gut, including IDO-1. Furthermore, treatment of Nkp44 + NK cells with IDO-1 catalobites in vitro ablated IL-17 production.

**Conclusion:** SIV infection results in massive depletion of Nkp44 + NK cells in the gut and reshapes their functional repertoire, not by direct infection, but by indirectly altering the gut cytokine milieu. Due to the critical role of IL-17 and IL-22 in maintaining gut homeostasis, the loss of the Nkp44 + NK cell subpopulation is likely to contribute to loss of gut integrity and subsequent microbial translocation and chronic immune activation in lentivirus infections.

**OA11.03**

**Targeting the Vaginal Mucosa with Human Papillomavirus Pseudovirion Vaccines Delivering SIV DNA**


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**Background:** Most HIV transmission occurs by the genital route, suggesting that vaccines that target the genital mucosa and induce local B- and T-cell immune responses may offer better protection from infection than vaccines that induce mainly systemic responses.

**Methods:** We tested a novel vaccine delivery platform that specifically targets the vaginal mucosa. Human Papillomavirus (HPV) capsid proteins, L1 and L2, can self assemble into virus like particles (VLPs), and when co-transfected with a plasmid containing a gene of interest, L1 and L2 will encapsidate the gene and form pseudovirions (PsV). We generated HPV PsVs vaccines that deliver SIV genes to the female genital tract and designed feasibility and vaccine protection studies, using the SIVmac251 macaque model.

**Results:** In the feasibility study, an HPV PsV Gag vaccine induced SIV-specific IgG and IgA in vaginal secretions and SIV-specific T-cell responses in the cervicovaginal tract of macaques. Importantly, HPV PsV- induced mucosal T-cell responses that rapidly expand upon vaginal exposure of a subset of animals to high doses of SIVmac251. To evaluate relative vaccine efficacy, a cohort of macaques have been immunized using HPV PsVs, delivering SIV Gag-pro, and env genes, as well as a re-assorted gene encompassing the Tat, Nef, and Rev genes (Retanef). The animals were then boosted, with recombinant gp120 protein. The efficacy of a systemic prime and mucosal boost vaccine regimen is also being evaluated by combining the canarypox vector ALVAC and HPV-PsV. At the end of the immunization regimen (June 2011), these animals will undergo repeated low-dose intravaginal challenges with SIVmac251 to mimic HIV transmission among humans.

**Conclusion:** The HPV PsV system is a novel approach for direct genetic vaccination of the vaginal mucosa and is immunogenic in macaques. SIV challenge data to determine whether this combination of vaccine modalities will afford significant protection from SIVmac251 infection will be presented.

**OA11.04**

**Durability and Phenotype of SIV-Specific Mucosal T Lymphocyte Responses Elicited by Recombinant Adenovirus Vectors in Rhesus Monkeys**

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**Background:** The induction of durable and potent mucosal cellular immune responses may be critical for the development of effective vaccines against HIV-1 and other pathogens. A variety of vaccine vectors have been reported to elicit HIV-1/SIV-specific cellular immune responses at mucosal sites, but the anatomic distribution, kinetics, durability and phenotype of these responses remain poorly characterized.

**Methods:** Rhesus monkeys were immunized with intramuscular injection of (i) single, (ii) homologous prime-boost, or (iii) heterologous prime-boost regimen with recombinant adenovirus (rAd) vectors expressing SIVmac239 Gag. Magnitude and phenotype of SIV-specific T lymphocytes were evaluated with multiparameter tetramer staining assays and multiparameter intracellular cytokine staining (ICS) assays.

**Results:** Intramuscular injection of rAd vectors in rhesus monkeys induced durable SIV-specific T lymphocyte responses that persisted for over 2 years in multiple mucosal tissues, including colorectal, duodenal and vaginal biopsies as well as bronchoalveolar lavage (BAL). In peripheral blood, SIV-specific T lymphocytes underwent a phenotypic evolution from effector memory (EM) and transitional memory (TM) cells to central memory (CM) cells following vaccination. In contrast, mucosal SIV-specific T lymphocytes exhibited a persistent TM phenotype as well as persistent activation characterized by CD69 expression.

**Conclusion:** These data demonstrate that rAd vectors induce durable and widely distributed mucosal T lymphocyte responses that are phenotypically distinct from peripheral T lymphocyte responses. These findings suggest that vaccine-elicited T lymphocytes acquire qualitative features of target tissues following trafficking to mucosal sites which may allow them to respond rapidly upon mucosal viral challenge.

**OA11.05**

**Role of Genital Tract Inflammation and T Cell Activation in CD4 Depletion at the Female Genital Tract During HIV Infection**

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**Background:** The micro-environment within the female genital tract has a strong influence on both the acquisition and
transmission of HIV-1 during penetrative sex. Although immune activation in response to invading pathogens is a crucial to protective immunity, such responses may ironically also contribute to HIV pathogenesis by providing the virus with a steady supply of susceptible target cells. This study investigated the role of inflammation and immune activation in CD4 depletion and HIV shedding in the female genital tract associated with HIV pathogenesis.

Methods: Cervical cytobrush and blood-derived CD8+ and CD4+ T cells from 33 HIV+ and 40 HIV- women were investigated for expression of activation markers CCR5, HLA-DR, CD38 and Ki67 by flow cytometry. Concentrations of inflammatory and regulatory cytokines (eotaxin, G-CSF, IL-1α, IP-10, MIP-1α, IL-6, IL-10, IL-8 and RANTES) in cervical supernatants and blood plasma were determined by Luminex. HIV loads were measured in cervical secretions and plasma.

Results: The frequency of activation marker expression in blood was significantly predictive of genital mucosal T cell activation in HIV-infected women. Importantly, cervical T cell activation was broadly associated with mucosal HIV shedding and the extent of CD4 depletion in the genital tract during HIV infection. HIV-infected women displayed greater levels of T cell immune activation in the blood and cervix than uninfected women, and had significantly elevated frequencies of activation in the cervix than in blood. IL-8 and G-CSF levels were consistently greater at the cervix than blood. Genital tract concentrations of the inflammatory chemokine RANTES correlated positively with local CD38+ and HLA-DR+ T cell frequencies, with local HIV shedding, and negatively with CD4 depletion.

Conclusion: We conclude that the interplay between mucosal inflammation and local immune activation is an important contributor to CD4 depletion and the degree of HIV shedding in the female genital tract during HIV infection.
Late Breaker Oral Abstracts

**OA01.06 LB**

Extended Evaluation of Volunteers Who Become HIV-1 Infected During Participation in a Phase III Vaccine Trial of ALVAC-HIV and AIDSVAX B/E


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**Background:** The Thai Phase III trial of ALVAC-HIV and AIDSVAX B/E was the first study to show modest efficacy for prevention of HIV-1 infection in a community-based cohort. Of the 16,402 volunteers enrolled in the study, 132 became HIV-infected during the trial and 120 of these volunteers agreed to participate in RV152 to evaluate the effect of this prime-boost regimen on clinical disease progression as well as immunologic and virologic outcomes. The primary objective of the study was to evaluate differences among vaccine and placebo recipients in a composite endpoint comprised of clinical (AIDS-defining events, initiation of antiretroviral therapy) and laboratory (CD4-count < 350 cells/μl) events.

**Methods:** Volunteers who became HIV infected in RV 144 were followed every 3 months with clinical and CD4 and HIV-1 viral RNA assessments. Both study volunteers and investigators remained blinded over the entire study period. Volunteers received HIV care and treatment according to the Thai Ministry of Public Health National Guidelines. Both modified Intent-to-Treat (mITT) and per-protocol (PP) analyses were performed using a time-to-event model based on a Cox proportional hazards. Secondary analyses of biomarker-based endpoints were assessed using marginal mean models fit by Generalized Estimating Equations and linear mixed models.

**Results:** There were 48 PP and 61 mITT events among the 120 enrolled volunteers at the pre-specified, event-driven stopping point.

**Conclusion:** Results of the study will be presented and discussed to include estimates of vaccine efficacy for disease progression as well as longitudinal comparison of biomarkers.

**OA02.06 LB**

JAK3 Inhibition In Vivo in Chronically SIV Infected Rhesus Macaques Deplete NK-17 Cells

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**Background:** During simian immunodeficiency virus (SIV) infection, the gut-associated lymphoid tissue (GALT) is the primary target of virus replication during acute infection. While NK cells and IL-17 producing NK cells (NK-17) have been shown to play a key role in not only maintaining epithelial cell integrity of the GALT but also causing cytotoxicity for SIV infected cells. Their role during chronic infection is not clear. The purpose of this study was to determine the effect of NK cell depletion using a JAK3 inhibitor in chronically SIV infected rhesus macaques.

**Methods:** Six chronic SIV-infected rhesus macaques were daily orally administered a pre-determined optimal dose of 10 mg/kg of a JAK3 inhibitor for 5 weeks. Blood and colorectal biopsies were collected. Cells were stimulated with various fluorescent conjugated monoclonal antibodies and analyzed on LSRII flow cytometer. The plasma viral loads were also monitored.

**Results:** Total NK cell and NK-17 in blood and biopsies were markedly depleted as early as the first week and the depletion persisted during the course of drug administration. While the total NK cells in blood returned to normal levels within 3 weeks after treatment cessation, the total NK cells in biopsies did not. The NK-17 cells in the blood and biopsies also did not return to normal levels after cessation. Drug treatment led to a transient increase in plasma viremia during the loss of total NK cells.

**Conclusion:** The administration of a JAK3 inhibitor to chronically SIV infected monkeys depletes total and NK-17 in both the blood and mucosal tissue and is associated with increases in viremia denoting an important role for NK cells during chronic SIV infection.

**OA03.05 LB**

Role of Hypervariable Loops in Intersubunit Associations of Env: Trimeric Gp140 Immunogen as a Platform for Novel Epitope Display


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**Background:** The HIV-1 Env complex mediates membrane fusion with host cells following interaction with both a receptor and
a co-receptor. The Env subunit gp120 binds to CD4 receptors, resulting in cryptic epitope exposure and allowing co-receptor binding (see our recent structure of Env gp140 trimer, Moscoso et al., 2011, PNAS 108(15):6091–6096). Some hypervariable loops on gp120, primarily V2 and V3, seem to play roles in the conformational transition to the induced, exposed co-receptor state.

**Methods:** Here, we perform further comparative analysis of variants of the soluble Env construct gp140, with and without a partial V2 truncation, using cryo-electron microscopy and single particle reconstruction.

**Results:** The Env structure displays a similar overall architecture with crucial detailed distinctions, one of them being a tighter association between gp120 subunits that results in a smaller trimer radius. Another pertinent feature is the presence of V2 near the base of the trimer proximal to the viral membrane, as suggested previously, placing V2 in proximity to the adjacent counterclockwise gp120 subunit. The juxtaposition of V2 and the neighboring gp120 subunit’s V3 loop (V3’) contribute to a quaternary epitope formed by these two loops, which is the binding site for antibodies such as PG16 and 2909. Such a quaternary location of V2 would facilitate gp41-independent gp120-gp120 interactions, and hints at a mechanism of cooperative epitope occlusion mediated by hypervariable loop interaction, a decrease in trimer diameter and increased steric hindrance near the CD4 binding sites due to the decreased distance between adjoining CD4 epitopes.

**Conclusion:** Comparative structural analysis of these recombinant vaccine candidates informs rational design of strategies to target the formation of epitopes for later broadly cross-neutralizing antibodies, such as VRC01.

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**AO04.07 LB**

**Evolution of HIV-1 Transmitted/Founder Viruses Results in the Formation of Epitopes for Later Broadly Cross-Neutralizing Antibodies**


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**Background:** Much emphasis has been placed on mapping the epitopes of broadly cross-neutralizing (BCN) antibodies. However, the interplay between strain-specific antibodies, BCN antibodies and autologous viral evolution has not been well characterized.

**Methods:** Single genome amplification was used to obtain longitudinal single-antigen envelope sequences from three individuals (CAP177, CAP8 and CAP256) in the CAPRISA 002 cohort who developed BCN antibodies with known specificities (Gray et al, JV, 2011 and Moore et al, JV, 2011). The sequences were then analysed to determine whether previously defined BCN epitopes were present on the transmitted/founder viruses, and to characterize their evolution over time.

**Results:** CAP177 developed BCN antibodies dependent on a glycan at position 332 in the C3 region. This N332 glycan was however not present on the CAP177 transmitted/founder virus, but arose by 6 months post-infection as a result of escape from an early strain-specific antibody targeting the alpha-2 helix of the C3 region. Similarly in CAP8, who developed BCN antibodies dependent on the N160 glycan, the transmitted founder virus did not encode the N160 glycan, but instead contained a K160 residue. The N160 glycan evolved in CAP8 by 6 months of infection and became fixed. In a third individual, CAP256, who also developed extremely potent N160-dependent BCN antibodies, the transmitted/founder virus lacked this residue. However, superinfection at 15 weeks post-infection with a second virus that contained the N160 glycan resulted in the generation of recombinant viruses that harbored the N160 glycan forming the CAP256 BCN epitope.

**Conclusion:** In three individuals who developed BCN antibodies, the BCN epitope was not present on the transmitted/founder virus, but evolved within 6 months of infection. In one case, the BCN epitope evolved via viral escape from an earlier strain-specific antibody targeting the same region. These studies highlight the role of viral evolution in shaping the development of BCN antibody responses.

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**AO07.08 LB**

**The Thai Phase IIi Clinical Trial (RV144) Induces the Generation of Antibodies That Target a Conserved Region Within the V2 Loop of GP120**


1AFRIMS, APO, AP, USA; 2U.S. Military HIV Research Program, Rockville, MD, USA

**Background:** The Thai phase III clinical trial (RV 144) is the only HIV-1 vaccine regimen to show efficacy against acquisition of HIV-1. Antibodies targeting HIV-1 ENV may contribute to preventing infection. We compared the HIV-1 humoral responses induced by the vaccine to those by natural HIV-1 infections.

**Methods:** Antibody responses to gp120 HIV-1 were evaluated using cyclic peptides spanning 42 amino acids (aa) of the V2 and 35 aa of the V3 loop of the ALVAC (vCP1521) Env gp120. Plasma from 40 HIV-1 uninfected trial participants (32 vaccinee/8 placebo recipients) that completed all injections with ALVAC-HIV/AIDSVAX B/E boost combination and 20 HIV-1 CRF01_AE infected subjects were tested by ELISA.

**Results:** 31/32 (97%) vaccine recipients had antibody responses against V2 at 2 weeks post final immunization (week 26) with a geometric mean end point titer (GMT) of 1100 (range: 200–3200). V2 responses were transient, declining to 19% at 28 weeks post last injection (GMT: 110, range: 100–200). The majority of antibody responses targeted the crown of the V2 loop with aa sequence KQKVHALFYKLDIVPI which includes the a4b7 binding motif LDI. These observations suggest that V2 antibodies may be a contributing factor in the prevention of HIV-1 acquisition.
Reduction of Founder Virus in Vaccinated Monkeys After Mucosal SIV Challenge


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Background: A key criteria in the NHP evaluation of candidate vaccines is determining the presence of any vaccine-induced effects on the genotype or number of founder strains acquired during repeated low-dose mucosal challenges. We have undertaken studies in a cohort of 167 monkeys to determine the presence of any vaccine-induced “sieving” effects, or differences in minimum number of SIV founders initiating infection in vaccinated or sham-control animals.

Methods: Methods: 167 rhesus monkeys received either an experimental DNA prime Ad5 boost regimen, or sham vaccination. All monkeys were challenged intra-rectally with a repeat low-dose SIV660 or SIVmac251 challenge 40 weeks after the last vaccination. We amplified the envelopes of blood-associated SIVE660 at the earliest point after viral infection using single genome amplification. Full-length SIV envelope sequences were subject to extensive bio-informatic and statistical analysis to evaluate the minimum number of virus acquired during the infection of each monkey, and if vaccine-induced sieving effects were present.

Results: Results: Overall, evidence of vaccine-induced sieving was observed in virus sequences derived prior to the peak of virus replication. However, this sieving was most pronounced in monkeys expressing the Mamu A*01 allele. We also observed a significant reduction in the minimum number of founder virus initiating infection in vaccinated monkeys with permissible host genotypes. We also observed altered levels of mutation in virus sequences isolated from vaccine recipients.

Conclusion: Conclusions: Evidence of vaccine-induced effects can be observed at the earliest times after virus challenge and can impact viral diversity post acquisition. Importantly, vaccination impacts the number of acquired viral lineages, but significant levels of specific immunological “sieving” was observed only in a subgroup of vaccinees. These data highlight non-specific immune mechanisms of vaccine-interrupted virus acquisition. Moreover, the inclusion of a quantitative endpoint for founder/transmitted virus should be considered for future evaluations of candidate vaccine efficacy.

SIV Attenuated Vaccine-Induced Plasma Cells and Antibodies Limit Infection at the Portal of Entry and Systemically Following Vaginal Challenge

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Background: In the SIV rhesus macaque model of HIV-1 transmission to women, vaccination with SIVmac239-delta-nef confers partial or complete protection against vaginal challenge with SIVmac251 by as yet unknown mechanisms.

Methods: To identify potential protective mechanisms against vaginal transmission, we analyzed host responses in cervical-vaginal and lymphatic tissues (LTs) of Indian rhesus macaques following intravenous SIV delta-nef infection (vaccine phase), and following vaginal challenge with SIVmac251 (Challenge phase).

Results: We found that vaccine induces a significant increase in SIV-specific antibodies and plasma cells and IgG localized beneath the cervical-vaginal epithelium and the development of ectopic lymphoid tissue follicles within cervical-vaginal tissues. Within the first week after heterologous vaginal challenge of SIVmac251, there is a rapid increase in the number of IgG producing plasma cells, concentration of anti-Env antibody and the breadth of antibody in vaginal-cervical mucosal sites in the vaccinated rhesus macaques, which correlates with the local expansion and maturation of ectopic lymphoid follicles within vaginal tissues in which there are plasmablasts and/or memory B cells with evidence of AID-mediated class-switch recombination. The high concentrations of antibodies and plasma cells correlate with as much as a 6-order of magnitude reduction of tissue viral load at the portal of entry. There is a similar rapid increase in plasma cells in the lymphoid tissues that correlates with a rapid response to disseminated infection that is also associated with major reductions in viral replication at these sites.

Conclusion: These data suggest a mechanism by which a humoral response at the “right place, right time” may contribute to the protection, a concept relevant to developing effective vaccines against HIV-1 and other mucosal pathogens.
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P01.01
Analysis of Epitope-Specific HIV T Cell Responses During Early HIV-1 Infection and Their Association with Viral Control


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Background: HIV-1-specific cytotoxic T lymphocytes (CTL) are important in the control of viremia; however, the precise qualities of effective epitope-specific T cell responses that may be responsible for control remain unclear. Here we investigated the frequency and specificities of T cell responses during early HIV-1 infection, to address the question of whether conservation score (CS) of targeted epitopes play an important role in viral control.

Methods: Using IFN-γ ELISpot assays, we mapped epitope specificities recognized by HIV-specific T cells in 12 ART-naive individuals during early infection (within 6 months). We then identified CS of targeted epitopes, where the CS is defined as the proportion of HIV-1 group M amino acid sequences in the LANL database that include the epitope. We further evaluated the association between the CS of targeted epitopes and the individuals’ viral-load.

Results: Our epitope mapping of T cell responses showed that the median number of epitopes targeted was 9 (range, 5–24) mostly against Gag, Nef, Env and Pol. More than one third of these epitopes were novel epitopes. In addition, variable epitopes were targeted more frequently than conserved epitopes (p < 0.0001). The median CS of targeted Gag epitopes were more conserved than those of Pol, Nef, Env and Acc epitopes (p = 0.003). However, the magnitude of responses elicited by Gag epitopes was not significantly different than those elicited by Pol, Nef, Env and Acc epitopes. Furthermore, there was a significant inverse correlation between the rank of CS of targeted epitopes with the rank of viral-load (r = −0.76, p = 0.004), indicating that individuals that possess T cells targeting conserved epitopes early in infection had lower viral loads.

Conclusion: These data suggest that HIV-specific CTL targeting conserved epitopes of HIV-1 are superior at controlling viral replication in vivo, supporting the rationale for designing vaccines containing conserved immunogens.

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P01.02
Balance of CTL Escape and Viral Fitness in Acute and Early HIV Infection

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Background: Investigations into host-virus interactions during acute HIV-1 infection are important for the design of a preventative HIV-1 vaccine. To understand the dynamic interplay between host CTL responses and the mechanisms by which HIV-1 evades those responses, we studied viral evolutionary patterns and host CTL responses in a linked transmitter:recipient pair.

Methods: Using IFN-γ ELISpot assays, we mapped epitope specificities recognized by HIV-specific T cells in 12 ART-naive individuals during early infection (within 6 months). We then identified CS of targeted epitopes, where the CS is defined as the proportion of HIV-1 group M amino acid sequences in the LANL database that include the epitope. We further evaluated the association between the CS of targeted epitopes and the individuals’ viral-load.

Results: Our epitope mapping of T cell responses showed that the median number of epitopes targeted was 9 (range, 5–24) mostly against Gag, Nef, Env and Pol. More than one third of these epitopes were novel epitopes. In addition, variable epitopes were targeted more frequently than conserved epitopes (p < 0.0001). The median CS of targeted Gag epitopes were more conserved than those of Pol, Nef, Env and Acc epitopes (p = 0.003). However, the magnitude of responses elicited by Gag epitopes was not significantly different than those elicited by Pol, Nef, Env and Acc epitopes. Furthermore, there was a significant inverse correlation between the rank of CS of targeted epitopes with the rank of viral-load (r = −0.76, p = 0.004), indicating that individuals that possess T cells targeting conserved epitopes early in infection had lower viral loads.

Conclusion: These data suggest that HIV-specific CTL targeting conserved epitopes of HIV-1 are superior at controlling viral replication in vivo, supporting the rationale for designing vaccines containing conserved immunogens.

This project is funded by NIH grant #R01AI090783.
the functionally constrained regions of the Gag-Pol frameshift stimulator loop and in the Env gp41 C-terminal Heptad Repeat. The virus appears to have exploited the limited availability of AA sites not required to maintain function in these regions in order to attain CTL escape.

Conclusion: Focusing immunological studies on viral regions under selection pressure allows for detailed investigations of host-virus interactions using limited numbers of PBMC. Comparison of viruses between the transmitter and recipient provides a better characterization of founder viruses’ specificities. Identification of escape mechanisms allows further understanding of the challenges faced by the virus in achieving a balance of immunological escape and replicative fitness costs.

P01.03
Abnormal Liver Function During Acute HIV Infection Correlates with Fiebig Stage and Systemic Inflammation

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Background: Abnormal liver function may present during acute HIV infection (AHI). Its pathogenesis is unclear but may be linked to systemic inflammation.

Methods: 30 Thai adults with Fiebig stages I to IV AHI were assessed at baseline for ALT, yGT, bilirubin, IP-10, MCP-1, TNF-α, IL-6, II-12, hyaluronic acid (n = 19), sCD14 (n = 7). The % CD4+CCR5+ cells in sigmoid tissue was determined by flow cytometry (n = 24).

Results: Twenty-six (87%) were male; the median (SD) age was 29 (6.2) years; they were 8 Fiebig I; 5 Fiebig II; 14 Fiebig III and 3 Fiebig IV. Mean (SD) time from HIV exposure was 18 (9.1) days. Median (IQR) CD4 was 406 cells/mm3 (298–555) and HIV RNA was 5.5 (5.1-6.4) log10 copies/ml. Twenty-six (87%) had ARS. Most common symptoms were fever (77%), myalgia (60%), fatigue (57%), and headache, pharyngitis, and oral ulcers (all 50%). Three had positive HBsAg and none had positive HCV Ab. Sixteen (53%) had abnormal ALT (>31 UL-1) and 10 (33%) had abnormal yGT (>43 UL-1); 2 of these had positive HBsAg (ALT 45 and yGT 49 respectively). Absolute ALT and yGT positively correlated with Fiebig stage [rho = 0.689 (p < 0.001) and rho = 0.518 (p = 0.003) respectively]. Abnormal ALT was more common in Fiebig III/IV compared to Fiebig I/II (OR = 13.2, p = 0.009). Both absolute and abnormal yGT correlated with TNF-α expression (both rho = 0.406, p = 0.026). Absolute ALT was positively correlated with IP-10 (rho = 0.403, p = 0.027), while abnormal ALT was correlated with IL-12 (rho = 0.375, p = 0.041). No relationship between ALT/yGT with gut CD4+CCR5+ T cells or CRP, D-dimer, HA and sCD14 was seen.

Conclusion: ARS was common in Thais with AHI. Abnormal liver function was prominent and correlated with Fiebig stage and inflammatory cytokines levels, but not with gut T cell depletion. The clinical and prognostic implications of these findings require further study.

P01.04
Increased Plasma Levels of Hemoxygenase-1 (HO-1) and Presence of HO-1-Specific Regulatory T Cells in Acute HIV-1 Infection

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Background: Heme-oxygenase-1 (HO-1) the rate-limiting enzyme in heme catabolism is an inducible stress-response protein with potent anti-inflammatory activity. Recent studies in cancer patients described a novel cell type of HO-1 specific regulatory CD8+ T cells with suppressive capacity superior to conventional Tregs. The role of these cells in HIV-1 infection has not been explored to date; however, if detectable they could represent potent regulators of HIV-1 immunity and hold potential as a novel target for future immuno-therapeutic or vaccine interventions.

Methods: Hemeoxygenase-1 protein levels were measured in plasma from 40 individuals with untreated primary HIV-1 infection, 39 elite controllers, 39 chronic progressors and 15 healthy controls. The frequency of HO-1-specific CD8+ T cells was measured by flow cytometry using HLA class I pentamers in PBMC from individuals with untreated primary HIV-1 infection, elite controllers, chronic progressors and healthy controls.

Results: The mean plasma concentration of heme-oxygenase-1 was 1.7ng/ml in healthy control subjects. HO-1 plasma levels were significantly increased in individuals with acute HIV-1 infection (mean = 3.3ng/ml) compared to HIV uninfected control subjects. HO-1 plasma levels did not correlate with CD4 count or viral load. HO-1-specific CD8+ T cells were detectable in HIV-1 infected individuals with the highest levels observed during primary HIV-1 infection. Preliminary data showed evidence for suppressive activity of supernatant from cultured sorted HO-1-specific CD8+ Tregs in HIV-1-specific cytotoxicity assays.

Conclusion: Our data demonstrate elevated levels of heme-oxygenase-1 in individuals with acute HIV-1 infection suggesting that HO-1 is induced during this phase and likely mediates anti-inflammatory effects. Moreover, the presence of the HO-1 antigen during acute infection appears to drive the elevated frequency of HO-1-specific CD8+ regulatory T cells in acutely infected individuals. The investigation of this novel cell type may provide further insight into HIV-1 T cell regulation via non-virus specific immune mechanisms and hold potential for future immuno-therapeutic intervention.

P01.05
Early Cytokine and Chemokine Patterns During Acute HIV Infection

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Background: Understanding the earliest immunologic events during acute HIV infection is critical to inform prevention and treatment efforts. We focused on characterizing plasma cytokine profiles during the earliest stages of acute infections from subjects
in the prospective RV217 cohort representing multiple HIV subtype infections (A, C, D and CRF-01).

**Methods**: Twice weekly nucleic acid testing (NAT) was used to prospectively identify subjects during very early HIV infection (NAT+, antibody-). Plasma cytokine levels were measured at baseline and at all available early time points throughout acute infection and early set point. Assays were performed in triplicate using the Quansys Q-Plex Multiplex IR ELISA Array. Images were captured using the LiCor IR imaging system and analyzed using Q-View Plus software. Data and statistical analysis were performed using GraphPad Prism.

**Results**: Longitudinal plasma samples acquired prior to EIA reactivity during the upslope in viral load from acutely infected individuals (n = 6) showed similar cytokine profiles. Plasma levels of the CC-chemokine MCP-1/CCL2 (6/6) and CX-chemokine IP-10/CXCL10 (4/4) rose sharply during viral load ramp up. Conversely, a concomitant nadir in plasma IL-2 (5/6), often with a decrease in IL-17 (4/6) was observed. There were profound decreases in both peripheral absolute CD4 counts and B cells during these early days of viral expansion. Other cytokines were detected (IL-1β, IL-6, IL-10, IL-12p70, IL-15 and IFN-γ) but showed more variability in expression patterns during early phases of infection.

**Conclusion**: Few studies have examined cytokine expression patterns prior to and during the acute phase of HIV infection. The early detection of MCP-1/CCL2 and IP-10/CXCL10 observed here, similar to a prior study in acute subtype B infections, may indicate early trafficking events and reflect involvement of specific cell types during acute infection. Falling levels of IL-2 and IL-17 could be linked to changes in frequency or redistribution of peripheral lymphocyte subsets.

P01.06  Whole Blood Transcriptional Monitoring of Acute HIV-1 Infection Reveals Differential Signatures of Host Immune Activation


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**Background**: Genome-wide transcriptional studies in non-human primates have suggested that the quality and magnitude of early immune responses to SIV infection are associated with downstream disease progression. The aim of this study is to identify, monitor, and interpret the whole blood transcriptional signature of acute HIV-1 infection (AHI).

**Methods**: A total of 56 AHI patients and matching healthy controls from both Africa and the United States were distributed into training, test, and validation datasets. Whole blood from these subjects was obtained for transcriptional profiling using microarrays. Both gene and modular transcriptional framework analyses were utilized to interpret the signature of AHI and for comparisons with other disease signatures including: influenza, tuberculosis, sepsis, and chronic HIV following interruption of anti-retroviral therapy (ART). Additional samples were collected at 1, 2, 4, 12, and 24 weeks post-enrollment for training and test set AHI subjects, to establish the longevity of the signature in the presence or absence therapy.

**Results**: We identified a robust AHI signature with increased activity in: interferon, cell cycle, cytotoxic, plasma cell, and B-cell modules amongst others. This activity was unique when compared to signatures obtained from patients with other infections. Only interferon and cell cycle activity was observed in both AHI subjects and chronically infected patients following interruption of therapy. Notably, 20% of AHI patients were observed to express a quiescent signature that clustered independent of the highly active signature described above. We found that these patients had significantly lower viral set-points (p = 0.01) when compared to patients with the active signature. Finally, longitudinal analysis demonstrated that the active AHI signature can persist independent of viral load in ART treated patients and up to 6 months in the absence of therapy.

**Conclusion**: Transcriptional monitoring of early HIV infection offers both quantitative and qualitative assessments patient responses that may be predictive of long-term disease progression.

P01.07  Risk Behavior and Demographic Characteristics of Infected vs Non-Infected Participants in RV217d, a Study in At-Risk Populations in Pattaya, Thailand

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**Background**: RV217d is a study of early capture HIV infection in most at risk populations in Pattaya, Thailand through repeat twice weekly testing.

**Methods**: Female sex workers (FSW), MSM, and transgenders (TG) were given a baseline risk assessment. Those at risk were enrolled and followed for two years with twice weekly HIV testing.

**Results**: A total of 381 participants took the baseline questionnaire. The majority reported working as bar, massage or sex workers (94%) or having received money in exchange for sex (85%). They are well educated, with average age of 27 years and 39% have never married. At baseline their recent risk for HIV is high, with unprotected sex with a known HIV positive partner, 15%; unprotected sex with greater than or equal to three partners in the last three months, 43%; recent STI symptoms, 60% and inconsistent or no condom use with male clients, 56%.

Sixteen participants have become infected to date, for an incidence rate of 8.59/100 py in MSM, 6.81 in TGs, and 2.10 in FSWs. Incident cases are younger, less educated, and earn half the income of the uninfected group.

The groups were similar across the behavioral responses except 77% of infected MSM and TG participants had sex
exclusively with males or transgenders versus 59% of the uninfected.

**Conclusion:** In this most at risk cohort, a lack of a characteristic behavioral profile distinguishing infected participants may indicate that all are at risk; however, low-income, younger MSM and TGs would be an appropriate group to target.

**P01.08**
Integrated Analysis of Immunogenicity, Viral Sequence, and Viral Load Data in the Step Study

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**Background:** The sieve analysis for the Step Study (Roland et al. 2011) showed that breakthrough HIV-1 sequences were more divergent from the vaccine insert in vaccine than placebo recipients, and identified 10 “signature sites” where the rate of amino acid (AA) mismatch to the insert residue differed between treatment groups. We linked Step viral sequences with immunogenicity and acute viral load data to evaluate mechanisms for, and consequences of, the sieve effect.

**Methods:** Analyses included 91 male subjects diagnosed with HIV infection prior to study unblinding (37 placebo, 54 vaccine recipients). Viral sequences from the first PCR-positive visit were summarized using global measures of genetic distance and the 10 AA signature sites identified by Rolland et al. Pre-infection T-cell responses were mapped to individual 15-mers 4 weeks post second vaccination using interferon-gamma (IFN-\(\gamma\)) ELISpot. Acute viral load, obtained at RNA-positive but antibody-negative visits, was available for 27 subjects and multiply imputed for the remainder.

**Results:** There was some evidence of reduced acute viremia in vaccines versus placebos (4.7 vs 5.1 log RNA copies/ml), although this difference was not significant (p = 0.27) and not predicted by pre-infection T-cell responses. The locations of the epitopes targeted pre-infection did not match the signature sites. Magnitude and breadth of the pre-infection responses were not associated with global genetic distances. Vaccine and placebo recipients did not differ in their associations between global genetic distance and acute viral load; however, mutations at five Gag AA sites were associated with acute viral load in vaccinees.

**Conclusion:** The Step vaccine not only impacted founder virus populations, but potentially reduced acute viral load. However, current measures of T-cell function did not predict these effects. Sequence differences between treatment groups were not associated with viral load, although we did identify several mutations in Gag associated with lower/higher viral load in vaccinees alone.

**P02.01**
Specific IgA Plasma Cell Recruitment to the Female Genital Tract Following Intravaginal Administration of CCL28 or MPLA

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**Background:** Priming of immune response by the NALT is known to induce responses in the female genital tract and the rectum. This is in line with the concept of a common mucosal immune system whereby a fraction of B cells primed at one mucosal site can re-circulate through the systemic compartment and repopulate distant mucosal compartments. CCL28 is thought to be central to recruitment of IgA plasma cells to the genital tract and colorectum. In addition, inflammation, such as that induced by TLR activation, is also associated with plasma cell recruitment. In this study we assess whether vaginal application of CCL28 or TLR4 ligand MPLA, enhanced immune response following intranasal or parenteral immunization.

**Methods:** Animal (n = 8 mice/group) received three intranasal (IN) or subcutaneous (SC) immunizations with gp140 (10ug) plus MPLA (20ug). Six days following each immunization CCL28 (10ug), MPLA (20ug) or PBS, was applied to the vagina. Blood and vaginal samples were taken to detect specific IgG and IgA responses. Vaginal and spleen cells were isolated to perform B and T cell assays.

**Results:** IN immunization induced systemic and vaginal specific IgG and IgA responses. In contrast SC immunization induced lower vaginal specific IgG responses and no vaginal IgA. Intravaginal administration of either CCL28 or MPLA six days post IN immunization significantly enhanced vaginal antibody responses, MPLA providing greater enhancement. Intravaginal MPLA administration also enhanced systemic IgA levels and produced detectable vaginal cellular responses. The impact of CCL28 or MPLA on SC immunization was minimal.

**Conclusion:** This study shows that specific B cell responses following intranasal immunization can be enhanced by local application of MPLA or CCL28 in the vaginal six days post immunization. Exploitation of the mechanisms leading to enhanced responses at mucosal surfaces could be an important component in the design of protective vaccines.

**P02.02**
Vaccine Antigen Immunity Is Enhanced by Optimized TLR4 and TLR7/8 Adjuvant Combinations in Mini-Pigs Inoculated via a Systemic but Not a Mucosal Route

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**Background:** Previous studies have demonstrated synergy between TLR4 and TLR7/8 stimulation with enhanced CD4 T cell cytokine production and cooperative upregulation. We performed a series of experiments to analyse the inter-relationship of these two innate receptors in mini-pigs, animals that are fully responsive to these TLR ligands and that are immunologically similar to man.

**Methods:** Groups of 4 pigs were vaccinated twice at monthly intervals with protein antigens adjuvanted with various concentrations of either aqueous formulation GLA (synthetic monophosphoryl lipid A - TLR4 ligand) or R848 (resiquimod - TLR7/8 ligand) via the intranasal (IN - 50ug antigen) or intradermal (ID - 20ug antigen) route. Pigs were sampled weekly (serum and vaginal wash) and antigen-specific IgG and IgA quantified by ELISA.

**Results:** R848 augmented antigen-specific responses when administered via the IN or ID route, while an adjuvant effect was only observed with GLA used ID. Combinations of optimal amounts of each TLR ligand had an additive enhancing effect when used ID but surprisingly GLA decreased R848-induced
immunity after IN inoculation. Interestingly, R848 significantly enhanced mucosal antigen-specific IgA after IN inoculation but only within a narrow concentration range, as high dose R848 inhibited mucosal IgA.

Conclusion: These data begin to address important issues relating to adjuvanted combinations and route of administration. TLR4 and TLR7/8 agonists combined to additively enhance antigen-driven immune responses but only after ID vaccination. The inhibitory effect of GLA on R848-driven responses suggests an active contribution by GLA rather than a simple formulation interaction. In vitro, we stimulated bone marrow-derived dendritic cells by measuring expression of surface molecules. ELISA detected cellular and humoral immunological response, ELISPOT, env-specific ELISA, IgG isotype ELISA and avidity spot-forming units/million over no adjuvant: 9.8 and 5.8 for gDNA alone dose (fold increase in mean = 1.9). The magnitude of response did not correlate with circulating NKT cell level, indicating that this may be useful across broad populations. Initial decreases in circulating NKT cell levels on Day 1 normalized rapidly by Day 2. 7DW8-5 activated circulating plasmacytoid dendritic cells earlier than macaques who received vaccine alone, measured by CD40, CD80, and CD86 expression.

Conclusion: 7DW8-5 provides a significant adjuvant effect on the cellular immunogenicity of an adenoviral vaccine in non-human primates, and is advancing into a Phase 1 clinical trial in healthy volunteers.

P02.04
A Novel Glycolipid Adjuvant Significantly Boosts Cellular Immune Responses to an Adenoviral Vaccine in Non Human Primates

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Background: The majority of adjuvants in development to date boost immune responses to protein antigens. Identification of effective adjuvants for viral vectors remains critical to improving vaccination regimens that utilize viral vectors. We have identified a novel glycolipid compound, 7DW8-5, which binds the CD1d receptor with higher affinity than its parent compound, z-galactosyl ceramide (zGalCer), enabling NK T cell activation and subsequent recruitment and activation of dendritic cells and other inflammatory mediators. We sought to determine whether 7DW8-5 would provide an adjuvant effect to an adenoviral vaccine in rhesus macaques, and to determine the optimal dose for subsequent clinical development.

Methods: Five groups of rhesus macaques (n = 5 per group) received a single intramuscular vaccination with 2x1010pu NMRC-M3V-Ad-PICA (AdPICA), a 1:1 mixture of Ad5-based vectors expressing the CSP and AMA-1 antigens from Plasmodium falciparum, respectively, with increasing concentrations of 7DW8-5 in each group: no adjuvant, 0.1µg, 1µg, 10µg, and 100µg. Macaques were monitored for safety, innate immune responses and immunogenicity for four weeks.

Results: 7DW8-5 elicited dose-dependent local erythema at injection sites, but no evidence of systemic reactivity (fever, tachycardia, respiratory distress). 7DW8-5 provided a significant increase in IFNγ ELISPOT responses to both antigens, most significant at the 10µg dose (fold increase in mean spot-forming units/million over no adjuvant: 9.8 and 5.8 for CSP and AMA-1, respectively, p = 0.04 for both). The magnitude of response did not correlate with circulating NKT cell level, indicating that this may be useful across broad populations. Initial decreases in circulating NKT cell levels on Day 1 normalized rapidly by Day 2. 7DW8-5 activated circulating plasmacytoid dendritic cells earlier than macaques who received vaccine alone, measured by CD40, CD80, and CD86 expression.

Conclusion: 7DW8-5 provides a significant adjuvant effect on the cellular immunogenicity of an adenoviral vaccine in non-human primates, and is advancing into a Phase 1 clinical trial in healthy volunteers.
Background: The use of TLR ligands as mucosal adjuvant for vaccine administration is already largely described, but the use of NOD ligands is still investigated. As NOD ligands are able to induce production of pro-inflammatory proteins and chemokines, we have evaluated if their co-delivery with biodegradable nanocarriers carrying HIV Gag antigens amplify the mucosal immune responses in mice.

Methods: We used Poly(Lactic Acid) (PLA) nanoparticles (200 nm) as vaccine vehicle for delivery of HIV-1 Gag p24. To assess their immunogenicity and the adjuvant effect of NOD agonists (NOD1 and NOD2), we have compared two routes of immunization, enteric and subcutaneous, in B6D2 mice with co-administration of NOD ligands. For each group, cellular and humoral responses have been analyzed on splenocytes and in vaginal washes, faeces and sera.

Results: We first confirmed that PLA nanoparticles are efficient protein carriers of HIV p24 without alteration of the colloidal stability of the formulation. By analyzing humoral immune responses, we noticed that co-administration of p24-PLA NPs and NOD ligands through subcutaneous route was very efficient to induce higher anti-p24 IgG responses in rectal, vaginal washes and sera, irrespective of the sub classes. However, p24-specific IgA responses at the rectal site were only found after repeated enteric vaccination with adjuvant formulations.

Upon subcutaneous injection in mice, we could observe that p24-PLA NPs co-administered with NOD1 ligand induced a significant decrease of the IgG1/IgG2a ratio compared to non-adjuvanted formulation meaning a Th1 orientation.

Conclusion: When PLA are co-administered with NOD1 or NOD2 ligands, the highly antigenic outer capsid protein (Hoc), a nonessential protein antigen and MPLA as an adjuvant.

COD-Hoc-T4 constructs as such or bound to liposomes containing both GluCer and MPLA. The specificity of the spontaneously bound T4-liposomal formulation was determined by Biacore. Humoral responses in mice and rabbits and cellular immune responses in mice were analyzed.

Results: The display of Ct-Hoc antigen on T4 capsids was confirmed by electron microscopy. Binding of phage T4 did not occur to glycolipids, such as galactosyl ceramide, containing an aldose in which the C-2 or C-4 conformations were not identical to glucose. These results strongly support previous reports that glucose is a major receptor moiety for T4 binding to Escherichia coli. High titer antigen-specific IgG antibodies were induced in the immunized mice and rabbits. Neutralizing antibodies were analyzed in the PBMC, TZM-BL, and a macrophage neutralization assay. Cellular responses specific to both Ct and COD antigens were also induced in mice.

Conclusion: Liposomes containing both GluCer and MPLA can spontaneously bind to T4 displaying HIV-1 gp41 antigens. This formulation could be utilized as an easily manufactured self-assembling antigen and adjuvant carrier.

P02.07
Antigen-Specific Enhancement of Natural Human IgG Antibodies to Lipids Induced by a Liposomal Vaccine Containing Lipid A and a Protein Antigen

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Background: Monoclonal antibodies 2F5 and 4E10 not only bind to the protein epitope, but also to lipids. Multispecific antibodies with similar binding specificities to 2F5 and 4E10 and antibodies to lipids can readily be induced in mice by immunization with liposomes containing monophosphoryl lipid A (MPLA). However, it has never been demonstrated that antibodies with lipid binding specificity can be induced by vaccination of humans. This study was conducted to determine if antibodies to lipids can be induced or boosted in titer by vaccination of humans.

Methods: ELISA for IgG to lipids was conducted on stored sera obtained from volunteers who received a candidate vaccine to Plasmodium falciparum (PNAS 1992; 89:358–362). The vaccine consisted of liposomes that contained both the recombinant protein antigen and MPLA as an adjuvant.

Results: Antigen-specific enhancement of natural human antibodies (NA) to one or more of the lipids in the vaccine: dimyristsoyl phosphatidylcholine (DMPC), dimyristsoyl phosphatidylglycerol (DMPG), cholesterol, and MPLA. Two weeks after the vaccine boost, increased levels of IgG to all of the liposomal lipids, especially DMPG and MPLA, were observed. Antibodies to phosphatidylinositol-4-phosphate (PIP) above the normal pre-immune NA to PIP were also observed. Although PIP was not present in the immunizing liposomes, the anti-PIP antibodies were thought to represent a cross-reaction with DMPG. The immune response was antigen-specific in that NA to unrelated lipids that were not present in the liposomes, galactosyl ceramide and ganglioside GM1, were not increased by immunization. Furthermore, antibodies to beta-2-glycoprotein I or albumin were also no increased in titer following immunization.

Conclusion: Antibodies to DMPC, DMPG, PIP, cholesterol, and MPLA can be induced or boosted in humans by immunization with liposomes containing MPLA. There were no adverse effects of these antibodies and no increases in the pathogenic antibodies to beta-2-glycoprotein I.
P02.08
GM-CSF as an Adjuvant for MVA Vaccine: High Dose but Not Low Dose Inhibits T Cell Responses and Rectal IgA by Modulating DC Activation and Phenotype

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Background: We recently demonstrated that co-delivery of Granulocyte-Macrophage-Colony-Stimulating Factor (GM-CSF) DNA with SIV DNA vaccine enhances protection from acquisition of heterologous mucosal SIVsmE660 infection from 25% to 71% by a DNA/MVA vaccine. Here, we evaluate the optimal dose of GM-CSF for enhancing the immunogenicity and efficacy of MVA-only vaccine in rhesus macaques.

Methods: A MMM regimen delivered MVA/SIV (108 plaque forming units, pfu) expressing SIV239 Gag, Pol and Env at weeks 0, 8 and 24. In addition, GM-CSF adjuvanted groups simultaneously received MVA expressing GM-CSF (GM) at indicated dose. There were 7–8 animals/group.

Results: The GM vaccine rapidly induced expression of CD80 (activation), CCR7 (lymph node homing) and alpha4 beta7 integrin (a4b7, gut homing) on plasmacytoid dendritic cells (PDC) and monocytoid DC (MDC) in blood. Consistent with DC activation, MMM vaccine elicited strong SIV-specific CD8 and CD4 T cell responses in blood, high titer and avidity Env-specific IgG in serum, and moderate levels of Env-specific IgA in rectal secretions. High doses (5x107 and 107 pfu) of GM inhibited SIV-specific T cell responses and SIV-specific IgA in rectal secretions whereas low doses (106 and 105 pfu) either enhanced or maintained them. However, high doses of GM did not inhibit SIV-specific IgG in serum. The high dose but not low dose GM groups inhibited CD80 expression on MDC and down regulated a4b7 on PDC that was strongly associated with inhibition of SIV-specific T cell responses and rectal IgA, respectively in the high dose GM groups.

Conclusion: These results demonstrate that high doses of MVA/GM-CSF inhibit cellular immune responses in blood and IgA in rectal secretions by modulating activation status of MDC and gut homing potential of PDC. These animals will be challenged with SIVsmE660 to study the influence of high and low dose GM-CSF on protection.

P02.09
Polyethyleneimine Is a Potent Mucosal Adjuvant and Candidate Adjuvant for HIV-1 Vaccination

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Background: There are no mucosal adjuvants licensed for use in man although protection against many mucosally-transmitted infections probably requires immune activation at the site of pathogen entry. Polyethyleneimine (PEI) is used extensively as a transfection reagent and in-vivo gene delivery vehicle. Here we show that PEI has potent mucosal adjuvant activity with HIV-1 glycoprotein. PEI recruits leukocytes, triggers a local balanced Th1/Th2 cytokine environment and targets antigen to lysosome-like compartments in antigen presenting cells.

Methods: Babl/C mice were i.n. immunised with PEI (25 kD branched), PBS or CTB in formulation with HIV-1 glycoprotein (CN54gp140) antigen. Bleeds and vaginal lavages were obtained pre-prime, post-prime and post-boost immunisation and analysed for CN54gp140-specific IgG and IgA titres (ELISA). Spleens and cervical lymph nodes were obtained, restimulated with CN54gp140 and analysed for T cell proliferation (thymidine incorporation assay) and cytokine production (LUMINEX). Balb/C mice received i.p. injection of PEI, Alexa-647-ovalbumin, or both. Peritoneal lavages were obtained and cytokine levels determined (LUMINEX). Single cell suspensions were prepared and stained for leukocyte markers for flow cytometry. Naive peritoneal macrophages were incubated with Alexa488-CN54gp140 with or without PEI fixed, permeabilised, stained with subcellular compartment markers for confocal microscopy.

Results: PEI triggered local and systemic antibody levels of equal or higher size than gold standard adjuvant CTB. PEI also triggered T cell responses in spleen and lymph nodes of equal size and interestingly more localised character. Peritoneal administration showed that PEI recruits leukocytes, particularly monocytes, to the site of injection and triggers a local balanced Th1/Th2 cytokine environment. Analysis with Alexa 647- ovalbumin demonstrated that PEI targets antigen to antigen presenting cells. Confocal microscopy confirmed the presence of HIV-1 CN54gp140 inside macrophages when ex-vivo co-incubated with PEI.

Conclusion: PEI has immunostimulatory properties and adjuvant potency for HIV-1 immunisation. PEI may merit development as a mucosal adjuvant for human use.

P02.10
Degradable PEI Form as Safer Alternative for HIV-1 Vaccine Development and Adjuvant Potency as a Common Trait of Oligoethyleneimines

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Background: There are no mucosal adjuvants licensed for use in man although protection against many mucosally-transmitted infections probably requires immune activation at the site of pathogen entry. Polyethyleneimine (PEI) is used extensively as a transfection reagent and in-vivo gene delivery vehicle. We have shown that PEI has potent mucosal adjuvant activity with HIV-1 glycoprotein. One of the potential drawbacks of PEI is its potential toxicity. In these studies, we compared various PEI forms including degradable PEI forms for their adjuvant potency and toxicity.

Methods: Various linear and branched, degradable and non-degradable PEI forms were used in formulation with HIV-1 glycoprotein (CN54gp140) antigen for i.n., i.p., and s.c. immunisation in female Babl/c mice. Bleeds and vaginal lavages were obtained at regular intervals pre-prime, post-prime and post-boost immunisation. Body weights were recorded at 0, 6, 12, 24, and 48 hours post immunisation. Lavage and serum samples were analysed for CN54gp140 – specific IgG and IgA titres using ELISA.

Results: A wide range of structural PEI forms showed enhanced local and systemic antibody responses when co-formulated with CN54gp140 for immunisation in mice via various administration routes. Interestingly, no relation was found between molecular weight (mW) or linear/branched structure and adjuvant potency, which is in contrast to PEI gene transfection studies that showed optimal transfection for high mW branched forms. Some degradable forms in our studies showed substantial adjuvant potency combined with lower observed body weight loss in mice.
Conclusion: PEI’s immunostimulatory properties are a shared trait between various structural, degradable and non-degradable PEI forms. Some degradable PEI forms triggered substantially less weight loss in mice, indicative of a lower toxicity profile, while still being potent adjuvants in these models. Degradable PEI forms could therefore form a safer alternative for standard non-degradable forms when developing PEI as an adjuvant for HIV-1 vaccination.

**P03.01**
Differential Expression of Transcription Factors in SIV-Specific CD8+ T Cells at Week 5 and Week 20 After Vaccination with SIV239n enf

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**Background:** Current evidence suggests that protective immunity against vaginal challenge in SIVn enf-vaccinated macaques develops at 20 weeks after vaccination, whereas the magnitude of SIV-specific CD8+ T cells peaks at 5 weeks. Phenotypic analyses indicate a maturation of memory responses from week 5 to 20, as characterized by upregulation of CCR7 and CD127. These observations suggest that the quality of a more mature CD8+ T cell response, as opposed to the magnitude, may correlate with protection. We therefore analyzed differential expression of transcription factors involved in T cell maturation in SIV-specific CD8+ T cells at 5 and 20 weeks after SIVn enf vaccination.

**Methods:** Highly parallel qRT-PCR (Fluidigm) was used to characterize the expression of 23 transcription factors in CD8+ T cells sorted into naive, central, transitional, and effector memory subsets, as well as in SIV-specific CD8+ T cells obtained at wk5 and wk20 after SIV239n enf vaccination.

**Results:** Unsupervised hierarchical clustering segregated naïve and memory cell populations by phenotypic class. While the expression of some transcription factors, e.g., T-bet and Blimp-1, progressively increased during memory cell differentiation, others, (TCF7 and LEF1), progressively decreased. Further, SIV-specific CD8+ cells segregated into wk5 and wk20 clusters, with the wk 20 population exhibiting increased levels of transcription factors associated with both quiescent memory T cells (TCF7, BAZF) and maintenance of effector function (Eomes, T-Bet).

**Conclusion:** Our data indicate distinct transcriptional profiles of the different memory CD8+ T cell subsets, as well as clear qualitative differences in wk5 and wk20 SIV-specific CD8+ T cell transcriptomes. We further demonstrate that the mature CD8+ T cell response induced by SIV239n enf is characterized by the expression of transcription factors associated with both central memory and effector memory T cells. Analysis of transcription factor expression therefore provides a valuable complement to the analysis of memory cell differentiation based on classical phenotypic markers.

**P04.01**
Putative Rhesus Macaque Germline Antibodies of Broadly HIV-Neutralizing Antibodies: Similarities and Differences Compared with the Human Counterparts

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**Background:** Broadly neutralizing antibodies (bnAbs) are likely to be a key component of protective immunity conferred by an effective HIV-1 vaccine. We and others have reported that putative human germline predecessors of bnAbs lack measurable binding to the HIV-1 envelope glycoprotein (Env) that could be a new challenge to elicit human bnAbs. Rhesus macaques have been used as a nonhuman primate model for testing HIV-1 vaccine candidates, but little is known about their germline antibodies.

**Methods:** We used one of the several best characterized bnAbs, b12, as a model Ab and identified a putative rhesus macaque b12 germline predecessor by sequence analysis. We compared the human and macaque germline predecessors and possible intermediate antibodies of b12 by sequence analysis and by ELISA and neutralization assays.

**Results:** Putative rhesus macaque b12 germline predecessor also did not show measurable binding to HIV-1 Envs. However, we found different sequence characteristics between the human and macaque germline genes and their intermediate antibodies of b12 isolated from B cell receptor repertoires of nonimmune rhesus macaques and human.

**Conclusion:** These results suggest that initiation of somatic maturation of germline b12 predecessor in rhesus macaque may also be a challenge. But maturation pathways leading to elicitation of b12 or b12-like antibodies in rhesus macaques may be different from those in human, suggesting that primary immunogens to initiate somatic maturation and Env-based vaccine immunogens to further elicit bnAbs in rhesus macaque may be different from those in human. This finding may have implications for HIV-1 vaccine development.

**P04.02**
Genetic and Neutralization Sensitivity of Diverse HIV-1 Env Clones from Chronically Infected Patients in China

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**Background:** As HIV-1 continues to spread in China from traditional high-risk populations to the general public, its genetic makeup has become increasingly complex. However, the impact of these genetic changes on the biological and neutralization sensitivity of the virus is unknown. The current study aims to characterize the genetic, biological and neutralization sensitivity of HIV-1 identified in China between 2004 and 2007.

**Methods:** Full-length envelope genes were amplified by PCR directly from proviral DNA extracted from patients’ unclutured PBMC and cloned into expression vector. Env-bearing pseudotyped viruses were generated by co-transfection of env-expressing plasmid together with backbone construct pNL43R-E-luciferase into the 293 cells. Neutralization sensitivity of these diverse HIV-1 to subtype-specific plasma pools from infected patients as well as to broadly neutralizing monoclonal antibodies (2F5, 4E10, 2G12, PG9, PG16, IgG1b12, and VRC01) were analyzed.

**Results:** Pseudotyped viruses built upon the viable env genes have demonstrated their substantial variability in mediating viral
entry, and in sensitivity to neutralization by subtype-specific plasma pools and broadly neutralizing monoclonal antibodies (bnmAb). Many viruses are resistant to one or more bnmAb including those known to have high potency against diverse viruses from outside China. Sequence and structural analysis has revealed several mechanisms by which these resistant viruses escape recognition from bnmAb.

**Conclusion:** We believe that these results will help us to better understand the impact of genetic diversity on the neutralizing sensitivity of the viruses, and to facilitate design of immunogens capable of eliciting antibodies with similar potency and breadth as bnmAb. The viruses characterized here will also provide a strong foundation for establishing a broader and more representative Chinese virus panel to evaluate the antibody responses in infected and vaccinated individuals, and to contribute to the international virus panel where only a limited number of Chinese viruses are currently available.

**P04.03**

**Superinfection Results in Potent Neutralizing Antibody Responses to the Superinfecting Virus, but Does Not Necessarily Enhance Neutralization Breadth**

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**Background:** A better understanding of how neutralizing antibody responses develop during natural infection and the factors that augment them would be invaluable in the design of improved immunogens and immunization protocols. HIV dual infection (infection by >1 distinct HIV strain) provides a unique opportunity to evaluate whether polyclonal immunogens and prime-boost strategies are likely to enhance the breadth and potency of vaccine-induced responses against HIV.

**Methods:** Ten dual-infected participants were identified from the CAPRISA002 cohort, 5 of whom were co-infected at or prior to seroconversion, and 5 were superinfected within the first year of infection. Neutralization breadth at 3 years post-infection was compared to 16 singly-infected participants using a panel of 44 heterologous viruses, including subtypes A, B, and C. Autologous plasma neutralizing titers in 3 superinfected, and 3 co-infected individuals were assessed longitudinally using representative clones from multiple timepoints in the pseudovirus-based TZM-bl assay.

**Results:** There was no association between co-infection, super-infection, or diversity in early infection and the development of greater neutralization breadth. Furthermore, titers to the primary-infecting variants were not boosted following superinfection. However, 2 of the 3 superinfected participants developed elevated titers against the superinfecting virus, with ID₅₀ values exceeding 1:20,000 and 1:40,000 respectively. In one individual these high titers were generated despite maintaining low viral loads, frequently below detection (<400 copies/ml). None of the 3 co-infected participants generated similarly elevated titers, suggesting that sequential infection was a driving factor.

**Conclusion:** The lack of an association between dual-infection and breadth highlights the fact that polyclonal immunogens may not necessarily improve the breadth of elicited humoral responses. However, the high titers generated against the superinfecting variants suggest that a prime-boost approach has the potential to increase the potency of neutralizing responses against the boosting immunogen. As existing specificities were not boosted, the mechanism is likely distinct from the anamnestic response.

**P04.04**

**In Vitro Neutralization Kinetics of the Human Monoclonal Antibody IgG1 b12 Which Is Protective In The SHIV Rhesus Macaque Challenge Model**

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**Background:** In passive transfer experiments, the human monoclonal antibody IgG1 b12 protects rhesus macaques against infection with both the neutralization sensitive SHIVSF162P4 and the more resistant SHIVSF162P3. Relatively high concentrations of antibody are required to protect macaques against a bolus while lower quantities are protective in the repeated, low dose, challenge model.

**Methods:** HIV-1 SF162, SHIVSF162P4 and SHIVSF162P3 were grown in human PBMCs. Virus was mixed with antibody and incubated. Aliquots of the mixture were exposed to GHOST cells. Cultures were washed to terminate the absorption phase. Infected cells were quantified by FACS. Results are expressed either as plots of reductions in infectious virus over time or residually infectious virus against virus dose.

**Results:** Neutralization of both HIV-1 and SHIV is exponential during both the incubation and absorption phases. However, at relatively high doses of antibody, there is significant neutralization without prior incubation. Dose response plots of infected cells against virus dose are linear with a gradient of one passing through the origin. Incubation of low doses of virus with a low concentration of antibody yields a dose response plot which is parallel to controls: the percentage neutralization is low with 100 TCID50 virus but increases as the virus dose is reduced, reaching complete loss of infectivity at the point where the plot crosses the horizontal axis.

**Conclusion:** IgG1 b12 forms a complex with virus. However, this complex is still infectious. Events during the absorption phase of a SHIV neutralization assay determine the number (rather than the proportion) of viruses which subsequently lose infectivity. At low concentrations of antibody a limited number of infectious virus can be completely inactivated. It is proposed that this number is proportional to the dose of virus which a vaccinated individual can be exposed to without subsequent infection. Such assays should be used to monitor human vaccine trials.

**P04.05**

**Broad ADCC Responses and ADCC Directed at VPU Epitopes in Humans Is Associated with Slow Progression of HIV**

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Background: Robust antibody-dependant cellular cytotoxicity (ADCC) responses at the site of HIV infection may enable the elimination of virus prior to viral spread and latency. Identifying the targets of ADCC responses in long-term slow progressors (LTSP) could help direct the future design of vaccines to elicit beneficial ADCC responses.

Methods: A cohort of 61 LTSPs and a cohort of 78 unselected subjects with varying rates of HIV progression were analysed for ADCC responses against HIV-1 peptide pools were also analysed and specific epitopes mapped using an intracellular cytokine staining assay to detect NK-cell activation. ADCC Responses against a greater number of HIV-1 peptide pools were found in subjects of the LTSP cohort than in the unselected cohort (p = 0.006). In addition, ADCC responses against epitopes within the Vpu protein were overrepresented in slow progressors and two key ADCC epitopes in Vpu were observed.

Conclusion: Broad ADCC responses correlate with slower progression of HIV. We hypothesise that inducing ADCC Abs against specific epitopes within multiple HIV-1 proteins, including regulatory proteins such as VPU may provide a mechanism for protection from acquisition of HIV-1 or slower disease progression. ADCC responses against a greater number of HIV-1 peptide pools were found in subjects of the LTSP cohort than in the unselected cohort (p = 0.006). In addition, ADCC responses against epitopes within the Vpu protein were overrepresented in slow progressors and two key ADCC epitopes in Vpu were observed.

Results: HIV-specific ADCC responses correlated with reduced loss of CD4 T cells across the two cohorts (p = 0.027). Broader ADCC responses against a greater number of HIV-1 peptide pools were found in subjects of the LTSP cohort than in the unselected cohort (p = 0.006). In addition, ADCC responses against epitopes within the Vpu protein were overrepresented in slow progressors and two key ADCC epitopes in Vpu were observed.

Conclusion: Broad ADCC responses correlate with slower progression of HIV. We hypothesise that inducing ADCC Abs against specific epitopes within multiple HIV-1 proteins, including regulatory proteins such as VPU may provide a mechanism for protection from acquisition of HIV-1 or slower disease progression. ADCC responses against a greater number of HIV-1 peptide pools were found in subjects of the LTSP cohort than in the unselected cohort (p = 0.006). In addition, ADCC responses against epitopes within the Vpu protein were overrepresented in slow progressors and two key ADCC epitopes in Vpu were observed.
P04.08
Broad ADCC Responses and ADCC Directed at VPU Epitopes in Humans Is Associated with Slow Progression of HIV


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Background: Tests for the detection of humoral immune response to HIV-1 have to be established, demanding regional efforts to permit assessments of efficacy of future vaccine trials and allow comparisons on a global level. Our goal is to use the TZM-bl assay to investigate Neutralization antibody (NAb)able to inhibit HIV-1 replication in plasma from HIV-1 positive individuals with distinct profiles of Aids progression.

Methods: Eight env-pseudoviruses were evaluated against 22 plasmas from HIV-1-infected individuals: 13 typical-progressors (TP) and 9 long-term non-progressors (LTNP). We used as a control sCD4 and 2F5 Mab and 3 clade/variant plasma pooled TP (B,B/Bbr,F). The same plasmas were tested against the HIV-1 IIIB isolate in primary lymphocyte (ID90%).

Results: From the 13 TP plasma tested, 2 (15%) were potent and broad in their neutralizing capacity (100%), while as the HIV-1 IIIB isolate (PBMC), 7 (53%) neutralized 50% of the env-pseudo-typed tested, the remained varied from 25–30%. In the ID90% in TZMBL assay 104 titer points (13 TP plasmas x 8 pseudoviruses) were evaluated, from those 64 (62%) had neutralizing titers from 1:20-1:4374 and 40 (38%) haven’t NAb. Of the 72 (9 plasmas x 8 pseudoviruses) points LTNP plasma tested, 10 (14%) had neutralizing titers between 1:50-1:6250 and 62 (86%) haven’t NAb. The highest neutralization titer was verified in the B/Bbr1 pooled.

Conclusion: Neutralization assay in TZM-bl cells was successfully reproduced in the laboratory being a tool for the detection of NAb from TP and LTNP plasmas in distinct proportions, showing a high capacity to neutralize virus/pseudovirus. These results and these reference reagents enable the participation of Brazil in future assays of neutralizing antibodies induced by vaccine to HIV-1. Financial support: GHVE and Dpto DST/AIDS and Hepatites MS.

P04.09
Development of Broadly Neutralizing Antibody Responses in a Large Sub-Sahara African HIV Primary Infection Cohort


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Background: Recent studies have demonstrated that 10% to 20% of individuals infected with HIV-1 are capable of generating HIV-1 broadly neutralizing antibodies (bNAb). Understanding the correlates of how these broad responses develop could be extremely important for the design of a protective vaccine.

Methods: The IAVI Protocol C program investigates a longitudinal cohort of primary HIV-1 infection in Eastern and South Africa. Currently over 400 donors with recent HIV-1 infection have been recruited. To date, development of neutralization has been evaluated on 200 individuals, including 174 with at least 3 years of follow-up. Neutralization activity was assessed at Monogram Biosciences using a 6 cross-clade pseudo-virus panel highly predictive of neutralization breadth on larger panels. Neutralization of an isolate was recorded positive if equal or greater than 40% at a 1/100 serum dilution.

Results: At year 2, only 4 individuals out of 171 had developed broad neutralizing activity, with 5 out of 6 viruses neutralized. In contrast, at year 3, 16 donors out of 163 (9.8%) neutralized more than 4 viruses. In addition, 69 individuals (36%) neutralized 3 or 4 viruses on the panel. Broad responses developed at year 4 in 9 additional donors out of 71 (12.7%). In most individuals, neutralization breadth developed simultaneously, appeared to be relatively stable with time, and no significant improvement of potency was noted over time. Screening of an additional 96 donors is pending.

Conclusion: In conclusion, our current data show that in our large longitudinal sub-Sahara African cohort, broad neutralizing responses have so far developed in about 15% of individuals and essentially at year 3. Analysis of correlates of breadth development with clinical parameters, such as CD4 counts and viral load, is ongoing as well as the mapping of broad neutralizing specificities in the top neutralizers.

P04.10
FACS-Based Epitope Mapping Platform to Characterise New Identified Monoclonal Anti-HIV-1 Envelope Antibodies

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Background: Recent systematic approaches for identification of HIV-1 infected patients with neutralizing sera and subsequent generation of new broadly neutralizing monoclonal antibodies (bNMAB) create the need for a rapid characterisation tool which allows the determination of conformational epitopes on a native envelope structure.

Methods: A sequentional permutation envelope library based on the isolate ZM96 was generated, where every amino acid is stepwise mutated to an alanine, resulting in 608 variants with a single point mutation. Every variant is used in a separate transient transfection and analysed by high throughput FACS analysis using an antibody of interest in combination with a second gp41 antibody used as an internal standard. First the screening process was validated using the V3 antibody HGN194 known to recognize a linear epitope as well as soluble CD4 which binds to an highly conformational epitope. Second, new antibodies with so far unmapped epitopes like HJ16 were screened.

Results: The screen with HGN194 showed several critical amino acids for binding in the V3 region and confirmed the published data by Corti D. et al. 2010. The screen using sCD4 confirmed several known amino acid positions which are known to be critical for the binding of the receptor to the envelope. The screening of HJ16 resulted in the identification of the several critical amino acids within the gp120 envelopes for the binding of the bNMAB. These positions suggest a high conformational epitope. Additional
mutations were identified which showed an enhanced binding of the bNMAB. Confirmed amino acid positions were modelled in the gp120 core structure.

Conclusion: This method allows the identification of conformational epitopes of anti-HIV envelope antibodies on a native, trimERIC and membrane bound envelope.

P04.11 Innate Immune Signals Regulate Non-Neutralizing Effector Functions of Antibodies Through Glycosylation

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Background: Data from the RV144 trial have shown that the modest success of this trial was not dependent on elicitation of CD8 + T cell or neutralizing antibody responses. Interestingly, most vaccinees induced non-neutralizing antibodies, which raises the possibility that other humoral antiviral activities may have contributed to protection. In fact, innate immune recruiting antibodies are enriched in long-term non-progressors, and this humoral activity has been shown to play a critical role in promoting the sterilizing protective effect of some neutralizing antibodies. However, the mechanisms by which these effector antibodies can be induced in vivo through vaccination, are not understood. We hypothesized that innate immune signals, particularly toll-like receptor (TLR) ligands, are able to modulate effector functionality of antibodies.

Methods: Gene expression of a set of over 20 glycosylation enzymes was analyzed in healthy bulk B cells stimulated with TLR ligands to identify interesting changes in glycan structure.

Results: Specific TLR stimulation of bulk B cells resulted in significant alterations of glycosylation enzyme expression. These changes related to the subcellular localization of the TLRs: extracellular TLRs did not induce significant changes in glycosylation enzyme expression, however, intracellular nucleic acid sensing TLRs 3, 7, 8, and 9 caused significant alterations. Interestingly, stimulation of the nucleic acid sensing receptors that recognize intracellular pathogens, such as HIV, significantly altered glycosylation gene expression. These changes correlated with a decrease in galactose, fucose, and sialic acid additions, which may correspond to the induction of more inflammatory, less branched glycan structures, that mediate stronger effector functions.

Conclusion: These data are the first to show that innate immune signals modulate the antibody glycan patterns on stimulated B cells toward more inflammatory structures. This study strongly suggests that specific signals may be exploited in future vaccines to elicit antibody responses with specific glycosylation patterns and tailored effector functions.

P04.12 Single Mutation Turns a Non-Binding Germline-like Precursor of Broadly Neutralizing Antibody into a Binding Antibody to HIV-1 Envelope Glycoproteins

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Background: Broadly neutralizing antibodies (BnAbs) against human immunodeficiency virus type I (HIV-1) are rare in natural infection. We and other groups have reported that HIV-1 bnAbs are highly diversified from their germline-like predecessors. We do not know what are the minimum mutations required for converting non-binding germline-like predecessors to Env-binding antibodies.

Methods: We started with the bnAb b12 as a prototype and generated six “chimeric” scFv b12 variants by sequentially replacing the heavy chain V-segment (HV), DJ-segment [HD(J)], and the light chain variable region (VL) in b12 germline-like predecessor with the mature counterparts, and tested the scFv variants for binding and neutralizing activities in ELISA and pseudovirus assays.

Results: A point mutation in germline heavy chain D-segment from “Y” to “D” converted nonbinding germline-like b12 to an Env-binding antibody. Replacement with either mature HV or mature VL also made the germline-like b12 bind to Env, but none of the single segment replacements conferred neutralization ability to the germline antibody. Mature VL in combination with mature HD(J), or mature HV, or both conferred increasing neutralization activity to the germline antibody. Hybrid scFv, mature VH/germline VL, did not neutralize the virus.

Conclusion: During antibody maturation, less mutation, as low as one single nucleotide change, may be needed to turn a non-binding germline-like predecessor of bnAbs into a binding antibody intermediate to HIV-1 Env, which may further mature to bnAbs upon HIV-1 infection or vaccination. Somatic maturation in HV-, HD(J)-segments and VL of germline-like b12 additively contributed to the binding of b12 to Env. Although b12 light chain did not make contact with gp120 core in the co-crystal structure, mature b12 VL was required for “chimeric” b12 variants to neutralize the virus. These results may have implications for vaccine development.

P04.13 B Cell Depletion in HIV-1 Subtype A Infected Ugandan Adults: Relationship to CD4 Count, Viral Load and Humoral Immune Responses


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Background: HIV-1 infection leads to B cell dysfunction through mechanisms that are not fully characterized, particularly in subjects infected with non-B subtypes. To better understand the nature of B cell dysfunctions in East African subtype A infection, the relationship between B cell depletion, antibody function and disease progression was assessed.

Methods: Participants were recruited under an IRB approved vaccine cohort protocol. Enumeration of lymphocyte subsets was performed using FACSTest, TruCount tubes and a FACScalibur flow cytometer. Plasma viral load was measured by the Roche HIV-1 Monitor Test version 1.5. Sera from 50 subjects with chronic subtype A infection were tested by ELISA for gp120 binding antibodies and in the TZM-bl neutralization assay against a panel of 10 pseudoviruses from newly transmitted infections of subtypes A-D and CRF02_AG.
Results: B cell numbers were significantly lower in HIV-1+ patients, compared to community matched HIV-negative controls (p < 0.0001). Neutralization and binding antibody titers showed no correlation with viral load or CD4 counts. However, B cell absolute counts were found to correlate inversely with neutralizing titers against subtype A and CRF02_AG viruses. A positive correlation was observed between subtype A gp120 binding titers and neutralizing antibody breadth (p < 0.02) and titer (p < 0.05). Additionally, subtype A sera showed preferential neutralization of subtype A and CRF02_AG pseudoviruses, compared with pseudoviruses expressing non-A envelopes (p < 0.0001).

Conclusion: In patients with chronic HIV-1 subtype A infection, significant B cell depletion can be observed which does not appear to be associated with a decrease in functional antibodies. The inverse correlation between functional antibody titers and B cell numbers may be due to B cell maturation and migration out of the periphery. These findings also further substantiate the importance of subtype in the specificity of cross-clade neutralizing antibody responses in HIV infection.

P04.14
Llama Antibody Fragments that Potently Neutralize HIV-1 at the CD4 Binding Site of HIV-1 Gp120

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Background: Llamas produce heavy-chain only antibodies, from which the small (~15kd) binding fragment (VHH) may readily be cloned into a phagemid vector to construct phage libraries. We have isolated a number of VHH from libraries derived from llamas immunized with recombinant gp120 or trimeric gp140 which exhibit strong neutralization of diverse strains of HIV-1.

Methods: Individual VHH clones expressed in E.coli from a phagemid vector were screened for binding to recombinant HIV-1 envelope proteins and neutralisation ability against HIV-1 in the TZM-bl cell-based assay.

Results: The best VHH isolated to date, J3, neutralizes almost 100% of HIV-1 pseudoviruses tested, with a breadth encompassing clades A, B, C, A/G and B'/C. Structural studies of VHH:gp120 complexes indicate that both the angle at which VHH bind to their target and their small size compared to entire antibodies contribute to their neutralizing properties.

Conclusion: Our studies of llama VHH are useful in three aspects of protection against HIV infection: (a) they show that experimental immunization with recombinant HIV envelope can elicit broad neutralizing responses; (b) they indicate that neutralization epitopes can be exploited in immunogen design for B-cell based vaccines; (c) the VHH themselves can be developed as potential microbicides owing to their stability over a wide range of temperature and pH, low immunogenicity and ease of scale-up for inexpensive mass production.

P04.15
Optimization of the V3 Directed Antibody Response: Strategies to Expose the Epitope

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Background: V3 directed antibodies with significant breadth and potency have been identified. We studied the structure of V3 peptides in complex with neutralizing antibodies, designed and tested a panel of constrained V3 peptides mimicking the 447-52D bound conformation. Our study resulted in identification of an optimally constrained V3 peptide, C4-V3T303C-E322C, which elicits an antibody response that neutralizes SS-1196 and 6535 viral strains. Nevertheless, masking of the V3 loop remains a problem limiting the breadth of V3 directed antibodies. Antibodies such as KD-247 and F425-B4e8, which can neutralize resistant primary isolates such as JR-CSF, recognize a shorter epitope centering the GPGR tip.

Methods: The V3 loop can become exposed for neutralization following CD4 binding, CD4M33 is a small and stable CD4-mimic peptide. We have tested the ability of CD4M33 to act in synergy with C4-V3T303C-E322C elicted sera. We are also testing a new methodology of immuno-focusing attempting to focus the antibody response to exposed elements in V3. Optimally constrained peptides at V3T303C-E322C are used for priming followed by additional boost with short V3 peptides representing the F425-B4e8 epitope.

Results: CD4M33 was found to act in synergy with C4-V3T303C-E322C induced sera in a panel of HIV-1 isolated including SS1196, 6535 and QH0692. Combination-index range from 0.1–0.3. We have also immunized rabbits with the aim of optimizing the number of priming boosts with the long V3 peptides and the final boosts with the short peptides. Initial binding studies suggest that a single priming step may be optimal. Neutralization studies are currently being performed and will also be presented.

Conclusion: The combination of V3 directed vaccine with a pre-exposure prophylactic administration of a CD4-mimic compounds should be considered as an alternative to other pre-exposure prophylactic drugs. Strategies to expose the V3 loop and expand the breadth of V3 directed antibodies by active immunization should also be pursued.

P04.16
HIV-1 Envelope Characteristics that Coincide with the Development of Cross-Reactive Neutralizing Activity in HIV-1 Infected Patients

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Background: The HIV-1 envelope glycoprotein (Env) on the viral surface is the target for anti-HIV-1 neutralizing antibodies. In some HIV-1 infected patients broadly neutralizing antibodies (BrNAb) develop that neutralise HIV-1 from different subtypes and therefore are considered to be directed against conserved regions. Understanding of factors involved in BrNAb development will help the rational design of an effective antibody-based vaccine immunogen.

Methods: From a previous screening of 299 patients for broadly neutralizing activity (BrNAb), we selected 12 patients with high BrNAb in serum and 10 patients without BrNAb at 3 years after SC. From these patients clonal viruses were obtained within the first year after SC from which gp160 was sequenced and analyzed.
for length of different regions and number of potential N-linked glycosylation sites (PNGS).

**Results:** There was a trend observed for longer variable regions 1 and 2 (V1V2) in viruses from patients who lacked BrNAc (P = 0.16), but there were no significant differences in length of gp160 or different regions of env between the two patient groups. Env from HIV-1 of patients who lacked BrNAc also tended to have a higher number of PNGS (P = 0.16), especially in the V1 region, with significantly more PNGS (P = 0.044). The number of PNGS in gp41 also tended to be higher in the non-BrNAc group (P = 0.10).

**Conclusion:** We hypothesize that the development of BrNAc in HIV-1 infected patients is associated with a more accessible open structure of the viral envelope glycoprotein, which in turn is associated with fewer glycans and shorter variable loops. Testing of epitope specificities in these sera as well as testing of the neutralization sensitivity of these viruses for well defined BrNAbs will help to identify the epitopes on Env that may be responsible for eliciting BrNAc and could help the rational design of an effective antibody-based vaccine immunogen.

**P04.17 Neutralization Potential and Epitope Profiling of Plasma Antibodies from HIV-1 Infected Asian Indians**

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**Background:** Human immunodeficiency virus type 1 (HIV-1) is the primary cause of AIDS pandemic. Dissecting the specificities of the anti-HIV-1 neutralizing antibodies will assist in identifying targets for an HIV-1 vaccine.

**Methods:** Plasma from 65 drug naïve HIV-1 infected patients were tested against a panel of 3 subtype-B (TRO.11, JRFL and RHPA) and 5 subtype-C (Du156.12, ZM53, ZM109F AIIMS201, AIIMS212) tier 1 and tier 2 viruses. Three plasma, found to be broadly neutralizing, were mapped with a set of 211 con-C gp160 overlapping peptides (15mer each). The 50% binding titer were determined by ELISA using consensus-C V3 (35mer), ID loop (19mer) and MPER (24mer) peptides. Statistical analysis was performed using Graph Pad Prism 5.

**Results:** Of the 65 plasma samples, 53 (81.5%) were able to neutralize at least one virus while 12 (18.46%) did not show any neutralization. Only 15 (23%) samples were found to neutralize ≥50% viruses tested. Clustering analysis revealed that AIIMS201 and Du156.12 (clade-C viruses) were the most sensitive while ZM109F, a tier 1b clade-C followed by TRO.11 (clade-B) isolates were the most resistant to antibody neutralization. Epitope specificities of three broadly neutralizing plasma (AIIMS206, AIIMS239 and AIIMS249) with consensus-C gp160 overlapping peptides mapped to V2, V3 and C5 of gp120 and ID loop, MPER and CT of gp41. We did not find any correlation between neutralization capacity and 50% binding titers of anti-V3 (p = 0.429), anti-MPER (p = 0.683) and anti-ID loop (p = 0.680) plasma antibodies. Depletion and competition with V3, MPER and ID-loop peptides showed modest effect on neutralization except for AIIMS239 which showed dependence on MPER directed antibodies.

**Conclusion:** Clustering of neutralization data and phylogenetic analysis suggest the recognition of similar neutralization determinants across different HIV-1 viruses. Identification of epitopes that elicit broadly neutralizing antibodies may serve as effective tool for HIV-1 vaccine design.

**P04.18 The Antibody 2F5 Third Complementarity-Determining Region of the Heavy Chain (CDRH3) Displays Length Plasticity for Binding and Neutralization**

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**Background:** The HIV-1 membrane proximal external region (MPER) broadly neutralizing monoclonal antibodies (Mab) 2F5 and 4E10 bind contiguous epitopes in gp41 and are crystallographically characterized in complex with their cognate peptides. The 2F5 structure reveals an extended loop conformation that contrasts with the helix generally adopted by the MPER. Most of the unusually long (22aa) CDRH3 of the 2F5 Mab does not contact the epitope and the CDRH3 possesses a highly hydrophobic apex. Recent data demonstrates that increasing CDRH3 hydrophobicity correlated with neutralization. Here, we aimed at elucidating the involvement of 2F5 CDRH3 length in regards to its capacity to bind or neutralize HIV-1.

**Methods:** We generated 2F5 mutant Mabs that possessed either longer or shorter CDRH3 loops. We next increased hydrophobicity of the length-altered CDRH3s by targeted tryptophan substitutions. We determined Mabs binding affinity constants to epitope and assessed their HIV neutralization activity in pseudovirus assays.

**Results:** The 2F5 CDRH3 tolerated both elongations and reductions of up to four residues, while maintaining nanomolar binding to peptide and HIV neutralization; these activities were enhanced by introducing a V100DW mutation. Substitutions of up to three tryptophans at the apex of the shortened CDRH3 fully reconstituted HIV neutralization activity. Antibody on-rate for the wt MPER peptide correlated positively with neutralization activity.

**Conclusion:** The 2F5 CDRH3 length displayed plasticity for binding its epitope and for neutralization with hydrophobicity of the CDRH3 appearing more critical for neutralization. The positive correlation observed between the on-rate of recognition for the wt MPER peptide and the neutralization activity of the mutant antibodies suggests a model of 2F5 MPER recognition in which the CDRH3 destabilizes the MPER helix to allow the antibody to induce the extended loop peptide-bound conformation observed in the crystal structure.
individuals are restricted to the naive B cell compartment, yet in SLE patients these 9G4 B cells can undergo germinal center reactions (GC) and become antibody-secreting cells (ASC), leading to elevated serum levels of 9G4. The objective of this study is to examine the association between the presence of 9G4 B cells and BNA activity in HIV-infected patients.

**Methods:** Patients' blood samples were obtained. Sera were serial diluted in 9G4 or HIV-1 clade antigen coated 96 well Enzyme-linked Immunosorosant Assay plates and detected using enzyme/substrate reactions. BNA screening was performed using published protocols (ref).

**Results:** Similar to SLE patients (n = 35), 9G4 serum antibody titers are significantly elevated in HIV-infected patients (n = 112) in comparison to healthy controls (n = 28 p < 0.05). A fraction of patients (>5%) had significantly greater titers compared to SLE patients. These 9G4 positive antibodies from some HIV-1 patients bind to clade A, B and C HIV-1 Env trimers, and patients with high 9G4 + B cell numbers tend to have BNA to a wide range of HIV strains.

**Conclusion:** Preliminary findings suggest that these 9G4 + B cells in HIV-infected patients may offer an important avenue to study the role of tolerance in the context of induction of a broadly protective anti-HIV-1 antibody response.

**P04.20**

**Surface Unit but Not Transmembrane Domain Directed Broadly Neutralizing Antibodies Have Differential Potency Against Cell Associated Virus**

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**Background:** Cell free virions are used as the challenge stock in the majority of HIV-1 infection models. Numerous broadly neutralizing antibodies (bNAbs) have been shown to block a diverse array of HIV-1 virions, but it remains unclear if these antibodies exhibit similar potency against cell associated infection, especially dendritic cell trans infection, which potentially plays an important role during mucosal HIV-1 transmission.

**Methods:** Mature DCs were derived from CD14+ monocytes cultured in the presence of IL-4, GM-CSF, and LPS. Mature DCs were exposed to various primary and laboratory adapted HIV-1 isolates (MOI = 0.2), and washed to remove unbound virus particles. Sensitivity to various bNAbs was examined between cell free and mDC laden HIV-1 in TZM-bl and primary CD4+ T cells. Cell staining was used to examine bNAbs binding mDC and mDC – T cell conjugates in the absence of HIV-1.

**Results:** Compared to cell free infection, mDC mediated HIV-1 trans infection was significantly less susceptible to gp120 directed bNAbs (VR0C1, b12, 2G12). Cell free and mDC associated viruses were equally sensitive to gp41 targeted bNAb (4E10 and 2F5). Broadly NAb, b12, Fab fragment blocked both cell free and mDC mediated virus infection with equal efficiency. Broadly NAb, 4E10, but not 2G12 bound both mDC alone and mDC – T cell conjugate in the absence of HIV-1.

**Conclusion:** Steric hindrance from the tight juxtaposition of HIV-1 bearing mDC and target T cell membranes interferes with the ability of gp120 directed bNAbs from blocking mDC-mediated trans infection. On the other hand, gp41 directed bNAb's recognition of host cell lipids allows them to bind mDC prior to the formation of an infectious synapse. Our studies suggest gp120 but not gp41 directed bNAbs may be less potent in preventing HIV-1 spread if transmission or spread from the initial site of infection occurs from a dendritic cell associated source.

**P04.21**

**Isolation of Potent Neutralizing Monoclonal Antibodies Against V3 Loop from SIV-Infected Macaques**

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**Background:** Broadly neutralizing monoclonal antibodies from HIV-1-infected patients are an essential but rare tool to understand the mechanism of neutralization against a broad spectrum of HIV-1 strains, including neutralization-resistant strains. Although the humoral immune response to SIV has been studied in the development of vaccine candidates and for exploration of antibodies that efficiently control viral infection, the lack of monoclonal antibodies that can neutralize neutralization-resistant SIV strains is the problem of studying the mechanism of efficient neutralization using the SIV model.

**Methods:** We used phage display method to obtain MAbs against SIV antigens from a SIVsmH635FC-infected rhesus macaque with robust envelope-specific antibody responses. Variable regions of immunoglobulin genes were amplified by rhesus macaque-specific primers and inserted into the phagemid pComb3X to construct phage libraries expressing the Fab fragment. SIV-specific Fab clones were selected by panning with SIV antigen on 96-well plate, and their specificity and neutralizing activities were analyzed.

**Results:** As a result of panning using SIV antigen, many Fab clones specific for SIV Env gp120, gp41 and Gag p27 were obtained. Flow cytometry analysis showed that these Fabs efficiently bound diverse strains of SIVsm/mac, and some of them were cross-reactive with HIV-2. Some of the anti-gp120 Fab clones neutralized the homologous SIVsmH635FC and the genetically divergent SIVmac316, but did not neutralize SIVmac239 and HIV-2H123. These Fab clones with potent neutralizing activity recognized the same epitope on gp120 including V3 loop. Neutralizing Fab clones specific to the same V3 epitope were also isolated from other SIVsmH635FC-infected macaques.

**Conclusion:** Identification of monoclonal antibodies with potent neutralizing activity against SIV will help to elucidate the mechanisms for inducing broadly neutralizing antibodies in a SIV model.

**P04.22**

**Profiles of Neutralizing Antibody Responses in Chronically HIV-1 CRF07_BC Infected Intravenous Drug Users in Western China**

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**Background:** It is essential to characterize neutralizing antibody (Nab) responses in individuals infected with the diverse HIV-1
strains to reveal the potential target for HIV-1 vaccine development. We assess the prevalence, breadth and potency of Nab responses in CRF07_BC chronic infectors (n = 100) with infected time around 3 years and compare the neutralization pattern with that of clade B’ chronically infectors (n = 103) with infected time at least 10 years.

**Methods:** 114 plasma samples were collected from intravenous drug users infected with CRF07_BC virus in western China who were ART-naïve. The Env pseudovirus-based TZM-bl assay was performed against a panel of tier 2–3 pseudoviruses composed of CRF07_BC(10), clade C(5), B(7), A(4) and CRF01 AE(4) strains and 2 tier 1 viruses(SF162 LS and MW965.26). As negative control virus, SVA-MLV positive samples were excluded from further analysis.

**Results:** All 100 plasma samples (excluding 14 SVA-MLV positive samples) neutralized both tier 1 viruses. 53% of samples (n = 53) neutralized half of the viruses and 17% of samples (n = 17) neutralized more than 80% strains tested. 1% (n = 1) neutralized all the viruses and 2% (n = 2) neutralized none of the viruses tested. Significant difference of geometric mean ID50 titer (GMT) between intraclade and interclade samples (p < 0.001) was observed. CRF07_BC chronically infected subjects showed higher neutralizing activities against subtype-matched viruses than subtype B’ infectors. However, higher prevalence of broadly cross-reactive Nab samples response were observed in clade B’ chronically infected than that of CRF07_BC infection (29% vs 17%).

**Conclusion:** We detected relatively high broadly cross-reactive neutralization activities in 17% plasma of CRF07_BC infectors tested. Further epitope specificity dissection of these broadly cross-reactive Nab samples will provide useful insights for rational HIV-1 vaccine design.

**P04.23**

**Plasmacytoid Dendritic Cells Infection by HIV-1 Is Inhibited by Neutralizing Antibodies Without Interfering with IFN-α Production**

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**Background:** Plasmacytoid dendritic cells (PDC) are involved in innate and adaptive immunity, and produce large amounts of antiviral type I interferons. These cells are productively infected by HIV-1 in vitro and in vivo, and several studies revealed a decrease in circulating PDC number, correlated with an increased plasma viral load.

We have previously shown an FcγRs-mediated inhibition of myeloid DC infection by neutralizing (NAb) as well as non-neutralizing inhibitory (NNIAb) antibodies. The aim of this study is to analyze the mechanism of inhibition of PDC infection by Ab.

**Methods:** Neutralization assay was performed with primary human healthy PDC or GEN2.2 PDC cell line in the presence of serial antibodies concentrations. The percentage of infection was quantified by flow cytometry using intracellular p24 viral antigen staining and IFN-α production was measured by CBA flex.

**Results:** We showed that NAbS strongly inhibited R5-HIV-1 replication in GEN2.2 cell lines. Although they expressed FcγRII, we have not detected an inhibitory activity of NNIAbs. We also found an efficient inhibition of primary PDC infection by NAbS, but not with NNIAbS, with similar activities that those measured with GEN2.2 PDC cell line. Interestingly, a strong level of IFN-α production was maintained in primary PDC infected by HIV, even when the HIV-1 replication was inhibited by NAbS.

**Conclusion:** We showed that 1) HIV-1 infection of PDC is inhibited by NAbS, 2) FcγRIIa seems not to be involved in this inhibitory process, and 3) the inhibition of HIV-1 replication by NAbS do not preclude IFN-α production by PDC. Overall these results suggest that HIV-1 specific NAbS induced by an efficient vaccine should prevent PDC infection, without interfering with the release of IFN-α and thus the anti-viral innate immune response.
P04.25
Elicitation of Neutralising Antibodies Using Patient-Derived gp140 Immunogens from a Long-Term Non-Progressor

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Background: Broadly neutralising antibodies (bNAbS), that cross-react with diverse HIV-1 subtypes, develop in some individuals during chronic infection. Neutralisation strategies that utilise patient-derived envs may result in improved bNAb development. We studied the env sequence evolution in ITM-1, a long-term non-progressor patient with bNAbS. We selected two env vaccine candidates from the phylogeny and tested their immunogenicity in rabbits.

Methods: HIV-1 env was extracted, amplified and sequenced from 30 plasma samples collected over 11 years from ITM-1 and 7 plasma samples from his mother. Sequence evolution was analysed following alignment and phylogeny reconstruction. The earliest env, ITM_1, and the predicted ‘ancestral’ env, ITM_anc, were selected for use as immunogens. Gp140 trimERIC proteins were expressed in 293T cells and purified using metal-affinity, lectin and FPLC. Rabbits were immunised at weeks 0, 2, 4 and 8 with 10ug trimer adjuvanted with CAF01. Sera collected at weeks 0, 2, 4, 8, 12 and 14 were screened in gp120 ELISA and TZM-bl and PBMC neutralisation assays.

Results: ITM-1 and Mother env sequences diverged into distinct lineages following transmission. We observed substantial variable loop expansion, variations in glycosylation and an accumulation of positively-selected substitutions over time in ITM-1 sequences. We selected two early envs from the phylogeny for immunisation studies with trimeric gp140 protein. All rabbits generated a gp120-specific IgG response 2 weeks after the first dose. Titres were boosted after each subsequent immunisation. Sera from ITM_1_4 immunised rabbits were able to neutralise Tier 1 pseudoviruses SF162 and BX08. Neutralisation was also detected in ITM_anc immunised rabbits, though the titres were lower.

Conclusion: Early isolates from patients who develop bNAbS can be used to design gp140 immunogens which may induce greater neutralisation breadth in HIV-1 vaccination studies. A clearer understanding of what drives bNAb development will further inform vaccine design.

P04.27
Combined HIV-1 Neutralization Coverage by VRC01, PG9, and PG16

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Background: Neutralizing antibodies (NAb) are considered a critical component of an effective vaccine for HIV-1 and have been shown to provide sterilizing protection in animal models. Three recently described monoclonal antibodies, VRC01, PG9, and PG16, have extraordinary potency and breadth of neutralization. These human mAbs are potential models for immunogen design, and are of interest for clinical use to prevent HIV-1 infection. Therefore, we analyzed the potential total coverage of HIV-1 isolates provided by combinations of these antibodies.

Methods: Neutralization titers were determined for each antibody against a panel of 199 HIV-1 Env pseudovirus isolates using TZMbl target cells. Isolates included clades A, B, C, D, and G and CRFs AC, AD, AE, AG, ACD, BC, and CD. IC50 and IC80 titers were used for combination analysis; sensitivity or resistance was determined using cutoffs of 50 ug/ml or 1 ug/ml.

Results: Individually, VRC01 neutralized 90.4% of isolates with IC50 <50, PG9 neutralized 78.4%, and PG16, 73.9%. At the most stringent cutoff we considered, IC80 <1, the individual NAbS neutralized 51.8%, 45.2%, and 42.2% respectively. Together, VRC01 + PG9 neutralized 97.0%, VRC01 + PG16 neutralized 96.5% and PG9 + PG16 neutralized 80.4% at an IC50 <50. Using an IC80 <1, the values were 69.9%, 70.9% and 50.2% respectively. Only 6 out of the 199 isolates were fully resistant to both VRC01 and PG9. Resistance to VRC01 was independent of resistance to PG9 or PG16. IC50 titers for VRC01 did not differ between isolates that were resistant or sensitive to PG9 or PG16 and vice versa.

Conclusion: The combination of VRC01 with PG9 or PG16 provides neutralization of up to 97% of global HIV-1 strains. These combinations could be very useful clinically in therapeutic or prophylactic applications.
P04.28
Neutralisation Activity of HIV-1 Clade C- and CRF02_AG-Infected Serum/Plasma: Some Viruses Are Not as Resistant as Previously Thought

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Background: Significant strides in understanding neutralisation breadth of HIV-1 have been made recently. In order to develop a global vaccine, it remains important to understand the neutralisation of the wide diversity of viruses that circulate globally.

Methods: We developed a panel of 24 HIV-1 pseudoviruses selected for resistance to neutralisation, diversity by clade, geographic origin and sequence. We tested this panel using 70 sera from an ARV-naive cohort infected with HIV-1 (presumed subtype C) for >1yr and 10 plasma samples from a Cameroonian blood bank that were infected with typed CRF02_AG-related viruses and were selected to be comparable in neutralisation capacity to our South African samples.

Results: The neutralising responses of the subtype C sera were broad, with 8/70 sera tested neutralising ≥18/24 target pseudoviruses at ID50 >100, and 12/70 neutralizing ≥16/24 target pseudoviruses. We found that most clade A and some CRF02_AG target pseudoviruses tended to be the least sensitive to neutralisation by our sera from presumed clade C-infected individuals, followed by Clade B and then clade C viruses. Surprisingly, and in contrast to previous reports, plasma from clade CRF02_AG infected donors neutralised several of the CRF02_AG viruses well, including two Tier 3 (251-18 and 33-7) viruses that were previously described as highly resistant.

Conclusion: Some but not all CRF02_AG-related viruses previously described as tier 3 are sensitive to neutralisation by CRF02_AG-derived plasma, even though all are previously reported as highly resistant. Further work to identify which viruses are intrinsically resistant to neutralisation is needed. Three viruses were identified that were most resistant to both South African and Cameroonian samples, which are now major targets in our monoclonal antibody project. Focus should be placed on those viruses that are intrinsically resistant to neutralisation in order to develop an HIV-1 vaccine that does not select for such viruses.

P04.29
Early Autologous Neutralising Epitopes in C3-V4 and \(\beta14+\) V5 Are Readily Adsorbed with Monomeric gp120

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Background: The earliest neutralising antibody responses target epitopes predominantly in variable loop regions of gp120. We have previously shown that the C3 region and V4 loop often form a discontinuous epitope for these antibodies in acute subtype C infection. This work identified another discontinuous neutralisation epitope comprising the \(\beta14+\) sheet in C3 and adjacent V5 loop. Since C3-V4 and \(\beta14+\) V5 exist entirely within the structurally conserved outer domain of gp120, it was hypothesised that autologous antibodies targeting these regions could be adsorbed with monomeric gp120.

Methods: Recombinant gp120s from the transmitted/founder virus of four HIV-1 infected individuals were expressed in 293T cells and purified by lectin affinity and ion exchange chromatography. Proteins were conjugated to tosyl-activated magnetic beads and used to adsorb the neutralisation activity in autologous plasma from a time point with peak titres for the epitope of interest. Chimeric viruses engineered by overlapping PCR were tested for neutralisation sensitivity to these plasmas, and recombinant chimeric proteins were expressed for adsorption studies. Potential escape mutations were introduced via site-directed mutagenesis.

Results: Similar to antibodies targeting C3-V4, neutralising antibodies targeting the \(\beta14+\) V5 region of gp120 developed to high titre during acute infection. In both instances these antibodies could be adsorbed with monomeric gp120; however this adsorption did not often account for the total neutralisation activity at a particular time point. These residual titres were shown to be the result of concurrent antibodies against quaternary epitopes in the V1-V2 domain. Escape from C3-V4 and \(\beta14+\) V5 antibody specificities were due to changes in charge or glycosylation within the epitope, with the exception of one individual that escaped C3-V4 neutralising antibodies through the acquisition of a glycan in V1.

Conclusion: Collectively this data identified two key immunogenic regions on monomeric gp120 often targeted by early neutralising antibody responses during acute subtype C infection.
In the current study, we conducted mouse experiments and protein may suppress T cell immune responses. The non-HIV vaccine field have suggested that co-delivery of DNA primary HIV-1 across different clades. At the same time, studies in important implications on the future design of prime-boost HIV vaccines. The above results indicated that DNA prime plays a critical role in immune activation and this finding will have important implications on the future design of prime-boost HIV vaccines.

**Conclusion:**

DNA prime-protein boost immunizations.

**Results:**

Our results showed that the protein alone approach was the least effective. While DNA/protein-DNA/protein was more effective than the protein alone approach in eliciting slightly higher antibodies, it was much less effective than the DNA-protein approach. Interestingly, DNA/protein-DNA/protein approach elicited lower antibody responses than the DNA-DNA approach, supporting the previous claim that adding a protein component to the DNA vaccine (coding for the same antigen) can be “suppressive” to the original immune responses elicited by a DNA alone vaccine. We further identified that such suppression is dose dependent, i.e. higher doses of co-delivered DNA/protein vaccines lead to lower antibody responses.

**Conclusion:**

The above results indicated that DNA prime plays a critical role in immune activation and this finding will have important implications on the future design of prime-boost HIV vaccines.

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**P04.32**

Antibody-Guided Exploration of V3 Exposure on HIV Subtype C


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**Background:**

Elucidating the exposure of antibody epitopes on HIV Env is important for vaccine immunogen design. While relatively much effort has been devoted to exploring accessibility of the V3 region on subtype B viruses, V3 exposure on subtype C viruses has yet to be fully understood. V3 is relatively more variable among subtype B than subtype C. However, the C3 region in subtype C exhibits greater sequence variability than in subtype B, suggesting different immunological pressures and possibly unique epitope exposure between these two subtypes. Investigating potential subtype-specific differences in V3 exposure may provide greater understanding of Env oligomeric structure and be insightful to immunogen design efforts.

**Methods:**

To assess the accessibility of V3 on subtype C viruses, anti-V3 monoclonal antibodies (MAbs) B4e8, 2219, and 268-D, isolated from subtype B infected individuals, and anti-V3 MAb 2557, isolated from a CRF02_AG infected individual, were tested for their ability to neutralize a panel of 10 subtype C primary isolates using a luciferase-based single-round pseudovirus assay.

**Results:**

Of the 10 subtype C isolates tested, 6 were not neutralized significantly by any of the 4 anti-V3 MAbs (IC50 <50% at 50 μg/ml). Unexpectedly however, the remaining 4 viruses (ZM53M, ZM249M, ZM233M and ZM197M) were neutralized with increasing potency at low antibody concentration (IC50 >50% at 2 μg/ml).

**Conclusion:**

The results suggest that in some subtype C viruses V3 might be more accessible to antibody than previously appreciated. To determine if neutralization of ZM53M, ZM249M, ZM233M and ZM197M at low antibody concentration is V3 specific, studies are ongoing to explore the sensitivity of these viruses to non-V3 MAbs. Studies are also ongoing to investigate the influence of the C3 region on V3 exposure by generating chimeric of V3 neutralization-sensitive and -resistant subtype C viruses. These chimeric viruses will be tested for neutralization sensitivity to V3 and non-V3 MAbs.

**P04.33**

Epitope Specificity and Envelope Diversity in an Elite Neutralizer from the San Francisco Bay Area


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**Background:**

In this report we describe an elite neutralizer (EN) with unusually potent broadly neutralizing antibodies. We have used swarm analysis of viral quasi-species to define the populations of antibodies responsible for this broad neutralizing activity, and to understand the characteristics of the envelope proteins that stimulated this unusual immune response.

**Methods:**

Sera from 17 volunteers were screened for broadly neutralizing antibodies (bNAbs) with pseudotype viruses from 5 different clades. Envelope genes were cloned from GSID001 plasma, and 11 with high infectivity were sequenced and tested for neutralization sensitivity/resistance. Additional neutralization studies were carried out with pairs of viruses from other donors.
Results: Sera from GSID001 potently neutralized 23 of 24 viruses in a panel of international isolates (GMT = 1179). While most of the autologous clones from subject GSID001 were resistant to neutralization, clone 029 was sensitive to neutralization. Sequence analysis identified 60 amino acid differences between clone 029 and neutralization resistant clone 012. Site directed mutagenesis was used to localize residues responsible for this difference. Testing of viral envelope clones from other donors yielded an additional 7 pairs of neutralization sensitive/resistant envelopes (5 clade B and 2 CRF A/E from Thailand) appropriate for epitope mapping. For example these studies showed that deletion of a glycosylation site at position 196 (N196H) in the V2 domain markedly enhanced neutralization sensitivity by GSID001 as well as another EN, Z23. Studies in progress have localized additional epitopes responsible for the broadly neutralizing activity.

Conclusion: The antibody response in GSID001 sera is an example of the type of broadly neutralizing immune response that we wish to elicit with a candidate HIV vaccine. Understanding the specificity of the antibody populations responsible for this broad neutralizing activity, and the antigenic structure of the envelope proteins that stimulated this unusual immune response, should allow us to build improved vaccine antigens.

P04.34 Mapping the Specificity of Broadly Neutralizing Antibodies from Thai Elite Neutralizers by Analysis of crf A/E Virus Quasi-Species

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Background: A major goal of HIV vaccine development is to identify immunogens that elicit broadly neutralizing antibodies (bNAbs) similar those found in rare HIV-infected individuals termed elite neutralizers. Although a few epitopes recognized by bNAbs have been defined for individuals infected with clade A, B, and C viruses, they have not been described for clade E (crf A/E). In this report we analyzed the specificity of neutralizing antibodies from Thai elite neutralizers using viruses collected from a cohort of injection drug users from Bangkok.

Methods: Envelope gene quasi-species were amplified from 30 individuals, and pseudotype viruses were evaluated for sensitivity and resistance to antibodies from 3 Thai elite neutralizers. Pairs of neutralization sensitive and resistant viruses were identified and amino acids responsible for neutralization sensitivity were localized by site directed mutagenesis.

Results: Analysis of mutations in Thai viruses that conferred neutralization sensitivity and resistance appeared to correspond to epitopes recognized by bNAbs. In contrast, mutations in North American viruses identified by this approach appeared to correspond to mutations that induced conformational changes affecting multiple epitopes. Moreover we found that different populations of neutralizing antibodies were present in different elite neutralizers. In some cases different antibody populations from different elite neutralizer sera could be mapped with virus quasi-species from a single patient. Mutations that conferred neutralization sensitivity/resistance included a unique site in the V1 domain, and a conserved residue in the V3 domain. Other sites recognized by different individuals in needle sharing transmission linkage groups are currently under investigation.

Conclusion: These studies identify unique amino acids that affect the binding of bNAbs from Thai elite neutralizers. Envelopes with these mutations potentially represent a new source of improved vaccine immunogens. It will be interesting to determine whether antibodies to the sites identified might represent potential correlates of protection in the RV144 vaccine trial.

P05.01 Enzyme Digests Eliminate Non-Functional Env from HIV-1 Particle Surfaces Leaving Native Env Trimmers Intact and Viral Infectivity Unaffected

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Background: HIV-1 viruses and virus-like particles (VLPs) bear aberrant Env species that could undermine the development of antibody responses against Env trimers, thereby blunting the ability of particles to elicit neutralizing antibodies. Here, we sought to better understand the nature of “junk” Env and to devise strategies for its removal.

Methods: VLPs were analyzed by blue native PAGE-Western blot, SDS-PAGE-Western blot and in infectivity assays.

Results: Native trimers are surprisingly stable in the face of harsh conditions, suggesting that junk Env does not arise from trimer dissociation. The limited gp120 shedding that can occur immediately after synthesis of primary HIV-1 Env was found not to be caused by aberrant cleavage at the tandem gp120/gp41 cleavage sites, which were cleave co-dependently. Our data suggest that glycosylation may influence gp120 shedding. A major VLP contaminant of particles was found to consist of an early, monomeric form of gp160, termed “gp160ER” that bypasses normal protein maturation and trafficks directly into particles from the endoplasmic reticulum. Gp160ER was found to bind two copies of mAB 2G12, consistent with its exclusively high mannosyl glycan profile. These findings prompted us to evaluate enzyme digests as a way to remove aberrant Env. Remarkably, sequential glycosidase-protease digests led to a complete or near-complete removal of junk Env from many viral strains, leaving trimers and viral infectivity largely intact.

Conclusion: “Trimer-VLPs” may be useful neutralizing antibody vaccine immunogens.

P05.02 Neutralising Antibodies After Immunisation with the Transmembrane Envelope Proteins of HIV-1, HIV-2, Two Foamy Viruses, and Three Gammaretroviruses

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Background: Broadly neutralising antibodies binding to the membrane proximal external region (MPER) of the transmembrane envelope (TM) protein gp41 of HIV-1 designated 2F5 and 4E10 have been isolated from HIV-infected individuals. All
Attempts to induce such antibodies failed until now. In contrast, in immunisation studies with the TM proteins of gammaretroviruses neutralising antibodies to the MPER of their TM protein were successfully induced.

**Methods:** Recombinant proteins corresponding to the ectodomain of the TM proteins of HIV-1, HIV-2, the feline foamy virus (FFV), and the gammaretroviruses feline leukaemia virus (FeLV), porcine endogenous retrovirus (PERV) and Koala retrovirus (KoRV) were used for immunisation of mice, rats and goats; binding and neutralising antibodies were determined. In the case of FeLV cats were immunised in addition and in vivo challenge experiments were performed.

**Results:** Immunisation with the TM proteins of FeLV, KoRV and PERV resulted in neutralising antibodies binding to the MPER as well as to the fusion peptide proximal region of the corresponding TM protein. One epitope in the MPER showed a sequence homology with the epitope of 4E10 in gp41. The neutralising antibodies induced by the TM protein of FeLV protected cats from FeLV challenge. In contrast, immunisation with the TM proteins of HIV-1, HIV-2, FFV and PFV did not result in neutralising antibodies and no antibodies binding to the MPER were found.

**Conclusion:** Whereas immunisation with the small TM proteins of different gammaretroviruses resulted in neutralising antibodies binding to the MPER, immunisation with the larger TM proteins of two lentiviruses and two foamy viruses failed. The reason for the difference is unclear and needs further investigation. Whereas the TM proteins of the gammaretroviruses are unglycosylated in the virus and those of the other viruses are glycosylated, all antigens used for immunisation were produced in bacteria and were non-glycosylated.

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**P05.03**

**Immunogenicity of a Stable Clade C HIV-1 gp140 Envelope Trimer in Different Adjuvants in Guinea Pigs**

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**Background:** The native envelope (Env) spike on the surface of human immunodeficiency virus type 1 (HIV-1) is trimeric, and thus trimeric Env vaccine immunogens are currently being explored in preclinical immunogenicity studies. We previously reported the binding and neutralizing antibody (NAB) responses in guinea pigs elicited by clade A (92UG037.8) and clade C (CZA97.012) Env gp140 trimer immunogens selected and engineered for optimal biochemical stability.

**Methods:** To improve the NAB responses elicited by the clade C gp140 trimer in Ribi adjuvant, we assessed its stability and immunogenicity in four formulations of Glycopyranosyl Lipid Adjuvant (GLA); aqueous, liposome, emulsion and alum, as well as CpG, Emulsigen, and R848 adjuvants. Stability was assessed by size-exclusion chromatography upon trimer re-purification from each adjuvant. Immunogenicity was determined by ELISA and TZM.bl NAB assays after immunizing guinea pigs 3 times with 100 μg of protein trimer in the various adjuvants.

**Results:** By size-exclusion chromatography, the clade C gp140 trimer exhibited monodisperse peaks when re-purified from GLA formulations, CpG, Emulsigen, and R848 indicating conformational stability in these adjuvants. Ribi adjuvant, however, showed evidence of protein aggregation. Comparable binding antibody ELISA titers of 6.5 logs were observed in all groups after two immunizations. The four GLA formulations and combinations of CpG with either Emulsigen or R848 adjuvants, all elicited potent, cross-clade NAB responses against select tier 1 viruses that were comparable to Ribi adjuvant. Against the more stringent tier 2 clade C viruses, the groups that received the GLA emulsion and aqueous adjuvants generated limited but clearly detectable tier 2 responses as compared with Ribi adjuvant.

**Conclusion:** These data demonstrate the immunogenicity of our stable, clade C gp140 trimer in different adjuvant systems. The effects of adjuvants on conformational structure and integrity may be important in the selection of adjuvants for optimal induction of Env specific NABs.

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**P05.04**

**Defects In B Cell Intrinsinc- & Extrinsic Factors Distinguish Novel 2009 H1N1 Vaccine Responders From Non-Responders Among HIV Infected Persons on ART**

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**Background:** This study investigated innate and adaptive immune responses associated with antibody (Ab) responsiveness to a single dose of non-adjuvanted H1N1/09 vaccine in 17 virologically suppressed HIV infected patients and 8 healthy controls (HC). H1N1 Ab titers of >1:40 units/4 fold rise developed in all HC but in only 9/17 patients.

**Methods:** PBMC and serum were isolated from venous peripheral blood pre-vaccination (T0) and post vaccination on day7 (T1) and day28 (T2). Immunologic assessments were made in fresh PBMC by multiparameter flow cytometry and in cryopreserved PBMC/serum by ELISPOT/ELISA.

**Results:** At T1, HC and vaccine responder (R) patients developed approximately 3 fold expansion of plasmablasts and >4 fold increase in spontaneous H1N1 antibody secreting cells (ASC). At T2, HC and R patients had expansion of memory B cells (HC, 1.4 fold; R, 2.5 fold) with increases in ex-vivo H1N1-stimulated IgG ASC, innate immune factors BAFF (B cells activating factor), APRIL (a proliferation-inducing ligand) and T cell cytokine interleukin (IL)-21. Concurrently IL-21R and TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) were upregulated and BAFFR downregulated on B cells. Frequency of CD4+ CXCR5+ T follicular helper like cells increased (HC L3 fold, R 1.4 fold). Vaccine non-responder (NR) patients did not manifest these immunologic responses. At T0, R and NR patients were equivalent in mean age, plasma HIV RNA, CD4 and CD8 T cell counts and CD20+ B cells. Phenotypic memory B cells were equivalent (but lower than HC) between R and NR patients but frequency of BAFFR+ and TACI+ B cells were higher in R patients.

**Conclusion:** Failure to mount H1N1 Ab responses was associated with multiple deficits of innate and adaptive immunity in association with altered B cell phenotype in otherwise stable HIV infected patients. These findings point to new avenues for investigation in future vaccine strategies.

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**P05.05**

**Predicted Antigenic Map of the V2 Loop**

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**Background:** The native envelope (Env) spike on the surface of human immunodeficiency virus type 1 (HIV-1) is trimeric, and thus trimeric Env vaccine immunogens are currently being explored in preclinical immunogenicity studies. We previously reported the binding and neutralizing antibody (NAB) responses in guinea pigs elicited by clade A (92UG037.8) and clade C (CZA97.012) Env gp140 trimer immunogens selected and engineered for optimal biochemical stability.

**Methods:** To improve the NAB responses elicited by the clade C gp140 trimer in Ribi adjuvant, we assessed its stability and immunogenicity in four formulations of Glycopyranosyl Lipid Adjuvant (GLA); aqueous, liposome, emulsion and alum, as well as CpG, Emulsigen, and R848 adjuvants. Stability was assessed by size-exclusion chromatography upon trimer re-purification from each adjuvant. Immunogenicity was determined by ELISA and TZM.bl NAB assays after immunizing guinea pigs 3 times with 100 μg of protein trimer in the various adjuvants.

**Results:** By size-exclusion chromatography, the clade C gp140 trimer exhibited monodisperse peaks when re-purified from GLA formulations, CpG, Emulsigen, and R848 indicating conformational stability in these adjuvants. Ribi adjuvant, however, showed evidence of protein aggregation. Comparable binding antibody ELISA titers of 6.5 logs were observed in all groups after two immunizations. The four GLA formulations and combinations of CpG with either Emulsigen or R848 adjuvants, all elicited potent, cross-clade NAB responses against select tier 1 viruses that were comparable to Ribi adjuvant. Against the more stringent tier 2 clade C viruses, the groups that received the GLA emulsion and aqueous adjuvants generated limited but clearly detectable tier 2 responses as compared with Ribi adjuvant.

**Conclusion:** These data demonstrate the immunogenicity of our stable, clade C gp140 trimer in different adjuvant systems. The effects of adjuvants on conformational structure and integrity may be important in the selection of adjuvants for optimal induction of Env specific NABs.
Background: The V2 loop of HIV-1 strains is immunogenic and exhibits an $\alpha_4\beta_7$ receptor binding site.

Methods: We inferred an antigenic map of the V2 loop by controlling for its length distribution across circulating strains 1) by multiply aligning only the sequences of the most common V2 loop length (40 amino acids) and 2) by taking length into account in deriving a consensus sequence. Ab initio folding was used to evaluate the local secondary structure preferences of a middle conserved block of amino acids spanning the $\alpha_4\beta_7$ receptor-binding site. The sequence conservation pattern, known glycosylation sites and known anti-V2 Ab binding sites were then mapped.

Results: The V2 loop exhibits an asymmetric distribution of chain lengths with few or no chains less than 38 amino acids and most chains being 40 amino acids in length or longer. There are three lengths with few or no chains less than 38 amino acids and most chains being 40 amino acids in length or longer. There are three blocks of conserved amino acids in the V2 loop, and most of the length variation occurs between the second (containing the second conserved block of amino acids in the V2 loop, and most of the length variation occurs between the second (containing the $\alpha_4\beta_7$ receptor binding motif) and third block. Ab initio folding demonstrated that the second block has a preference for alpha-helical secondary structure. Mapping the amino acid conservation pattern of the second conserved block onto an alpha-helix clusters secondary structure. Glycan chains or residue positions with poor amino acid conservation among circulating strains are predicted to obscure most of the remainder of the molecular surface.

Conclusion: A 3D map of the second sequence conserved block of the V2 loop is an antigenic map exhibiting an exposed site complementary to the $\alpha_4\beta_7$ receptor binding motif that is poorly conserved among circulating strains. The second block has a preference for alpha-helical secondary structure. Mapping the amino acid conservation pattern of the second conserved block onto an alpha-helix clusters secondary structure. Glycan chains or residue positions with poor amino acid conservation among circulating strains are predicted to obscure most of the remainder of the molecular surface.

Results: We have identified HIV clade C early transmitted virus 16055 Env that binds with nanomolar affinity to PG9, VRC01 and CD4lgG. 16055 Env when converted to gp140 trimers binds trimers-specific PG9, PG16, CD4 binding site-specific VRC01, PGV04, b12 and gp41-specific 4E10 bnAbs. High affinity binding of 16055 gp140 to trimmer specific PG16 antibody suggests that these molecules present conformation of the variable loops 2 and 3 representative of functional HIV-1 Env. Importantly, 16055 virus shows binding and neutralization by germ line precursor mimic for PG9. Mutagenesis of asparagine (N) at position 160 and glutamic acid (E) at position 168 abrogates PG9 and PG16 binding on the 16055 Env but do not effect CD4lgG binding. Furthermore, the PG9 and PG16 binding and neutralization property of 16055 was transferred to 16936 virus by V1V2 and V3 loop swap. 16936 virus, in the absence of loop swap, is resistant to PG9 and PG16.

Conclusion: 16055 soluble Env proteins mimic some conformational characteristics of the native spike as indicated by PG16 antibody binding. 16055 Env will help understand the V1V2V3 loops conformation and as novel HIV immunogen.

P05.07
Native HIV-1 Sequential Envelope Quasispecies as a Novel B Cell Vaccine Concept


Background: HIV-1 evolves rapidly within the host, resulting in the development of diverse HIV-1 variants called a viral “quasispecies” population. Envelope (Env) is the only target of neutralizing antibodies (NAbs), which can prevent infection of target cells. NAbs increase in titer and affinity over time as Env diverges. A major goal of HIV-1 vaccine efforts, so far elusive, is the design of Env-based immunogens effective at eliciting broad NAbs (BNAbs). We hypothesize that B cells are programmed to develop NAbs by exposure to Env presented by the viral quasispecies variants.

Methods: We cloned 15 env genes from different timepoints in a macaque that developed BNAbs following SHIV-SF162P4 infection. Rabbits were immunized (gp160-DNA prime, gp140-protein boost) with: (1) sequential env clones (Sequential); (2) a cocktail of env clones (Mixture); or (3) a single env variant (Clonal). We identified HIV+ subjects with BNAbs and developed DNA and protein vaccines based on these quasispecies variants.

Results: All groups immunized with the SHIV envs generated strong ANAbs and modest HNAbs against Tier-1 viruses. Late autologous clones were more difficult to neutralize than early ones. Specific PNGs in V1, V2 and V4 are involved in neutralization resistance, as are key amino acid residues. The Mixture and Sequential strategies replicated the epitope targeting of the V3 loop seen in the macaque. The Sequential and the Clonal strategies elicited higher antibody affinity maturation than the Mixture. Vaccines based on the human HIV Env quasispecies are in testing.
by exposing it to a native HIV-1 quasispecies derived from an individual with BNAbs.

P05.08
Robust Multi-Clade Cellular and Humoral Immune Responses in Rhesus Macaques Following Optimized Consensus DNA Vaccination

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Background: Advancements to the DNA vaccine platform have improved the magnitude and quality of the immune response achieved in both small and large animal models. Data presented at the recent Keystone HIV Vaccine meeting by the HVTN from our HVTN 080 trial demonstrates that the CD4 and CD8 cellular responses induced by the combination of a highly optimized, cytokine adjuvanted, HIV-1 DNA vaccine (PENNVAX) delivered by EP (E-DNA) in humans at are as good or better than those induced by viral vector systems alone, or in prime boost combinations. Recently, we have observed that E-DNA outperforms adjuvanted envelope protein for induction of neutralization antibody titers, results improved with a DNA prime protein boost. Here we sought to define the potential of a DNA prime for inducing both cellular and humoral responses in non-human primates prior to a protein boost.

Methods: Three groups of five rhesus macaques received 1.0 mg consensus pVax HIV-1 M gag, HIV-1 M pol, and HIV-1 B either IM 0.5 Amps, ID 0.2 Amps, or ID 0.1 Amps. A Fourth group of five macaques included HIV-1 Env A, C, D, and A/E IM. DNA was delivered at weeks 0, 6, 12 and 18.

Results: CD8+ responses greater than 1% were observed following only two doses of E-DNA in all groups. Multi-envelope vaccination induced multiclade T-cell responses as well as a ten-fold increase in antibody binding titers to HIV-1 Env compared with single clade groups. Antibody titers greater than 1:1000 were consistently achieved with E-DNA alone.

Conclusion: These data demonstrate that optimized E-DNA can induce robust immune responses in the NHP model. The inclusion of multiple envelope vectors induces cross-reactive responses that increase the breadth and magnitude of vaccine induced responses. Additionally, E-DNA priming can induce potent antibody response that may be boosted by a recombinant protein boost.

P06.01
Motivators of Enrollment in HIV Vaccine Trials: A Review of HIV Vaccine Preparedness Studies

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Background: HIV vaccine preparedness studies (VPS) are important precursors to HIV vaccine trials. As well, they contribute to an understanding of motivators and barriers for participation in hypothetical HIV vaccine trials. Motivators can take the form of altruism and a desire for social benefits. Perceived personal benefits, including psychological, personal, and financial well-being, may also motivate participation.

Methods: We performed a systematic review of HIV VPS using the Cochrane Database for Systematic Reviews, Medline/Pubmed, Embase, and Google Scholar. Two people independently searched the literature for individual HIV VPS that examined motivators of participation in a hypothetical HIV vaccine trial. The denominators employed in the literature varied across studies, and these were standardized to the number of respondents per survey item, regardless of their willingness to participate (WTP) in an HIV vaccine trial. The Organization for Economic Co-operation and Development (OECD) countries and the non-OECD countries were compared with respect to these motivators.

Results: We retrieved eight studies on social benefits (i.e., altruism) and 11 studies on personal benefits conducted in the OECD countries, as well as 19 studies on social benefits and 20 studies on personal benefits in the non-OECD countries. Various different forms of altruism were found to be the major motivators for participation in a hypothetical HIV vaccine trial in the OECD and the non-OECD countries. In a large number of studies, protection from HIV was cited as a personal motivator for participation in a hypothetical HIV vaccine trial in the OECD and the non-OECD countries.

Conclusion: This is the first comprehensive review examining motivators of participation for an HIV vaccine trial. Knowledge of motivators can inform and target recruitment for HIV vaccine trials, though it must be remembered that hypothetical motivators may not always translate into motivators in an actual vaccine trial.

P06.02
Barriers to Enrollment in HIV Vaccine Trials: A Review of HIV Vaccine Preparedness Studies

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Background: Barriers to participation in an HIV vaccine trial have been examined in many HIV vaccine preparedness studies (VPS). These barriers can be understood in terms of the locus of the barrier (personal vs. social) and the nature of the barrier (risk vs. cost). Another type of barrier is perceived misconceptions.

Methods: We performed a systematic review of HIV VPS using the Cochrane Database for Systematic Reviews, Medline/Pubmed, Embase, and Google Scholar. Two people independently searched the literature for HIV VPS that examined barriers of participation in a hypothetical HIV vaccine trial, using the same search strategy. We categorize these barriers, and compare barriers between the Organization for Economic Co-operation and Development (OECD) countries and the non-OECD countries. Risk was operationalized in terms of possible outcomes; costs in terms of very probably outcomes.

Results: In the OECD countries, we retrieved 18 studies reporting personal risks (PR), 7 studies reporting social risks (SR), and 15 studies reporting misconceptions. In the non-OECD countries, we retrieved 22 studies reporting PR, 16 studies reporting SR, 14 studies reporting PC, 1 study reporting social costs (SC), and 19 studies reporting misconceptions. Important PR were “adverse effects” and “vaccine-induced seropositivity”, and “temptation to have unsafe sex” in men who have sex with men (MSM). “Discrimination” was a common SC. “Fear of needles” and “time commitment” were important PC, and “family commitments” were a SC in one non-OECD country. “Distrust of institutions” and “HIV infection from the vaccine” were common misconceptions.
Conclusion: This is a comprehensive review to participation in an HIV vaccine trial. Both the OECD and non-OECD countries have similar barriers, and people’s decisions to participate in a clinical trial involve multiple barriers. However, these barriers apply to hypothetical HIV vaccine trials, and barriers for actual vaccine trials need more assessment.

P06.03 Development of CAVD/EQAPOL Long-Term PBMC Repository (‘07-’10) @ DUMC; T-Cell Functionality as Measured by ELISpot and Flow Cytometry Is Maintained Over Time


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Background: Testing of clinical samples to evaluate vaccine-induced responses by endpoint laboratories requires extensive standardization and validation of the assay and corresponding controls to monitor assay performance. Samples with well-characterized functionality are needed for assay development and comparison to preexisting assays. To provide these samples, we have established a PBMC repository in which cells are processed and stored to retain viability, recovery and functionality. The repository includes HIV seropositive and seronegative donor samples with a wide range of CEF-, CMV-, and HIV-specific functional responses.

Methods: PBMCs were collected by leukopheresis from 223 donors, isolated by density gradient separation, and cryopreserved using a rate-controlled freezer. For all samples, viability, recovery, and functionality were assessed by IFN-γ ELISpot within 4 weeks of freezing. We evaluated viability, recovery, and functionality every 6 months in 5 samples with different levels of responses and monthly in 1 low/moderate responder. Finally, in a subset of 70 samples functionality was measured by both ELISpot and IFN-γ/IL-2 ICS assays.

Results: Viability of 97.7% of the samples was above 80%, and the recovery of 90.5% was above 66% after thawing and resting the cells overnight. For the 5 low/moderate and high CEF and CMV responders, there was no specific trend suggesting a loss in viability or recovery over time. Functionality was retained in 4 of the 5 samples. No specific trends suggesting degradation for any parameter were observed for the low/moderate responder measured monthly. A significant correlation was observed for CEF (R2 = 0.91, p < 0.0001) and CMV (R2 = 0.93, p < 0.0001) IFN-γ responses measured by ELISpot and ICS. Since 2007, approximately 4,300 PBMC vials have been distributed to 23 laboratories, worldwide, to perform assay development, validation & quality assurance.

Conclusion: In conclusion, we have developed PBMC repository for use by endpoint laboratories for assay validation and development across different networks.

P06.04 Prevalence, Incidence, Risk Factors and Willingness to Participate in HIV Vaccine Trials Among Gay and Bisexual Men and Transgender Persons Seeking HIV

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Background: HIV prevalence among men who have sex with men (MSM) and transgenders (TG) persons is high and increasing in Chiang Mai, northern Thailand.

Methods: In 2008-09, gay and bisexual men and transgender women attending HIV testing and counseling at an MSM friendly clinic in Chiang Mai (PIMAN Clinic) were interviewed and tested for HIV and STI. We sought to investigate demographic, socioeconomic, sexual behavior and interest in future HIV prevention trials among these participants and to conduct pre-screening for the iPrEx pre-exposure chemoprophylaxis trial. Univariate and multivariate regression analyses were done to assess associations with HIV infection.

Results: A total of 551 MSM clients (56.1% gay, 25.4% TG, and 18.5% bisexual (BS)) were enrolled. The mean age was 23.9 years. HIV prevalence among MSM overall was 12.9% (71/551; 16.5% among gay men, 9.3% among TG, and 6.9% among BS. Consistent use of condom was low, 33.3% at last insertive anal sex and 31.9% in last receptive anal sex. Most, 55.4%, had prior HIV testing, and 64.6% had VCT within 1 year. Sex role segregation was marked, with nearly all TG reporting only receptive anal sex; nearly all BS reporting exclusive insertive sex, and most gay men reporting both roles. Interest in participation was high at 69.7%, for HIV vaccine trials. Interest in trial participation was not associated with HIV infection or risks. HIV was independently associated with being gay identified, aOR 2.7, p = 0.037; and with being aged 25–29, aOR 2.7, p = 0.027. Among repeat testers, HIV incidence was 8.2/100 PY, 95% CI, 3.7/100PY to 18.3/100PY.

Conclusion: These young and at risk populations are in urgent need of novel HIV prevention strategies and are willing to participate in HIV vaccine research.

P06.05 Challenges in Consenting Sex Workers, Transgenders and Men Who Have Sex with Men into an HIV Acute Infection Study in Pattaya, Thailand

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Background: The acute stages of HIV-1 infection influence disease progression. Understanding the earliest host-virus interactions is critical to vaccine development. The Armed Forces Research Institute of Medical Sciences is conducting an acute infection study in Pattaya. Starting mid-2009, it recruited among sex workers, transgenders, and men who have sex with
Careful screening and an ongoing process that un-

Conclusion:

the main consent was long and technical.

respondents and “want to do good” 82. Most volunteers reported

pation reported at screening are “want to know HIV status” 201

ology during counseling sessions. Primary reasons for partici-

formance is given to review and an appointment is made to return.

For those determined ready, a one-on-one review of the main

ment followed by a test of understanding is done. Once

view key points of the trial and discuss factors affecting their participation.

Results: 910 people were seen, of whom 455 were screened. All

passed the test of understanding. 322 were enrolled and could

date the trial’s objective and rationale for the research methodology
during counseling sessions. Primary reasons for participa-

tion reported at screening are “want to know HIV status” 201

respondents and “want to do good” 82. Most volunteers reported the

main consent form was long and technical.

Conclusion: Careful screening and an ongoing process that under-

lines the trial’s objective and research methodology can help

volunteers maintain understanding of their participation. How-

ever, common factors related to sex work affect level of under-

standing and rationale for participation.

Results: 237 subjects were enrolled in Lima. The loss of follow-up

was 20% in the first year (the majority lost within the first 6

mouths) and then stabilized for year 2 (10%) and 3 (12%). HIV

incidence was 4%, with most of the new infections (87%) occur-

ring between visits 2 and 4, resulting in a seroconversion rate of

9% in the first year. In the Barcelona cohort, 160 individuals were

recruited thus far, with 3 seroconversions observed to date (all

occurring within the first three visits). Retention rate was 92%.

Conclusion: Prospective tailoring of cohort-specific retention

strategies in consultation with community members and applying

initial questionnaires to assess high risk helps to establish

cohorts with elevated seroconversion rates. The concentration of

new infections at earlier time points may facilitate cohort man-

agement (in terms of duration, number of enrollees) to capture

new infections and, importantly, may reflect an educational effect

among the enrollees during their participation in these studies.

Factors Influencing Recruitment, Retention and

Seroconversion Rates in MSM at High Risk for HIV

Infection in Lima and Barcelona

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Background: The availability of well-defined cohorts with high

retention rates is crucial for HIV vaccine development. Aside from

representing suitable cohorts for vaccine efficacy trials, closely

monitored cohorts of individuals at elevated risk for HIV infec-
tion also offer access to individuals with acute HIV infection

and highly exposed persistently seronegative subjects (HEPS), for

the study of factors associated with increased or reduced sus-
cceptibility to HIV acquisition.

Methods: Men who have sex with men (MSM) were recruited in

Lima and Barcelona by advertising in community-based HIV

detection centres, MSM venues and magazines. Individuals at

elevated risk were selected through an initial “risk-assessment”

questionnaire. Participants were followed on a quarterly basis for

HIV infection. In the Barcelona cohort, participants were also

screened for the main sexually transmitted infections. Data from

questionnaires and blood samples were collected at each visit.

Background: The GAIA Vaccine Foundation (VF) has been col-

aborating with Malian HIV clinicians, scientists and community-

based organizations to prepare a site for Phase I-III HIV vaccine

trial in Sikoro, a multi-ethnic neighborhood of Bamako, Mali

where more than 40,000 people live; 90% are illiterate and

roughly 50% are unemployed.

Methods: GAIA VF has worked to improve HIV prevention

through MTCTP, access to HIV treatment and peer education.

HIV specialists working at the Hope Center Clinic (HCC), a

community-based infirmary located in Sikoro, have improved

prevention and set up a comprehensive clinical and biological

cart review of patients treated with ARV. The HCC was built in

2007–08 and became the first village-based clinic with an HIV care

program in February 2009.

Results: 8,210 pregnant women at the village clinic have been

tested for HIV; 174 were diagnosed with HIV. MTCTP was of-

ered to all women; 99% accepted. Until 2009, HIV + patients at

the clinic were transported to off site for treatment. > 120 patients

have enrolled since 2009 at the new on-site care center, of which

59 are on treatment. GAIA VF has also performed three KAP

studies in Bamako, in collaboration with the DRS, to evaluate

baseline levels of HIV knowledge and willingness to participate

in an HIV vaccination trial. Of 399 persons surveyed in Sikoro,

78% were willing to participate (WTP) in an HIV vaccine trial,

65% were WTP in a Malaria vaccine trial and 61% were WTP in a

TB vaccine trial. Women were more WTP (81%) than men (76%).

Conclusion: Providing HIV care and education allowed GAIA

VF to reinforce the rapport between clinic staff and community

members, assess HIV knowledge and misconceptions and WTP

in a vaccine trial. This groundwork will help GAIA VF set up a

Phase-I-III HIV vaccine trial site in this region of Mali, West

Africa.
P06.08
Education and Research Initiatives for the Mobilization of African-American Faith Communities for HIV/AIDS Prevention and HIV Vaccine Awareness

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Background: The African-American (AA) community bears a disproportionate burden of HIV in the United States and the AA church must assume a leadership role in stemming the epidemic. However, AA clergy may feel ill-equipped to engage the issue without HIV education that aligns with their faith tradition. We established a collaborative program to provide AA clergy with the understanding needed to lead discussions on HIV-related issues, including vaccine research, with their congregations.

Methods: Capacity building for AA church leadership in Rochester, NY was provided through a series of seminars/group discussions between local clergy and a CDC-supported spiritual leader, Rev. Edwin Sanders II, from MICTAN in Nashville, TN. Additional support was provided by the NIAID-funded HVTN Legacy Project, the UR HVTN site and 2 local community-based organizations with faith initiatives. Clergy participated in four monthly seminars presented by nationally-recognized AA leaders in the church/academia who addressed barriers to clergy engaging in HIV ministry. Participants were required to attend at least 3 seminars to be eligible to attend the annual MICTAN “Faith Academy” meeting in Nashville; those who completed written assignments received certification from MICTAN.

Results: The program was well-received by Rochester’s AA clergy. Fifty-one clergy attended the introductory seminar, 20 fulfilled requirements to attend the “Faith Academy”, 19 travelled to Nashville and 12 received certification. Pastors embraced universal core values for engaging in inclusive HIV/AIDS ministries and expressed eagerness to continue collaborating with UR and MICTAN.

Conclusion: Many AA clergy in Rochester, NY were willing to address HIV/AIDS issues when provided spiritually-grounded HIV/AIDS training. The monthly sessions created a safe environment for ongoing dialogue and an opportunity for relationships to develop. The program’s success hinged on sustained collaborative efforts between CDC, NIH, state and locally-funded partners to build the trust required for AA clergy to become invested in HIV vaccine research.

P06.09
Reference Values for Clinical Laboratory Parameters Among Youngs in Maputo City, Mozambique

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Background: Common practice in Mozambique is to use reference values from Europe or US provided by clinical laboratory assay systems. A planned phase I/II HIV vaccine trial in youths necessitated the establishment of reference values for clinical laboratory parameters.

Methods: A cross sectional study was conducted at a youth clinic in Maputo City, Mozambique. A total of 196 participants (18–24 years old) were enrolled, of which 144 were female and 52 male. Medical staff collected clinical histories using standardized forms. Immunophenotyping was performed using a single-platform lyse-no-wash procedure with Trucount tubes and MultiTest (CD3FITC/CD8PE/CD45perCP/CD4APC) reagents on a FACSCalibur cytometer (all from Becton-Dickinson). A complete blood count and differential was performed using a Sysmex KX-21N Hematology Analyzer. Serum chemistry was performed using a Vitalab Selectra Junior (Vital Scientific). Median and 2.5th-97.5th percentile reference ranges were established for immunology, hematology and chemistry values.

Results: Median levels were as follows: hemoglobin, 13.75 (male) and 11.2 (female) g/dL (p = 0.0001); platelets, 220x103/?L (male) and 264x103/?L (female) (p = 0.0028); leukocyte (WBC) counts, 4.9x103/?L (male) and 5.45x103/?L (female) (p = 0.018); absolute lymphocyte counts, 1869 cells/?L (male) and 2070 cells/?L (female) (p = 0.018); absolute CD4 T cell count, 698 cells/?L (male) and 828 cells/?L (female) (p = 0.0001); absolute CD8 T cell count, 467 cells/?L (male) and 474 cells/?L (female) (p = 0.8821); bilirubin, 0.65 (male) and 0.26 (female) mg/dl (p = 0.0001); albumin, 5.02 (male) and 4.78 (female) g/L (p = 0.0001); creatinine, 0.99 (male) and 0.74 (female) mg/dl (p = 0.0127); aspartate aminotransferase (AST), 25.05 (male) and 20.60 (female) U/L (p = 0.0001); alanine aminotransferase (ALT), 15.40 (male) and 11.00 (female) U/L (p = 0.0001).

Conclusion: We have reported reference values for hematology, immunology and biochemistry parameters in youths (18–24 years) which will inform inclusion criteria and evaluation of adverse events in planned HIV vaccine trials in Maputo, Mozambique.

P06.10
Incidence of HIV Among Youths in Maputo City, Mozambique: A Cohort Study

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Background: HIV prevalence in 15–49 year old individuals in Mozambique is 11.5%, being 16.8% in Maputo City. Across the country, HIV prevalence is higher in women than in men (13.1% vs 9.2%), peaking at ages 25–29 and 35–39 in women and men, respectively. This study aimed at determining the incidence of HIV and prevalence of other sexually transmitted infections in young adults, and at creating a cohort of youths for future HIV vaccine trials in Maputo City.

Methods: Young adults from both genders aged 18–24 (n = 1380) were recruited at a youth clinic and followed-up for 12 months. Clinical, demographic and behavioural data were collected using standardised questionnaires. HIV testing was conducted at screening and every four months using rapid antibody and PCR.
assays. Serological testing for hepatitis B (HBV) and syphilis was performed at screening. Viral load was measured in acute HIV cases using real-time PCR.

**Results:** Volunteers had a median age of 20.9 years and were mostly female (77.3%). All volunteers had some formal education, with 98.6% having at least the secondary level. Almost all (98.8%) of study participants reported at least one previous sexual intercourse, with a median age of 17 years at the first intercourse.

At screening, the prevalence of HIV, HBV and syphilis was 5.1% (95%CI 3.97%–6.31%), 11.1% (95%CI 9.31%–12.91%) and 0.51% (95%CI 0.13%–0.89%), respectively. The prevalence of HIV-HBV and HIV-syphilis co-infections was 0.59% (95%CI 0.16%–1.04%) and 100%, respectively.

In youths that tested HIV-negative at screening (n = 1309), the HIV incidence was 1.14/100 person-years (95%CI 0.57–2.27). All acute HIV infections (n = 8) occurred in female individuals. HIV plasma viral load in these patients ranged from 9370-188,419 copies/mL.

**Conclusion:** HIV incidence in this cohort of youths in Maputo City is relatively low. This cohort is suitable for the conduct of phase I/II vaccine trials.

**P06.11 The Impact of a Multi-Faceted Training and Mentoring Program in HIV Risk Reduction Counseling at Global HIV Vaccine Trial Sites**

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**Background:** HIV risk reduction counseling (RRC) is an essential component of HIV vaccine trial conduct; strategies to sustain high quality counseling at trial sites are needed. We sought to evaluate the impact of a novel training and support program for site RRC mentors to enhance local RRC quality assurance efforts.

**Methods:** In November 2008, the HVTN implemented an RRC mentor training program for NIAID-sponsored sites and those contracted by Merck, Inc. to conduct the Step trial. Thirty-eight sites designated RRC mentors to participate in a multi-faceted program including an RRC protocol/worksheets, Training of Mentors workshop, monthly webinar/call series, training at HVTN Conferences, personalized and small group consultations with an RRC training expert, an online clearinghouse of RRC resources, and a newsletter. In June 2010, an anonymous internet survey was administered to 203 counseling staff from participating sites.

**Results:** The overall response rate was 60% (n = 121). Approximately half (47%) of respondents were clinicians (physician/nurse). Ongoing RRC mentoring programs were reported by 91% (30/33) of currently active sites; virtually all mentors (97%) participated in the HVTN-organized program. At sites, mentors most frequently facilitated individual or group discussions about challenging counseling issues, provided targeted technical support, and addressed counselor fatigue. Mentors cited time constraints and scheduling problems with staff as the most common barriers to mentoring. A substantial majority (79%) of RRC staff indicated their counseling skills improved over time due to on-site mentoring/training activities with 75% directly attributing an increase in skills to the HVTN program.

**Conclusion:** Survey respondents reinforced that a centralized, multi-faceted training program to support local RRC mentors can facilitate RRC quality assurance efforts at global vaccine trial sites.

**P06.12 Hepatitis B Virus (HBV) Immunity After Vaccination in a Preparatory Study Among Men Who Have Sex with Men, Bangkok, Thailand, 2006–2009**

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**Background:** To develop clinical research infrastructure and assess hepatitis B (HBV) prevalence, vaccination uptake, and development of vaccine-induced immunity among men who have sex with men (MSM) in Bangkok with high HIV incidence in preparation for HIV prevention trials.

**Methods:** Prospective cohort study, follow-up at 4 monthly intervals for a minimum of 3 years. Study recruitment targeted community-based organizations, MSM entertainment venues and the Internet. We asked men to come to the clinic for eligibility evaluation, provide informed consent, complete a behavioral questionnaire using audio-computer-assisted self-interviewing, and undergo medical evaluation and HBV and HIV testing with pre- and post-test counseling. Baseline and 12 month HBV infection status was determined by serology. Those who were susceptible to HBV infection were offered the series of three HBV vaccinations free (ENGERIX-B®; 1 mL (20 mcg) given at months 0, 1 and 6).

**Results:** Between April 2006 and January 2008, 1,292 men (mean age 26 years) were enrolled (enrollment rate 98.6%). At baseline, 494 (38.2%) men had immunity from natural infection (anti-HBc+/-anti-HBs), 120 (9.3%) had acute or chronic infection (HBsAg); and 103 (8.0%) had vaccine-induced immunity (isolated anti-HBs). Of the remaining 572, 506 (88.5%) consented and received the 1st, 488 (96.4%) received the 2nd, and 453 (89.5%) received the 3rd dose. Of 433 vaccinees that completed 12 months of follow-up, 347 (80.1%) developed vaccine-induced immunity (anti-HBs), 60 (13.9%) developed no antibodies, 17 (3.9%) had evidence of immunity due to natural infection (anti-HBc/anti-HBs), 8 (1.8%) showed evidence of acute infection (anti-HBc, no anti-HBs), and 1 (<1%) was indeterminate.

**Conclusion:** High HBV prevalence, high HBV vaccination uptake and high rates of vaccine induced protection were observed in this preparatory cohort of MSM in Bangkok. HBV vaccination may serve as an appropriate model for evaluating and providing access to HIV vaccines among Bangkok MSM.

**P07.01 HLA Targeting Efficiency and Its Applications in Predictions of HIV Viral Load and HIV Disease Progression**


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**Background:** To assess the ability of HLA binding predictions to identify HLA targets associated with high viral loads and progression to AIDS.

**Methods:** A database of HLA class I peptides was used to predict HLA binding. Peptides associated with viral load were identified using a machine learning approach. The ability of these predictions to identify HLA targets associated with high viral loads and progression to AIDS was tested using a blinded validation set.

**Results:** The prediction algorithm was able to accurately identify HLA targets associated with high viral loads and progression to AIDS. The predictions were also able to identify HLA targets that were not associated with high viral loads or progression to AIDS.

**Conclusion:** HLA binding predictions can be used to identify HLA targets associated with high viral loads and progression to AIDS. These findings have implications for the development of new HIV vaccines.

**References:**

Background: The diversity of HLA binding preferences has been driven by the sequence diversity of short segments of pathogenic proteins, presented by HLA molecules. To identify commonalities in HLA binding preferences, we define a novel measure termed ‘targeting efficiency,’ which captures the correlation between HLA-binding affinities and the conservation of the targeted proteomic regions. Analysis of targeting efficiencies for 95 HLA Class-I alleles over thousands of human proteins and 52 human viruses indicates that HLA molecules preferentially target conserved regions in these proteomes. We predicted that ranking HLA alleles by their efficiency scores for a given virus would be predictive of the ability to control infection.

Methods: We analyzed the effect of efficiency scores for HIV-1 proteins on viral load in a study population of 191 HIV-1 clade-B infected, treatment-naive individuals from the Western Australian HIV cohort. We computed HLA targeting efficiency for each individual using their HLA-A and HLA-B alleles, thus approximating the aggregate ability of patient-specific HLA alleles to differentiate between conserved and variable targets.

Results: Overall HLA efficiency scores toward HIV-1 proteins were negatively correlated with viral load (ns, p = 0.24). However, HLA-B locus efficiency in targeting Gag was more strongly correlated with viral load (r = −0.19, p = 0.009), consistent with experimental evidence that HLA-B CTL responses to Gag play a significant role in control of HIV infection. Using multivariate regression to combine efficiency scores of all HIV-1 proteins and proteasomal cleavage, we found that efficiency scores accounted for 7% of variance in viral load (r = 0.27, p < 0.0004). We then analyzed the distribution of HLA efficiency scores for HIV-1 Gag and found that protective HLA alleles tend to rank more highly then alleles associated with rapid progression.

Conclusion: Ranking HLA alleles or individuals by their efficiency scores is predictive of clinical phenotypes and may assist in HIV vaccine design.

P07.02
TRIM5α Does Not Affect SIVmac251 Replication in Vaccinated or Unvaccinated Indian Rhesus Macaques

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Background: The SIVmac251 macaque model is widely used to evaluate the relative efficacy of HIV vaccine candidates. Thus, the understanding of natural factors that confer resistance to SIVmac251 replication in Rhesus macaques is key to minimize the over estimation of vaccine efficacy. HIV-1 does not infect macaques and the restriction of HIV replication in these old world monkeys is mediated at the post-entry level by the interaction of TRIM5α and the viral capsid (CA) protein. In vitro TRIM5α restriction depends on the dose of SIVmac251 used in the infectivity assay, suggesting the importance of the stoichiometry between the capsid and the TRIM5α proteins. However, TRIM5α in rhesus macaques is highly polymorphic. Recent studies have shown polymorphisms in the B30.2/SPRY domain of rhesus TRIM5α are associated with reduced efficiency of SIVmac251 replication in vivo.

Methods: We determined the distribution of polymorphic TRIM5α alleles in a cohort of macaques by DNA sequencing and assessed whether these alleles affected SIVmac251 virus levels and virus induced CD4+ T-cell depletion in naïve macaques or in macaques immunized with a combination of DNA-SIV/ALVAC-SIV/gp120 or ALVAC-SIV/gp120 vaccines. The synergistic effect of protective MHCClass-I alleles and the role of the dose of virus used in the challenge experiments, was also evaluated.

Results: Surprisingly, our results on a cohort of eighty two macaques, forty three vaccinated and thirty nine naive, demonstrated that previously-identified restrictive alleles of TRIM5α, did not appear to have a significant effect on SIVmac251 replication in vivo, regardless of prior vaccination for challenge dose. Furthermore, the presence of the protective alleles MamuA011, B081, or B0174 did not synergize with vaccination or TRIM5α.

Conclusion: Polymorphic TRIM5α alleles do not appear to influence the result of vaccination when the SIVmac251 is used in intra-rectal challenge experiments.

P08.01
Humoral and Cellular Immune Pressures May Converge at the Base of the V3 Loop During Rwandan Subtype A HIV-1 Infection

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Background: Despite continual rounds of viral escape, the human immune system deftly targets and eliminates circulating virions during HIV-1 infection. Better understanding how this targeting is accomplished could inform mechanisms to exploit the envelope’s vulnerabilities and to design vaccine immunogens that would irreversibly cripple the virus, disallow escape, and force viral clearance.

Methods: Along with that of the transmitted founder virus, envelopes from twenty longitudinal neutralizing antibody escape variants were cloned out of plasma from a Rwandan, subtype A-infected HIV-1 subject. Envelope sequence analysis revealed three mutational hot spots in gp120: C2, the alpha2 helix, and V5. Single mutations from the escape variants were introduced into the founder virus by site-directed mutagenesis, and resultant envelopes were assayed against autologous plasma and an autologous monoclonal antibody (mAb) to map sites of immune evasion. Peptides representing certain regions of mutation were deduced partial plasma resistance but rendered envelopes sensitive to the autologous mAb. Collectively, these residues responsible for
neutralization escape coalesced at a V3-proximal epitope. Of note is the phenomenon that, though the 340 mutation appeared to introduce a humoral vulnerability early during infection, it became fixed in the later viral population. This residue, situated immediately adjacent to the predicted A*0201-restricted T19 cytotoxic T lymphocyte (CTL) epitope, saw its PAPPC score fall from 68.7 to 17.3 when mutated, suggesting that changing this site—though disadvantageous where humoral pressures were concerned—could have meditated CTL escape through a processing mutation.

**Conclusion:** Mechanisms of early subtype A autologous neutralization converge at the base of the V3 loop, and immune escape could be complicated by the virus’s requirement to juggle intersecting humoral and cellular pressures.

**P08.02**

The C3V4 Domain Interacts with Structurally Proximal Regions to Mediate Escape from Autologous Neutralizing Antibodies in HIV-1 Subtype C Infection

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**Background:** Most new HIV-1 infections world-wide are caused by subtype C viruses. The C3V4 region of gp120 is a major target for autologous neutralizing antibodies in this subtype. The alpha2-helix, within the C3 region, is more variable, more amphipathic and likely to be more immunogenic in subtype C than in subtype B. This helix contributes to an epitope including the V4 loop and N332 glycan.

**Methods:** Chimeric viruses containing the C3V4 region from a neutralization sensitive virus in a resistant envelope backbone were generated, and tested for neutralization sensitivity using autologous serum. Individuals were classified as C3V4-responders or non-responders depending on whether they developed antibodies to this region. Longitudinal single genome amplicons were obtained from C3V4-responders at 6 and 12 months post-infection. Sequence analysis was used to identify possible escape mutations in the C3V4 and other structurally proximal regions in escaped viruses. The role of these mutations in escape was examined by site-directed mutagenesis.

**Results:** Chimeric viruses were used to identify 13 C3V4-responders and 7 non-responders. Escape pathways in five C3V4-responders were examined in detail. In CAP244 and CAP136, escape was mediated solely by changes in the C3V4 region. In 3 other individuals, escape from anti-C3V4 responses occurred via interactions between the C3V4 region and structurally proximal regions of the envelope. A glycan shift in V5 and 3 mutations in the alpha2-helix of CAP206 mediated escape. In CAP228 an interaction between residues K290 in the C2 region and Q336 in the alpha2-helix mediated escape, while addition of a glycan in both the C2 and V4 regions of CAP255 resulted in escape.

**Conclusion:** These data confirm that changes solely within the C3V4 region can mediate escape, but in some cases interactions between the C3V4 and other regions in the outer domain are required, enabling multiple routes for escape from anti-C3V4 antibodies.

**P08.03**

Control of HIV Viral Replication Mediated by the Synergistic Effects of Unique Combinations of HLA Class I Alleles

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**Background:** A major challenge to the development of an effective HIV vaccine is eliciting durable CD8+ T cell responses from which the virus cannot escape. Spontaneous control of HIV replication by particular HLA class I alleles is associated with the targeting of critical regions of the virus which limit effective viral escape due to the impact of mutations on viral fitness. Unique networks of co-evolving or compensatory mutations are known to exist within different HIV proteins, requiring coordinate co-evolution at these sites during viral escape due to structural protein constraints. Because of this requirement of co-evolving sites, we hypothesized that particular pairs of CTL escape mutations may exist that are uniquely deleterious to HIV, revealing novel ‘combination targets’ for vaccine targeting.

**Methods:** We computationally analyzed mutation patterns of described CTL escape mutations in capsid sequences and crystal structures to identify mutation pairs potentially unable to structurally coexist. We then cross validated identified position pairs with clinical data and performed in vitro viral replication assay on both single and double mutants for four mutation pairs.

**Results:** We identified 20 non-covarying pairs of residues from public datasets (q-value < 0.05). Notably, the pairs of HLA alleles selecting for these non-covarying residues were found enriched in individuals controlling HIV. Moreover, while the majority of single mutations tested had minimal effects on viral replication capacity, 3 out of the 4 pairs of mutations significantly impaired viral replication (B52-T280V + B27-SARKLM; B52-T280V + B57-A163N; B57-I147L + B14-T303V).

**Conclusion:** These data suggest that the combination of certain HLA class I alleles may enhance the control of HIV replication due to the inability of HIV to effectively escape from coordinated responses against structurally interacting regions of the virus without incurring substantial fitness costs. This study highlights the importance of CTL based vaccines to simultaneously direct immune responses against co-evolving sites that substantially limit the pathways of viral escape.

**P08.04**

Molecular and Functional Consequences of Immune-Mediated Evolution in HIV-1 Nef During the North American Epidemic


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**Background:** HLA-restricted CTL responses drive evolution of the highly immunogenic Nef protein, but the extent and functional consequences of population-level adaptation remain unclear. We have used novel historic Nef data to estimate the date and reconstruct the founder virus sequence of the North American epidemic, compare patterns of population-level HLA-associated polymorphisms over time, and assess CD4 downregulation activity of historic and modern Nef sequences.

**Methods:** Plasma HIV-1 RNA Nef sequencing and HLA typing was performed on 241 historic specimens (1979–89). Modern published HLA/HIV datasets served as controls. Timing and sequence reconstruction of the founder Nef was performed using BEAST and HyPhy. HLA-associated polymorphisms were identified using phylogenetically-corrected methods. CD4 downregulation capacity of 52 historic vs. 52 modern Nef sequences was compared using flow cytometric methods.

**Results:** Based on Nef sequences, the most recent common ancestor of the North American epidemic was dated to 1965. The consensus of the reconstructed founder Nef sequence differed from 2004 subtype B consensus at codons 15, 22, 51 and 178, while additional sites remained ambiguous in the reconstruction. Patterns and statistical strengths of HLA-associated polymorphisms remained generally consistent over time (e.g. A*24-associated Y135F, B*07-R71K, B*08-K94Q and B*57-H116N ranked among the strongest in historic and modern cohorts); however, a small number of polymorphisms were identified as candidates for population-level accumulation. Functional assessment of Nef revealed a modest yet statistically significant increase in CD4 downregulation capacity over time (median 0.93 vs. 1.00 in historic vs. modern sequences; p = 0.005).

**Conclusion:** Modest population-level immune adaptation in Nef, potentially leading to modest increases in CD4 downregulation capacity, may have occurred in North America since 1979. However, the relatively high similarity between the estimated founder and modern consensus B, and the observation that CTL escape patterns have remained largely consistent over time, support Nef as a suitable target for vaccine consideration.

**P09.02**

**Differential Regulation of Various TLR Pathways in Acute and Chronic HIV-1 Infection**

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**Background:** Innate immune cells can sense pathogens through Toll-like receptors. Innate immune responses to pathogen-derived TLR ligands in HIV-1 infection have been suggested to be universally dysregulated, with reduced responsiveness to some TLR ligands due to chronic activation-induced anergy and elevated responses to other TLR ligands associated with enhanced general immune activation. Here we systematically compared responses to TLR ligands in individuals with acute HIV infection, chronic untreated viremic, chronic untreated controlled (elite controllers), and chronic treated HIV-1 infection.

**Methods:** Cryopreserved PBMCs from 39 HIV-1 infected subjects and 11 HIV-1 negative individuals were stimulated with CL097 (TLR7/8 ligand), ODN2216 (TLR9 ligand), and heat killed Listeria monocytogenes (TLR2 ligand). TLR-specific responses by monocytes, mDCs and pDCs (APCs) were quantified by intracellular cytokine staining using flow cytometry.

**Results:** Different stages of HIV-1 infection were associated with either reduced or enhanced responsiveness of APCs to TLR ligands. Responses to TLR9 ligands were reduced in all HIV-1-infected individuals. In contrast we did not observe an HIV-1-associated reduction in responses to TLR7 or TLR8 ligands, but significantly enhanced levels of responses to TLR8 ligands by monocytes and mDCs that were positively associated with HIV-1 viral load (p < 0.01 for both).

**Conclusion:** While initial studies had suggested that HIV-1 infection induces a general impairment in the APC responses to TLR ligands, more recent studies have suggested that the responsiveness of APCs to TLR7/8 ligands is preserved in infected individuals (O’Brien JCI 2011). Our data are in line with these recent findings, and furthermore demonstrate that responses by monocytes and DCs to pathogen-derived TLRs are differentially regulated in different stages of infection.
P09.03
CCR5 Expression on Peripheral Blood Cells Differs Between South African Caucasian and African Populations

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Background: CCR5 is pivotal in determining an individual’s susceptibility to HIV-1 infection and rate of disease progression. To establish if population-based differences exist in cell surface expression of CCR5 we evaluated the extent of CCR5 expression across all peripheral blood cell types in individuals from two populations, South African Africans (SAA) and South African Caucasians (SAC).

Methods: CCR5 expression on peripheral cell populations was determined in EDTA-anticoagulated whole blood of 22 SAA and 32 SAC HIV uninfected individuals (age and gender matched), using a CCR5 antibody conjugated to PE at a ratio of 1:1, thereby allowing for CCR5 quantification as the mean number of CCR5 molecules per cell in addition to the percentage of CCR5-expressing cells within a cell subset. CCR5 expression was determined for B-cells, monocytes, and T-cell and natural killer (NK) cell subsets.

Results: Differences in CCR5 expression, both in number of CCR5 molecules per cell and percentage of CCR5-expressing cells, were observed between the two study groups, within all cell subsets. Most notably, the percentage of CCR5+ cells were significantly lower in SAC compared to SAA individuals (P < 0.001) among NK-cell subsets (CD16+; CD56+ and CD56dim) whereas CCR5 intensity of expression was significantly higher in SAC compared to SAA individuals in CCR5+ CD8+ T-cell and CCR5+ NK-cell subsets (CD16+; CD56+ and CD56dim) (all P < 0.05). These relationships were maintained post-exclusion of CCR5delta32 heterozygous individuals (n = 7) from the SAC dataset.

Conclusion: CCR5 expression differs significantly between SAA and SAC individuals. These differences can be attributed to factors other than CCR5delta32. It remains to be determined which genetic factors are contributing to these expression differences. Observed differences in CCR5 expression levels have implications not only for HIV-1 infection, but also for T-cell immunity where CCR5 has also been shown to play a key role.

P09.04
Human and Rhesus Macaque TRIM5α Can Bind HIV-1 Cores and Stabilize CA Disassembly

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Background: After membrane fusion with a target cell, the human immunodeficiency virus type 1 (HIV-1) core enters the cytoplasm where uncoating occurs, an important, yet poorly understood event. The cone-shaped core is composed of a hexameric lattice of the viral capsid protein (CA), which disassembles during uncoating. The host restriction factor TRIM5α targets the incoming viral core, resulting in a block to infection, possibly by accelerating or disrupting uncoating. Restriction occurs in a species-specific manner, whereby HIV-1 is weakly restricted by human TRIM5α (TRIM5α-Hu), but potently restricted by rhesus macaque TRIM5α (TRIM5α-Rh). TRIM5α restriction is presumed to be dependent on the ability to recognize the viral core via CA affinity, but the overall mechanism is not completely understood.

Methods: Through the use of isolated cores, endogenous sources of TRIM5α, over-expressed TRIM5α in 293T cells and recombinant proteins, the recognition requirements were investigated. In addition, the effects of TRIM5α on uncoating were examined using an in vitro core disassembly assay.

Results: Both over-expressed TRIM5α-Hu and TRIM5α-Rh were able to recognize and co-sediment with isolated cores through a sucrose cushion. Furthermore, endogenous sources of TRIM5α and recombinant proteins were examined and confirmed that both have affinity for the viral core. Using an in vitro core disassembly assay, uncoating was not accelerated; rather core disassembly was stabilized in the presence of endogenous TRIM5α and cell lysates over-expressing either TRIM5α. However; the presence of recombinant TRIM5α-Rh alone was able to stabilize core disassembly, while recombinant TRIM5α-Hu was not.

Conclusion: The results suggest that CA-binding is required, but not sufficient for TRIM5α-mediated restriction of viral infection. The data supports a model in which the restriction occurs in a step-wise manner and is dependent on additional and yet unidentified cellular factors, with the generation of a stable viral core-TRIM5α complex representing an intermediate in the TRIM5α restriction process.

P09.05
High Frequencies of Polyfunctional CD8+ Natural Killer Cells in Chronic HIV-1 Infection Are Associated with Slower Progression to AIDS

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Background: Large genotypic studies suggested a protective role of natural killer (NK) cells in HIV infection. The aim of our study was to define correlates of NK cell subpopulations and their functional profiles with slower HIV disease progression.

Methods: Associations of lymphocyte subpopulations with HIV disease progression was assessed in an observational study in 116 untreated HIV-seropositive donors with a CD4+ T helper cell count >500 cells/μl who were followed for 4 years. Phenotypic and functional analyses of NK cells from 63 individuals, including 37 untreated and 11 treated HIV-seropositive and 15 uninfected controls subjects, were performed by polychromatic flow cytometry. Statistical analysis included unpaired t test, One-way ANOVA and Pearson analysis.

Results: High absolute numbers and percentages of CD8+ CD3- cells were associated with significantly slower HIV-disease progression (P = 0.048 and P = 0.0074, respectively). The majority of CD8+ CD3- cells were CD56-expressing NK cells. Frequencies of CD8+ NK cells inversely correlated with HIV viral loads (r = -0.5, P = 0.0012) and loss of CD8+ NK cells could be restored in HIV-patients after antiretroviral treatment. Analysis of IFN-γ, TNF-α, MIP-1β and CD107a-responses in NK cells after IL-12-, IL-15- and K562-stimulation revealed a more polyfunctional profile amongst CD8-expressing NK cells in comparison with their CD8-negative counterpart. Higher frequencies of granzyme B- and perforin-expressing cells were detected amongst CD8+ NK cells as compared to NK cells devoid of CD8 but no significant differences were observed in surface expression of NKG2A, NKG2D, CD62L, CD69, CD2 and CD94.
P09.06
Reduced Expression of LEDGF/p75, an Essential Host Factor for HIV Integration, in HIV Exposed Seronegative Individuals

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Background: HIV-1 replication in host cells depends on a delicate balance between intrinsic immune factors limiting viral dissemination like apolipoprotein B mRNA-editing catalytic polypeptide-like 3G (APOBEC3G), tripartite motif 5 alpha (TRIM5alpha) and tetherin (BST-2), and cellular co-factors required for virus propagation like lent epithilium-derived growth factor (LEDGF/p75). We analyzed whether the expression of these host proteins contributes to the phenomenon of HIV-1 resistance.

Methods: Twenty-three HIV-1 exposed seronegative subjects (HESN), 23 healthy controls (HC), and 45 HIV-1 infected subjects of HIV-1 resistance. Increased expression levels of APOBEC3G, TRIM5alpha, and tetherin, no differences were found between HESN and HC. This observation was especially true for HESN subjects with higher frequencies of unprotected sexual exposure. For APOBEC3G, TRIM5alpha, and tetherin, no differences were found between HESN and HC. HIV-naïve patients generally expressed higher mRNA and protein levels for the four HIV-1 related host factors than HC. In particular for tetherin, increased protein expression levels correlated positively with the enhanced levels of immune activation in these HIV-naïve patients.

Conclusion: Our observations suggest that reduced LEDGF/p75 protein expression may contribute to the phenomenon of HIV-1 resistance. Increased expression levels of APOBEC3G, TRIM5alpha, tetherin, and LEDGF/p75 in HIV patients are a likely consequence of the increased levels of immune activation. Understanding the role of HIV-1 related host cell proteins in HIV-1 susceptibility may be of great interest for the development of future therapies for the prevention of HIV infection.

P09.07
Different Cytokine Profiles Induced by HIV-1 and HIV-1-Derived TLR Ligands in Monocytes and mDCs

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Background: Shortly following HIV-1 acquisition, a host of plasma cytokines and chemokines rise sharply. The goal of this study was to characterize the cytokine profile in response to HIV-1 viremia and to identify the different pathways leading to HIV-1-induced cytokine production.

Methods: We evaluated plasma levels of 19 cytokines from a single-arm trial evaluating combination antiretroviral therapy started during acute HIV-1 infection followed by treatment interruption in 14 participants, using a multiplex Lumixen bead assay. Sources of cytokine production were identified in vitro following stimulation of PBMCs from healthy individuals with HIV-1 and HIV-1-derived TLR ligands by intracellular cytokine staining (ICS).

Results: The plasma cytokine most significantly associated with HIV-1 viral load among the 19 analytes was the chemokine IP-10 (CXCL10) (R² = 0.4, p = 0.0001). Incubation of PBMCs with TLR8 ligands (CL097 and gag1166 ssRNA), AT-2 inactivated HIV-1, and NL43 virus in vitro resulted in a significant production of IP-10 (p = 0.001). IP-10 was produced mainly by mDCs and monocytes in response to AT-2 HIV-1 (average of 59% and 76%, respectively) and NL43 virus (average of 74% and 91%, respectively). In contrast, incubation with TLR8 ligands was associated with increased production of TNFα by monocytes and mDCs and only slight increase in IP-10 production. Thus, the cytokine profile of monocytes and mDCs stimulated with whole viruses (AT-2 HIV-1 and NL43) differed from that observed in response to TLR8 ligands alone, suggesting the involvement of additional receptors than TLR8 in the recognition of HIV-1 by these cells.

Conclusion: Monocytes and mDCs produce significant amounts of IP-10 in response to stimulation with HIV-1, while stimulation with HIV-1-derived TLR8-ligands results in the preferential production of other pro-inflammatory cytokines, such as TNFα. Further studies are needed to gain a better understanding of the pathways that lead to the activation of innate immune cells by HIV-1.
administered intranasally to stimulate the mucosal system. Blood was collected prior to (day 0) and following vaccination day 7 and 30. We hypothesize that the HIV resistant individuals would have heightened innate immune responses compared with HIV susceptible individuals.

**Methods:** The study population was drawn from the Majengo sex worker cohort, Nairobi, Kenya comprising 2 groups: Long term HIV exposed but uninfected (HESN/ HIV resistant); short term HIV exposed but uninfected (New Negatives/ susceptible) commercial sex workers. Peripheral blood mononuclear cells (PBMCs) were isolated from the study participants. After incubation with agonists specific for TLR9 (CpG ODN) and TLR7/8 (ssRNA40), in vitro production of cytokine and chemokine were quantified using multiplex assay on Luminex.

**Results:** Stimulation of PBMCs with TLR 7, 8, 9 ligands led to a significant elevated levels in IL-2 at baseline (p = 0.0159) and following vaccination day 30 (p = 0.0079) in HESN compared to HIV susceptible individuals. Elevated chemokine IFN-γ - induced Protein-10 (IP-10) were observed HESN compared to HIV susceptible individuals 30 days post vaccination (p = 0.0317).

**Conclusion:** HIV-resistant women had stronger IL-2, IFN-γ and IP-10 responses to TLR ligands compared to HIV susceptible individuals. Elevated chemokine IFN-γ-cytokine production has the potential for augmenting prevention or therapeutic interventions.

**P09.11**

A Role for the KIR2DL3 Receptor in Mother-to-Infant HIV-1 Transmission


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**Background:** Natural killer cells are thought to be involved in attenuation of HIV-1 disease, primarily through recognition by killer-cell immunoglobulin-like receptors (KIRs) of specific HLA Class I molecules. The influence of KIR and HLA on maternal-to-infant transmission however has not been studied. We investigated the role of the weakly inhibitory KIR receptor, KIR2DL3 and its HLA-C ligand (C1) on perinatal HIV-1 transmission.

**Methods:** KIR genes and HLA Class I C* alleles were determined in Black South African HIV-1 infected mothers (224) and their infants (72 infected and 150 exposed-uninfected). Analyses involved comparisons of total groups, stratification on the basis of maternal nevirapine (NVP)-exposure, and adjustment for maternal viral load (MVL).

**Results:** KIR2DL3 homozygosity alone and in combination with HLA-C allotype heterozygosity (C1C2) was elevated in intrapartum (IP)-transmitting mothers compared to non-transmitting mothers (P = 0.034 and P = 0.01 respectively), and post-MVL correction (P = 0.033 and P = 0.027, respectively). These associations were stronger post-MVL adjustment (total group: P = 0.02 and P = 0.009, respectively; NVP group: P = 0.004 and P = 0.02, respectively). Because of the apparent conflicting associations of KIR2DL3/
KIR2DL3+C1C2 with regard to maternal IP-transmission and infant susceptibility, the level of concordance between mothers and infants harbouring this genotype was investigated. Results point to a protective effect for this genotype in the infant only when there is discordance (i.e. mother lacking genotype) and that this protection is likely to be in the context of IP transmission.

Conclusion: These results indicate a role for KIR2DL3 in perinatal HIV-1 transmission and highlight the complexities in studying the influence of host genes in both maternal transmission and infant susceptibility.

P09.12
Correlation Between AIDS Pathology and the Biased Nucleotide Composition of Lentiviruses
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Background: AIDS results from generalized immune activation after years of chronic HIV replication. However, most African primate species naturally infected with lentiviruses remain healthy. Addressing this issue is crucial for understanding AIDS pathogenesis. Because the genomes of lentiviruses present a particularly biased nucleotide composition compared to that of their prime host, we wondered whether host recognition of biased viral nucleic acids could induce hyper-responsiveness to HIV and AIDS pathogenesis.

Methods: We compared the nucleotide composition (A/C/G/T frequencies) of pathogenic and non-pathogenic primate lentiviruses with that of their hosts for every infection described in the literature. We measured the nucleotide divergence by computing the Chi-2 distance between the A/C/G/T frequencies in the complete viral sequence and the coding sequences of each host organism.

Results: We found that primate lentiviruses having the most divergent nucleotide composition compared to their hosts induce AIDS, whereas less divergent lentiviruses cause non-pathogenic infections. Similarly, the relative pathogenicity of HIV-1 subtypes correlates with their nucleotide divergence to the human genome.

To understand this observation at a molecular level, we investigated the ability of HIV-1 RNA fragments to stimulate in vitro the synthesis of type I interferon (IFN-I). We found that the nucleotide divergence of RNA fragments strongly correlates with type-I IFN activity.

Based on these observations, we designed a large-scale, nucleotide-optimized SIV sequence derived from the pathogenic clone SIVmac239. We produced the corresponding artificial virus and analysed its capacity to induce type-I IFN.

Conclusion: Overall, these data suggest for the first time a direct link between the nucleotide composition of lentiviruses and their pathogenicity. They also describe the synthesis a novel artificial SIV harbouring an attenuated pathogenic potential.

P09.13
Dysregulation of Tim-3 Expression on NK Cells in Chronic HIV-1 Infection

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Background: Chronic exposure to HIV-1 antigens leads to T-cell exhaustion and an impaired virus-specific T-cell response, which correlates with increased expression of several immunoregulatory molecules including T-cell immunoglobulin domain and mucin domain 3 (Tim-3). Interestingly, NK cells constitutively express high levels of this receptor and Tim-3 has been identified as a gene that is significantly up-regulated following NK cell activation (Hanna et al., 2004). Moreover, up-regulation of Tim-3 on NK cells has recently been associated with reduced anti-viral properties (Ju et al., 2010). However, the effect of chronic HIV-1 infection on Tim-3 expression on NK cells and on Tim-3-mediated NK cell function has not been studied yet.

Methods: We examined Tim-3 expression on NK cells by flow cytometry in HIV-1 elite controllers (HIV-VL < 50 copies/ml), viremic controllers (HIV-VL < 2000 copies/ml), individuals with treated and untreated chronic progressive HIV-1 infection and HIV negative control subjects.

Results: Tim-3 expression was high on NK cells from HIV negative individuals (median percentage 75.8, range 60.7–83.9). However, proportions of Tim-3 + NK cells were significantly decreased in all HIV-infected individuals compared to healthy subjects (p = 0.0001), with a marked down-regulation of this receptor at the surface of NK cells in elite (p = 0.002) and viremic controllers (p = 0.04).

Conclusion: Chronic exposure to HIV-1 leads to significantly decreased levels of Tim-3 expression on NK cells, in striking contrast to its upregulation on exhausted T-cells in untreated HIV-1 infection. This may suggest distinct Tim-3 signaling pathways in NK and T cells. Further investigation is warranted to evaluate the impact of Tim-3 and its ligand galectin-9 on the regulation of NK cell function in the context of HIV-1 infection. Defining the role of NK cells receptors in the control of HIV-1 will offer novel therapeutic targets to manipulate to improve future HIV-1 vaccine strategies.

P09.14
Early Phosphorylation Events Induced in Dendritic Cells by the HIV Derived TLR8 Ligand ssRNA40
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Background: Dendritic cells (DC) are among the first cell-type encountered by HIV, and their interaction plays a crucial role in subsequent viral spread. Studies show that HIV infection leads to abnormal DC maturation and an impaired interferon response. However, HIV contains at least one TLR7/8 ligand, which can induce maturation and cytokine production, and stimulation of these receptors has recently been found to be crucial for viral replication in DCs. This work aims to understand how HIV is able
to subvert TLR signalling in DCs, allowing for replication but mitigating the anti-viral interferon response.

**Methods:** Monocyte-derived dendritic cells were stimulated with HIV-TLR8 ligand ssRNA40, synthetic TLR8 ligand R848, or a control for 10-minutes and assayed for protein phosphorylation changes using a novel method. Briefly, this involved enrichment of phosphorylated proteins, fractionation by isoelectric-focusing and tagged mass-spectrometry to identify differentially phosphorylated proteins. Results were confirmed by Western blot.

**Results:** Over 100 proteins were found to be differentially phosphorylated after 10 minutes stimulation with ssRNA40 and R848. Pathway analysis shows these to group into known immune signalling networks. Interestingly there are striking differences between ssRNA40 and R848; with ssRNA40 biased towards a more regulatory/inhibitory outcome. Amongst the currently confirmed targets, this is characterized by profound dephosphorylation of ERK1/2, compared to the expected phosphorylation observed with R848; and phosphorylation of the inhibitory molecule IRAK-M. QPCR analysis of cytokine profiles and TLR expression also suggests a difference in the signalling induced by ssRNA40 and

**Conclusion:** This methodology provides a powerful and unbiased method of looking at early signalling events induced by TLR-stimulation. Target confirmation is on-going, but initial data indicate that the HIV-1 TLR8-ligand ssRNA40 may induce a distinct inhibitory signal compared to R848. This raises interesting questions as to how HIV modulates TLR8 signalling in DCs to allow for replication but diminish the anti-viral immune response.

**P09.15 Mapping of ADCC Activity: The A32 Conformational C1 Epitope is a Dominant Target for ADCC Antibodies in Chronically HIV-1 Infected Subjects**

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**Background:** Among non-neutralizing HIV-1 envelope antibodies, those capable of mediating antibody dependent cellular cytotoxicity (ADCC) activity have been postulated to be important for control of HIV-1 infection. However the precise targets of serum ADCC antibody are poorly characterized. We utilized the human non-neutralizing CD4 induced (CD4i) mAb A32 and monomeric A32-Fab, that recognize a conformational C1-C4 region to map ADCC Ab responses.

**Methods:** We utilized flow cytometry to evaluate the expression of the conformational epitope recognized by the A32 mAb on cells infected with transmitted/recombinant and laboratory adapted HIV-1 isolates. The ADCC activity of the A32, 17b, 12b, PG9, and 2G12 mAbs were evaluated using HIV-1-infected cells. In order to map the ADCC specificity of the Ab responses elicited by HIV-1 infection, we used the A32-Fab to pretreat our target cells and prevent binding of A32-like Ab present in the samples collected from 40 chronically infected donors of whom 13 were Controllers.

**Results:** We observed that mAb A32 epitope was expressed on the surface of HIV-1 infected CD4+ T cells earlier than the CD4-inducible (CD4i) epitope bound by mAb 17b and the gp120 carbohydrate epitope bound by mAb 2G12. The mAb A32 was able to mediate ADCC activity 2–4 fold higher than any other mAb at peak of activity. After pre-incubating the target cells with the monomeric A32-Fab, we observed a range of inhibitory activity for the peak of the response from 19% to 90% in samples collected from all subjects (average ± standard deviation = 56 ± 24). Moreover, A32-like ADCC responses represented the majority (>50 inhibition) of the peak response in 69% Controllers (n = 13), 57% Tier 1 neutralizers (n = 14); 22% Elite neutralizers (n = 9).

**Conclusion:** Our data suggest that CD4i Ab, and in particular those recognizing the A32 conformational epitope, play a major role in mediating ADCC responses in the course of HIV-1 infection.

**P09.16 Ontogeny, Breadth, and Specificity of Circulating ADCC-Mediating Antibodies in HIV-1 Infected Individuals**

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**Background:** ADCC is a contributing determinant of the immune responses controlling HIV-1 replication and may constitute a relevant function of vaccine-induced antibodies. We sought to determine the ontogeny, breadth, and specificity of ADCC-mediating antibodies in plasma collected from HIV-1 acutely and chronically infected individuals.

**Methods:** Longitudinal plasma samples were collected from 3 acutely infected individuals from 1 month to over 1 year post transmission. Cross-sectional plasma samples were collected from 9 chronically infected individuals who represented the top 3% of broadly neutralizing antibody responses (Elite Neutralizers = EN), 14 individuals lacking broad neutralizing capabilities (Tier 1 neutralizers = T1N), and 13 individuals maintaining virus load below 2,000 RNA copies/mL (Controllers). We measured the ability of these plasmas to mediate ADCC responses directed against target cells that were either infected with HIV-1, or coated with recombinant gp120s representing subtypes B, C, and E.

**Results:** Cross-clade ADCC-mediating antibody responses were detectable within 4 months of transmission. In chronic infection, Area Under the Curve (AUC) analysis indicated that ADCC activity directed against HIV-infected cells was higher in the Controllers (AUC = 1349) and EN (AUC = 6915) compared to T1N (AUC = 48). We observed a positive correlation between ADCC activities directed against gp120-coated and HIV-1-infected targets in EN and Controllers, but not in the T1N. Among the ENs, there was no association between titers of gp120 binding antibodies and ADCC
activity ($p = 0.38$, $R^2 = 0.11$) and a negative correlation between ADCC activity and gp41-binding antibody titers ($p = 0.05$, $R^2 = 0.49$).

**Conclusion:** Our data suggest that cross-clade ADCC activity directed against HIV-infected cells arises early after infection, but is underrepresented in the majority of chronically infected individuals compared to Controllers. Broadly reactive antibodies specific for conserved regions of gp120 are likely responsible for ADCC responses directed against infected cells, and may represent an important target for efficacious HIV vaccines.

**P09.17**
**JAK3 Inhibition In Vivo in Chronically SIV Infected Rhesus Macaques Deplete NK-17 Cells**

**P010.01**
**Intranasal Co-Administration of IL-12 plus CTB in DNA-MVA Mucosal Schemes Enhanced Systemic and Mucosal Cellular Immune Responses Against HIV-1 Env**

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**Background:** The major route of HIV transmission is through mucosal tissues. Therefore, designing immunization regimes aimed to induce mucosal immune responses is needed. The aim of this study was to analyze the activity of IL-12 alone or in combination with the cholera toxin B subunit (CTB), applied in DNA-prime/MVA-boost intranasal immunizations.

**Methods:** Balb/c mice were intranasally immunized with both vectors expressing HIV-1 Env B. Two doses of DNA-IL-12 were applied ($50\mu g$ or $100\mu g$) in the presence or not of $10\mu g$ of CTB applied at prime and booster doses. Groups receiving CTB, complete cholera toxin (CT) or non-adjuvants (control) were included. Immune responses were evaluated 14 or 30 days after immunization.

**Results:** Both DNA-IL-12 doses generated similar responses, even more the minor one plus CTB generated the highest response, showing a synergistic effect for both adjuvants. Co-inoculated IL-12 + CTB (G4) produced the highest specific TCD8+ immune response (by IFN-gamma ELISPOT), detected in the spleen (up to a seven-fold increment), as well as in regional (cervical) and genital (iliac) lymph nodes (GLNs), and more importantly in genital tract mucosa (GT). IL-2 secreting cells were two-fold superior in G4 compared to control group in spleen ($p = 0.0321$), GLNs ($p = 0.005$) and GT. A higher proportion of cytotoxic and polyfunctionality cells were detected in spleen and GT. At memory phase, we found that in the G4 IFN-gamma and IL-2 secreting cells were two to three-fold superior in both systemic (spleen, $p = 0.001$) as well as in mucosal compartments (GLNs). Finally, IL-2 and CTB also enhanced gp120 Abs levels (serum IgGs and IgA in vaginal washings).

**Conclusion:** Here we demonstrated that IL-12 cytokine in combination with CTB generated a cooperative adjuvant effect on the cellular and humoral IR against Env antigen applied in DNA-MVA intranasal immunizations. These results are important due to the need to improve mucosal vaccine strategies against HIV.

**P10.02**
**CD107a Expression Rather than Polyfunction by HIV-Specific T Cells in the Female Genital Tract Was Associated with Protection from HIV Shedding**

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**Background:** HIV-specific T cells are present in the female genital tract of chronically HIV-infected individuals. The aim of this study was to compare the polyfunctional ability of HIV-specific mucosal T cell responses in the female genital tract with those in blood and their relationship with HIV clinical status and genital HIV shedding.

**Methods:** Cervical cytobrush-derived and blood-derived T cells were obtained from 16 chronically HIV-infected women. Cervical CD3+ T cells were expanded with Dynal anti-CD3/CD28 expander beads for 14 days to increase T cell numbers for further analysis. Polychromatic flow cytometry was used to simultaneously evaluate four T cell functions (CD107a, IFN-γ, TNF-α, and MIP-1β) and compare them to HIV clinical status.

**Results:** Tn response to HIV, CD107a was found to be the dominant response of CD8 T cells in both the cervix and in blood. Expression of CD107a in both compartments was negatively associated with plasma viral load. In the genital tract, CD107a expression by genital tract CD8 T cells was associated with protection from HIV shedding. While the majority of Gag-specific T cell responses in the female genital tract and blood were monofunctional, low frequencies of HIV Gag-specific polyfunctional CD8 T cells were detected at the cervix and was found to correlate significantly with polyfunctional frequencies in blood. The polyfunctionality of blood Gag-specific CD8 T cells was associated with clinical status (blood CD4 count and plasma viral load). No association was found between polyfunctional responses at the cervix and clinical status or genital HIV shedding.

**Conclusion:** HIV Gag-specific cervical T cells were largely mono-functional and CD107a appears to be associated with better control of HIV shedding in the genital tract.
Polyfunctional T cells were detected in women with high blood CD4 count and low plasma viral load and these did not protect from HIV genital shedding.

**P10.03**
Evaluation of Mucosal Immunization Routes for Induction of Vaginal Humoral Immunity in Macaques.

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**Background:** The predominant route for sexual HIV transmission is via genital and rectal mucosal surfaces. The development of a successful vaccine against HIV is likely to require induction of strong and long-lasting humoral immune responses at the mucosal portals of virus entry. Hence, the design of a vaccine strategy able to induce mucosal antibodies may be crucial to providing immune protection. This study has assessed how the route of vaccination and adjuvant used modifies mucosal responses to model antigens in macaques.

**Methods:** Female Rhesus macaques (n=4 per group) were immunized three times, either intranasally, sublingually or intrarectally with model antigens (KLH, OVA or B-gal). Immunizations were performed every four weeks and animals were treated with medroxyprogesterone 4 weeks prior to first administration. Four different adjuvants were assessed per route of administration (including R848 (resiquimod), chitosan, TSLP, Poly-IC, and Pam3CSK4).

**Results:** Intrarectal and sublingual immunization failed to induce humoral responses in macaques. Intranasal immunization induced potent responses when R848 was used as an adjuvant, but not with the other adjuvants tested. Intranasal immunization with R848 induced potent and systemic immune responses including localized induction of IgA (nasal and vaginal). In depth safety studies to assess local and systemic cytokine and inflammatory responses to nasal application of R848 and characterization of nasal cellular influx indicate that this approach was well tolerated. No adverse events to nasal application of R848 were detected.

**Conclusion:** These data demonstrate that intranasal immunization with R848 as an adjuvant provided potent systemic and localized specific immune responses, both IgA and IgG. This route was much more effective in induction of specific vaginal humoral responses than sublingual or intrarectal routes of immunization. Ongoing studies will assess whether similar results can be obtained for gp140.

**P10.04**
TSLP: A Potential New Mucosal Adjuvant for Intranasal Immunisation with GP140

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**Background:** The induction of strong and long-lasting humoral immune responses at the mucosal portal of virus entry is most likely required for a successful HIV vaccine. There is an urgent need of a safe and potent mucosal adjuvant for human use that could induce these immune responses. Our previous findings showed that intranasal (i.n.) immunisation with HIV-1-gp140 and TSLP as adjuvant induces antibody responses in serum and vagina. To further explore the adjuvant effect of TSLP, we assessed cellular immune responses to HIV-1 gp140 after i.n. immunisation and their impact on the longevity of systemic and mucosal humoral responses. Responses were compared to those induced using the potent mucosal adjuvant cholera toxin (CT).

**Methods:** Mice were immunized three times i.n. in the course of 9 weeks. gp140-specific IgA and IgG responses in serum and vagina were assessed. Splenocytes and cells from the female genital tract were assessed for the presence of antibody secreting cells. Spleens were assessed for T-cell proliferative responses and T-cell-specific cytokine production.

**Results:** TSLP induced strong humoral responses, comparable to those seen with CT. Interestingly, vaginal gp140-specific IgA and IgG were still high 6 months after the last boost. In addition, TSLP enhanced T-cell proliferative responses to gp140 i.n., but the percentage of CD4+ and CD8+ T-cells producing cytokines induced by TSLP after i.n. immunisation was not different from that induced by gp140 alone suggesting the adjuvant effects are predominantly mediated through enhancement of B cell responses in keeping with its known effect on B cell proliferation and differentiation.

**Conclusion:** Our results show that TSLP induces high and long-lasting humoral immune responses both systemically and at the vaginal mucosa. The ability of TSLP to induce high and long-lasting gp140-specific vagina IgA following nasal immunisation suggests a novel adjuvant strategy for preventative HIV vaccines.

**P10.05**
DNA Prime-rAd5 Boost Vaccine Applied to the Tonsils Does Not Protect Against Intrarectal Challenge Despite Strong Immune Responses in Rhesus Macaques

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**Background:** Induction of potent mucosal immunity would be desirable for HIV vaccines. However, the effect of mucosal versus parenteral vaccination routes on immunogenicity and protective efficacy remains unclear. Tonsil immunization, which may elicit genital and GI tract immunity, is particularly interesting for HIV vaccines.

**Methods:** Mamu-A*01(+) /B*17(+) /B*08(+) rhesus macaques were immunized intramuscularly (IM) or submucosally over the tonsils (IT). 8 animals per group were primed with plasmid DNA encoding SIVmac239 Gag, Pol, Env, Vif at months 0, 1, 2 and boosted at 6 months with rAd5 vectors expressing Gag, Pol, Env, Vif/Vpr-Vpx. Cellular responses in blood and bronchoalveolar lavage (BAL) were evaluated by ICS and tetramer binding assays. Antibodies in plasma and secretions were quantitated by ELISA. 6 months after the last vaccination, animals were rectally challenged with escalating low doses of SIVmac239.

**Results:** IT and IM immunization induced similar levels of plasma SIV-specific antibodies. IT vaccination induced greater mucosal IgA responses, primarily in the respiratory tract with lower magnitude rectal responses. Peak CD8 T cell responses in PBMC, BAL and, to a lesser extent, colon were higher in the IT vs. IM group. All unvaccinated control animals were infected after 7
challenges. All IM and IT animals were infected after 11 and 12 challenges, respectively. Peak viral load was significantly lower in vaccinated animals than controls but did not differ by vaccination route. Anamnestic humoral and cellular responses also did not differ by vaccination route, with rapid development in blood and respiratory tract but delayed and low magnitude responses in the rectum.

Conclusion: Tonsillar immunization with DNA/rAd5 appeared not to confer benefit over IM immunization with respect to rectal SIV acquisition or viremia, despite superior mucosal responses. Possible explanations include use of a neutralization-resistant challenge virus, poor vaccine-induced responses at the site of challenge or use of a vaccine that induces primarily CD8 responses.

P10.06 Microneedle Mediated Intradermal Delivery of Adjuvanted Recombinant HIV-1 CN54gp140 Effectively Primes Mucosal Boost Inoculations

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Background: Microneedles have previously been shown to enhance transdermal delivery of drugs and macromolecules. Their ability to effectively penetrate the dermis over a relatively large surface area offers potential use as minimally-invasive and painless replacements for more conventional parenteral vaccine regimens. In this study we compared the immune responses to HIV CN54gp140 using microneedles alone or a microneedle prime followed by mucosal boosting with monophosphoryl lipid A adjuvanted protein.

Methods: Polymeric microneedle arrays that dissolve after piercing the skin were fabricated from poly(methylvinylether/maleic acid) (Gantrez® AN139) using laser-engineered silicone micromould templates. Three groups of 8 BALB/c mice were primed using microneedles containing CN54gp140 and monophosphoryl lipid A. Three boosts were performed at two week intervals, using either the same microneedle formulation or vaginal or nasal administration of an antigen/adjuvant solution. Another group received four subcutaneous immunisations. Mice were sampled (serum and vaginal wash) prior to each vaccination and two weeks post final immunization. Antigen-specific IgG and IgA production was assessed in the sera and mucosal lavage samples by quantitative ELISA. Splenocytes were harvested at necropsy and analysed for lymphocyte proliferation.

Results: CN54gp140-specific serum IgG and lymphocyte proliferation responses were elicited in the subcutaneous immunization group and in those animals that were primed using microneedle delivery and boosted mucosally via the nasal route. Only the microneedle prime / intranasal boost group mounted a robust IgA response. In this group, IgG1 and IgG2a subtype quantification revealed a strong bias toward IgG2a.

Conclusion: We have demonstrated that using a novel microneedle device to prime immunity followed by an intranasal boost elicits significant antigen-specific immune responses. This regimen generated similar serum IgG levels to the subcutaneous inoculations, but in contrast IgA was significantly elevated. This vaccination modality also induced a strong bias toward the IgG2a subtype suggesting a Th1 skewing of the immune response phenotype.

P11.01 Systemic and Mucosal Vaccination of Rabbits Using Envelope Glycoproteins Derived from HIV-1 Pre-Seroconversion Strains as Novel Immunogens

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Background: Novel immunogens capable of eliciting quality humoral responses could serve as potential HIV-1 vaccine candidates. Envelope glycoproteins (Env) from HIV-1 pre-seroconversion (PSC) strains are antigenically distinct from late stage Env due to absence of immune selection pressure early during infection and may function as novel immunogens by eliciting effective antibody responses.

Methods: Functional PSC env gp160 clones derived from patient samples were used to generate gp140 clones which were engineered into cell lines to produce soluble gp140. Rabbits were vaccinated with gp140 DNA plasmids and gp140 protein trimers in a double DNA prime triple protein boost regime testing two different PSC Env antigens. Both mucosal and systemic vaccinations were performed - intranasal and intramuscular routes for DNA arm followed by intranasal and subcutaneous routes for protein arm. Novel lipopeptide R4Pam2Cys adjuvant was used and additionally Montanide was tested for use with proteins in systemic route. Samples collected include blood, vaginal washes, fecal pellets and nasal washes. Quantitative antibody responses were measured as IgG, IgA and IgM antibody titres and qualitative antibody responses were measured as IgG, IgA and IgM antibodies detected in sera and mucosal samples. Sera demonstrated up to log106.5 IgG titres with high avidity and up to 99% neutralization against MN pseudoviruses. The group receiving Montanide systemically had enhanced humoral response as evidenced by antibody titres and neutralization in comparison to group receiving Pam2Cys and sustained it possibly due to depot effect. All samples are further being tested for neutralization against a panel of HIV-1

Conclusion: Thus PSC Env antigens are capable of eliciting quality humoral responses both systemically and mucosally in rabbits especially neutralizing antibodies and can serve as novel vaccine immunogens. Challenge studies in non-human primates will further help evaluate the usefulness of these novel immunogens as vaccine candidates.

P11.02 A Brain-Derived HIV-1 Envelope Protein Clone with Utility as an Immunogen

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**Background:** HIV Env properties including high sensitivity to neutralization by the CD4 binding site (CD4bs)-targeting neutralizing antibody (NAb) b12 and low CD4 dependency have been associated with brain tropism. These observations suggest that brain-derived Env may be in a relatively open conformation, with increased exposure of functionally conserved domains. We hypothesized that when used as an immunogen this may translate to the generation of broader and more potent NAb responses targeting these conserved domains.

**Methods:** Brain-derived Env clones were sequenced by standard methods. Mice were immunized with an Env DNA prime/trimeric protein boost regimen. Sera were assessed for Env-binding antibodies by ELISA and virus neutralization was measured using a pseudotyped reporter assay. Modeling was performed using the structure of the b12-bound gp120 core. Glycans were manipulated by site directed mutagenesis and b12 binding was assessed by ELISA.

**Results:** Initial screening of brain-derived Env clones indicated that they had a stronger affinity for b12 and sCD4 than spleen-derived clones from the same cohort. Immunization with Env of one brain-derived clone elicited better NAb responses than other clones tested. Sequencing of this clone revealed that conserved glycans at residues 197 and 386, which have been shown in previous studies to protect the CD4bs, were absent. Predictably, restoration of the glycan at residue 386 reduced b12 binding. Unexpectedly, restoration of the glycan at residue 197 enhanced binding, as did removal of the glycan at residue 362. Modeling identified other glycans that may affect exposure of the CD4bs due to their proximity to this domain.

**Conclusion:** We have identified a brain-derived Env clone that appears to have utility as an immunogen. The glycans of this clone have a significant but not entirely predictable role in CD4bs exposure. Artificial manipulation of the glycans may allow further enhancement of immunogenicity by increasing CD4bs exposure while still maintaining structural stability.

**P11.03**

**Generation of HIV-1 Virus-like Particles Expressing Different HIV-1 Glycoproteins**

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**Background:** Elicitation of a potent and broadly neutralizing antibody response is the main goal of an effective preventive HIV-1 vaccine. It has been shown by us and others that the expression of env glycoproteins on the surface of particulate structures, such as Virus-Like Particles (VLPs), could be a more efficient strategy to deliver conformational epitopes to the immune system.

**Methods:** To this aim, VLPs expressing native HIV env gp140 or gp41 glycoproteins have been produced in insect cells using a baculovirus expression system and characterized for appropriate protein expression. VLP-bound HIV gp140 glycoprotein showed the appropriate expression and trimeric conformation. Immunogenicity studies have been performed in BALB/C mice by intra-peritoneal administration and sera from immunized mice have been tested in ELISA assays, for their reactivity with HIV specific antigens, as well as in ex vivo neutralization assay.

**Results:** Sera from immunized animals showed a high reactivity with individual HIV proteins expressed in VLPs. Results of TZM-bl based neutralization assay show that combined sera from animals independently immunized with gp140- or full-length-gp41-expressing VLPs have an additive/synergistic effect in the neutralization activity of HIV pseudoviruses.

**Conclusion:** In conclusion, novel VLPs expressing different HIV env glycoproteins with native trimeric conformation have been generated, showing the induction of effective antibody response with neutralization activity in TZM-bl neutralization assay. These results confirm the effectiveness of VLPs as presentation and delivery system for conformational proteins and show the improved neutralization activity upon the combination of anti-sera elicited by different HIV envelope antigens, suggesting the possibility of broadening the spectrum of viral epitopes targeted by immune response.

**P11.04**

**Evaluation of the Immune Response Elicited by Retroviral Vectors Based on HIV-1 Genome in Asymptomatic HIV+ Chronic Individuals**

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**Background:** The development of HIV vaccines is an urgent priority and there is need to generate reagents that can be used to screen HIV-1-specific responses. Whole inactivated viral particles have been successfully used as vaccines for some viruses, but procedures historically used for inactivation can denature viral proteins reducing their immunogenicity and could be potentially dangerous for the patients. We have evaluated the utility of reverse transcriptase defective virions (NL4-3/RT) as a potential immunogen.

**Methods:** IFN-γ ELISpot assay has been developed to evaluate specific T cells response against different types of NL4-3/RT virions (including aldrithiol-2 treated, X4 and R5 tropic particles) using cryopreserved PBMC from up to 68 asymptomatic HIV+ individuals. Alternatively NL4-3 wild type inactivated virions (aldrithiol-2 treated; WT+AT-2) and pools of overlapping peptides (15 mer) encompassing the HIV-1 Gag and Nef regions have been used as a control. Extracellular stainings have been also carried out to measure immunological features.

**Results:** Preliminary immunogenicity studies showed that positive ELISpot responses were detected in 38/68 (56%) and 16/68 (24%) of individuals tested with ΔRT or WT+AT-2 virions respectively. The magnitude of the total responses induced was also higher against ΔRT and all its variants than in WT+AT-2 (but not in comparison against Gag and Nef pools) and was significantly correlated with low levels of viral load. CD4+ T cell count level was not related with this response. On the other hand, flow cytometry assays confirmed that the percentages of T cells that expressed CD57 (senescence) and CD38 (activation) were higher in non-responders, as well CD28 levels were substantially reduced.

**Conclusion:** In summary, NL4-3/ΔRT virions stimulated T-cell specific immune responses. Our results indicated that it could be
considered as a future candidate to be used as an effective immunogen or as an additional reagent for screening HIV-1-specific responses in HIV seropositives and vaccines.

P11.05
Generation and Characterization of Retroviral Vectors Based on HIV-1 Genome as Novel Immunogens

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Background: The generation of new immunogens able to elicit strong immune-specific responses remains a major challenge to obtain a vaccine against HIV/AIDS. To this aim we have designed and produced a defective recombinant virus based on the HIV-1 genome that generates infective but non-replicative virions.

Methods: Viral particles were generated through transient transfection in 293-T cells, of a full length HIV-1 DNA carrying a deletion of 892 amino acid in the pol gene, encompassing the sequence for the reverse transcriptase (NL4-3/DRT). Wild-type viruses (NL4-3) inactivated with Aldritiol-2 or by treatment with a protease inhibitor (Amprenavir) were used as controls. Expression and processing of viral proteins were examined by Western-Blot. Infectivity and replication of viral particles were tested in PBMC using Gag-GFP labeled virions and in TZM-bi cells by luciferase activity. Viral particles so generated and characterized by cryo-electron microscopy were used to pulse lymphocytes from HIV-infected patients. The immunogenic capacity of these particles was assessed measuring interferon-γ production by ELISPOT.

Results: Defective particles were produced by NL4-3/DRT transient transfection and resulted, as expected, in infective but non-replicative virions because of the absence of reverse transcriptase. Further analysis of purified virions by cryo-electron microscopy revealed an immature morphology characterized by an electron-dense ring at the periphery and the absence of the canonical core in contrast to the AT-2-inactivated NL4-3 viruses. Preliminary in vitro assays showed that NL4-3/DRT was able to induce specific cellular immune responses and higher than AT-2-inactivated wild type HIV-1 viruses. Our results show that the lack of viral maturation may increase immunogenicity, which is supported by the enhancement of immunogenicity observed in immature virions generated by protease inhibitors treatment.

Conclusion: Immature HIV-1 virions generated from NL4-3/DRT represent effective novel immunogens displaying a safer and stronger capacity to induce HIV-specific cellular responses than wild-type viral particles.

P11.06
Generation of a Collection of HIV-1 Envelopes to Be Used as Immunogens with Increased Affinity for Broadly Neutralizing Antibodies

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Background: Antibody-based HIV-1 vaccine strategies rely on the elicitation of broadly neutralizing antibodies (bNAbs). Considering that the ability of an epitope to elicit antibodies will depend on its exposure on the virion, we took a new approach to optimize the HIV-1 envelope protein (Env) as an immunogen based on the selection of variants with increased affinity for the bNAb 4E10 from a library of virions with randomly mutated Env.

Methods: We used the full-length env gene from HIV strain AC10 to generate the library of randomly mutated envelopes. Cloning was performed into NL4-3 context and virions were produced by transient transfection in 293T cells. Selection of viruses with increased affinity to 4E10 was carried out by an improved in-solution virion capture assay. RNA extraction and reverse transcription PCR of the env gene was performed from the virus population captured for further sequencing and cloning into NL4-3 context.

Results: After a single round of selection we succeeded in selecting an envelope with a 4-fold increased affinity to 4E10 compared to AC10. Sequencing of the full-length envelope revealed 10 amino acid substitutions across the env gene, including the loss of 3 potential N-linked glycosylation sites. Mutation C131Y is especially relevant because it disrupts the architecture of the V1/V2 loop.

Conclusion: We have selected, in a single round, an Env variant with increased affinity for 4E10 which harbours the kind of mutations that have been previously associated with increased neutralization sensitivity and increased induction of neutralizing antibodies. The immunogenicity of this variant will be tested next in a mouse model. We propose the usage of the present new approach for the selection of envelopes with increased affinity for different bNAbs and the generation of a collection of envelopes to be used as immunogens for eliciting a broadly neutralizing antibody response.

P11.07
A Prime-Boost Immunization with an Auxotrophic BCG Expressing HIV-1 Gag and Gag VLPs Induces Broad, Polyfunctional Memory T Cell Responses in Baboons


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Background: We previously reported a BCG panthothenate auxotroph expressing HIV-1 subtype C Gag (panBCG) that is immunogenic in baboons. In this study, we assessed the breadth, polyfunctionality and memory phenotype of T cell responses.

Methods: Two groups of baboons (n = 6 each) were primed or mock-primed twice with either panBCG or a control BCG and boosted with Gag VLPs. The breadth of Gag-specific responses was measured by IFN-γ ELISPOT method, using HIV-1 Gag peptides. The proportion of Gag-specific cells was measured by polychromatic flow cytometry after staining for IFN-γ, IL-2 and TNF-γ. CD8+ and CD95 were used to delineate central and effector memory T cells.

Results: PBMC from panBCG-primed animals responded to an average of 11 peptides per animal, targeting 3 peptides each in p17 and p24, and 5 peptides in p15 domains. In contrast, PBMC from controls targeted an average of 4 peptides per animal in either p24 or p15 domains. All panBCG-primed animals generated Gag-specific polyfunctional T cells, with the majority of responding CD4+ T cells producing all 3 cytokines simultaneously. The majority of CD8+ T cells produced IFN-γ and TNF-γ only. In contrast, only 2 of 6 controls mounted Gag-specific responses. In addition, panBCG-primed animals generated significantly higher frequencies of total cytokine positive T cells than the controls. Over 97% of the mean frequency of responses in the CD4 compartment was of a central
membrane phenotype. A more balanced central and effector memory Gag-specific CD8+ T cell phenotype was generated, with a mean of 69% and 31% in each compartment, respectively. Measurable CD4 and CD8 responses persisted in 2 of 6 and 1 of 6 animals respectively over a 12-week period after the last Gag VLPs inoculation. Conclusion: These data demonstrate that these candidate HIV vaccines are capable of inducing broad and polyfunctional T cells that may be long-lived.

P12.01
HIV Neutralization in Breast Milk Mirrors that of Plasma and Is Mainly Mediated by IgG Antibodies

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Background: Despite months of mucosal virus exposure, the majority of breastfed infants born to HIV-infected mothers do not become infected, raising the possibility that immune factors in milk inhibit mucosal transmission of HIV. HIV envelope–specific antibodies are present in the milk of HIV-infected mothers, but their functional role has not been fully investigated.

Methods: The HIV envelope–specific antibody binding, heterologous virus neutralization and antibody-dependent cell toxicity (ADCC) responses were measured in the breast milk and plasma samples of 40 HIV-infected, lactating women. In addition, the ability of breast milk and plasma to neutralize autologous breast milk and plasma viruses was determined in 7 women, including 3 who transmitted HIV to their baby during breastfeeding.

Results: HIV envelope–specific IgG antibodies were more abundant than HIV-specific IgA in milk, although IgA is the predominant antibody isotype in milk. The concentration of anti-gp120 IgG was directly correlated in milk and plasma (r = 0.75 p < 0.0001), yet the response in milk was two logarithms (logs) lower than in plasma. Autologous virus neutralization was detected in all plasma samples and in one of the transmitter breast milk. Heterologous virus neutralization and ADCC activity in milk were directly correlated to that in the systemic compartment, but were two logs lower in magnitude (neutralization r = 0.48, p = 0.018, ADCC r = 0.64 p < 0.0001). Moreover, IgG purified from milk and plasma had equal heterologous neutralizing potency (r = 0.65 p < 0.0001), and the maximal ADCC potency was similar in the two compartments (r = 0.60, p < 0.0001). Milk heterologous virus neutralization titers also correlated with HIV gp120 envelope-binding IgG responses, but not IgA responses (r = 0.70, p < 0.0001 and r = 0.13, p = 0.34). No neutralization was detected in the non-IgG fraction of milk.

Conclusion: These results suggest that plasma-derived IgG antibodies play a major role in the low level HIV neutralization and ADCC activity of breast milk.

P12.02
PedVacc Infant HIV-1 Vaccine Trials: First Stage Towards a Vaccine Against Breast Milk Transmission of HIV-1


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Background: Over 60% of the global HIV-1-infected population lives in Africa and about half of the infected adults are women of childbearing age. Approximately half of mother-to-child transmission is due to breast-feeding, but formula feeding is an option for many HIV-1 infected mothers. The best hope for protecting newborns and infants in developing countries against HIV-1 from their infected mothers while breastfeeding, is through development of a safe, effective, accessible vaccine.

Methods: Two PedVacc infant HIV-1 vaccine trials examine the safety of a novel HIV-1 vaccine, MVA.HIVA, in infants. The trials are taking place in The Gambia and Kenya and will recruit in total 120 healthy, HIV-1-negative infants born to healthy, either HIV-1-positive or HIV-1-negative mothers. Both trials entail a single injection into the muscle of infants aged 20 weeks of MVA.HIVA. Any HIV-1-positive women in this study are provided with antiretroviral drugs and extensive feeding counselling during pregnancy and while breastfeeding to reduce the risk of HIV-1 transmission to their infants. Half of the infants in the study are randomised to receive the MVA.HIVA study vaccine in addition to their regular childhood immunizations. The other half only receive their regular immunizations, but not the study vaccine, and they serve as a control to the vaccinated infants.

Results: Initial MVA.HIVA vaccine safety and immunogenicity data will be presented.

Conclusion: The two PedVacc trials contribute to the capacity building for infant vaccine trials in Africa and represent the first stage towards a more complex heterologous prime-boost vaccine regimen.
Nucleic Acid Kit (Roche) for manual specimen preparation according to manufacturer’s instructions. Cell-free HIV-1 viral load was measured in breast milk samples using the COBAS® TaqMan Analyzer (Roche Diagnostics Corporation, Indianapolis, USA). The lower limit of detection was 40 copies/ml HIV-1 RNA.

**Results:** No significant differences in breast milk HIV-1 RNA levels were observed between EBF and MBF groups at either 2 weeks, 2 months, or 5 months postpartum (p = 0.57, p = 0.35, p = 0.88, respectively).

**Conclusion:** We report no observed relationship between MBF and measured breast milk viral RNA load. It appears unlikely that the increased transmission risk associated with MBF is mediated by breast milk HIV RNA concentration.

**P13.01 Safety and Immunogenicity of a HLA Supertype Restricted CTL Epitope Based HIV-1 Therapeutic Peptide Vaccine in CAF01 Adjuvant**

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**Background:** We designed a peptide based vaccine based on 18 peptides together with a novel adjuvant CAF01. CAF01 is a synthetic two-component liposomic adjuvant comprising the quaternary ammonium dimethyl-dioctadecyl-ammonium (DDA) and the immune modulator trehalose 6,6’-dibehenate (TDB). The peptides were 15 CTL epitopes plus three T helper epitopes, in order to induce a balanced CD4+ and CD8+ cellular immune response. The selected CTL epitopes were derived from conserved regions of the HIV-1 and were subdominant (i.e. infrequently targeted) within natural infection. Moreover, the CTL epitopes were predicted to be restricted to five different HLA supertypes, thereby the vaccine should theoretically cover >90% of any population worldwide.

**Methods:** In a single blinded phase 1/II therapeutic HIV-1 vaccine trial 9 treatment naïve HIV-1 infected Danish individuals where immunized intramuscular (week 0, 2, 4 and 8) with peptides (18 times 0.3 mg) together with the adjuvant CAF01 and 2 individuals received placebo (NaCl). Novel T cell responses were evaluated by IFNγ ELISpot two weeks and three months after the last immunization.

**Results:** Previously undetected T cell responses specific for one or more epitopes were induced. The immunizations were safe and well tolerated. No allergic or autoimmune reactions were observed. No haematological, hepatic, muscular, pulmonary or renal toxicities were observed by blood testing. No overall or sustained change in viral load or CD4+ T cell counts was observed.

**Conclusion:** In this study we show that it is possible to generate new T cell responses in treatment-naïve HIV-1-infected individuals using peptides in adjuvant and thereby redirect immunity to target selected subdominant CTL epitopes. Although possible during untreated infection and high viral loads, therapeutic immunization during successive antiretroviral therapy where the immune functions are better preserved might lead to more potent and durable cellular responses.

**P14.01 Long-Term Consequences of Participation in HIV Preventive Vaccine Trials Among Healthy Uninfected Subjects: The ANRS COHVAC Cohort**

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**Background:** ANRS COHVAC cohort gathers data of healthy low HIV risk volunteers who participated in phase I and II trials in France since 1992; one of its objectives is to explore long term psycho-social consequences of participation in HIV vaccine trials and vaccine-induced seropositivity (VISP).

**Methods:** Vaccine candidates were a recombinant envelope protein (rgp160) [1992–1993], Alvac vectors (vCP) expressing Env, Gag, Pro and CTL domains of Pol [1994–2001], and/or HIV-1 lipopeptides representing CTL epitopes of Gag, Pol and Nef proteins [1997–2007]. Personal and social consequences of participation were collected by an anonymous questionnaire and an interview with a clinician. VISP was defined as HIV positivity in at least one licensed enzyme immunoassay, regardless of Western-blots results.

**Results:** 422 volunteers were eligible for the cohort study. Questionnaires and blood samples were collected from 250 (59%) and 248 subjects at inclusion, respectively. The median age was 52 years (25–71), 52% were male. Most subjects had graduated from high school (86%), were employed (84%) and 102 (40%) were blood or tissue donors. VISP was observed in 11% (27/248) of subjects: 66% (19/29), 16% (7/44) and 0.6% (1/151) of rgp160, vCP and lipopeptides recipients, respectively. Western-blots showed presence of bands included in the vaccine products (mainly gp160, p25, p55) in 10 of these subjects. The median VISP durability was 16.6 years (range, 15.6 to 18.3) for rgp160 and 9.7 years (range, 8.5 to 15) for vCP. Five subjects reported problems with insurance and 32 with blood or tissue donation after participation had stopped. Other social negative consequences were expressed by 13% of interviewed subjects.

**Conclusion:** Negative consequences were seldom reported except regarding blood donation. VISP was maintained 16 years in a majority of rgp160 recipients and over 8 years in a minority of Alvac recipients. Volunteers should be informed of possible VISP several years after active participation.

**P14.02 Multifunctional Fusion Proteins of the Human Engineered Antibody Domain m36.4 with Human Soluble CD4 and Fc as Potential Prophylactics**

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**Background:** Multifunctional fusion protein represent a new class of therapeutic proteins that combine one or more functions. They can act at the cell surface or inside cells to overcome biological barriers that limit the accessibility of conventional proteins. Examples of such proteins include fusion proteins that combine a soluble CD4 domain with an antibody domain (m36.4) to achieve several biological effects, including inhibiting HIV-1 entry and neutralizing HIV-1 infection.

**Methods:** To develop a novel therapeutic protein for HIV-1, we generated a series of fusion proteins that combine soluble CD4 (sCD4) and m36.4 domains. These fusion proteins were expressed in mammalian cell lines and evaluated for their ability to inhibit HIV-1 entry and neutralize HIV-1 infection.

**Results:** The fusion proteins showed potent inhibition of HIV-1 entry and neutralization of HIV-1 infection. The fusion proteins were also shown to be capable of crossing biological barriers and accessing HIV-1-infected cells.

**Conclusion:** These results suggest that multifunctional fusion proteins of the human engineered antibody domain m36.4 with human soluble CD4 and Fc have potential as prophylactic agents for HIV-1.
P14.03
Safety and Reactogenicity of ALVAC-HIV (vCP1521) and HIV-1 gp120 AIDSVAX B/E Prime-Boost Vaccination Regimen in a Community-Based Efficacy Trial

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Background: A prime-boost vaccination regimen with ALVAC-HIV (vCP1521) administered intramuscularly at 0, 4, 12, and 24 weeks and gp120 AIDSVAX B/E at 12 and 24 weeks demonstrated modest efficacy of 31.2% for HIV acquisition in HIV-uninfected adults in a phase III trial in Thailand.

Methods: Reactogenicity was recorded for 3 days following vaccination. Adverse events were monitored every 6 months for 3.5 years, and pregnancy outcomes were recorded.

Results: Of the 16,402 volunteers, 69% of the participants reported an adverse event any time after the first dose. Only 32.9% of events occurred within 30 days post vaccination, 3.2% attributed to vaccine, and rates did not differ between groups. The frequency of serious adverse events was similar in vaccine (14.3%) and placebo (14.9%) recipients (p = 0.33). None of the 160 deaths (85 in vaccine, 75 in placebo) were related to vaccine. The most common cause of death was trauma and accident. Approximately 30% of female participants reported a pregnancy during the study. Abnormal outcomes were experienced in 17.1% and 14.6% (p = 0.13) of vaccine and placebo recipients with pregnancies, respectively. Among pregnancies with estimated dates of conception within 3 months of a vaccination, the large majority of these abnormal outcomes were spontaneous or elective induced abortions reported in 22.2% and 15.3% of vaccine and placebo pregnant recipients, respectively (p = 0.08). Local reactions (mostly pain and tenderness) occurred in 88.0% of vaccine and 61.0% of placebo recipients (p < 0.001) and were more frequent after ALVAC-HIV than AIDSVAX B/E vaccination. Systemic reactions (mostly fatigue, myalgia, headache) were more frequent in vaccine than placebo recipients (77.2% vs. 59.8%, p < 0.001). Local and systemic reactions were mostly mild to moderate, resolving within 3 days.

Conclusion: The ALVAC-HIV and AIDSVAX B/E vaccine regimen was found to be safe, well tolerated and suitable for large-scale use in Thailand.

P14.04
HIV-Specific CD4 and CD8 Proliferative Responses and Effector Memory Profile in Swedish Vaccinees Immunized with HIV-1 DNA and HIV-1 MVA and HIV-1 MVA

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Background: We used a Flow-Cytometric Lymphoproliferation Assay (FC-LPA) to monitor HIV-1-vaccine-induced responses. Healthy volunteers were immunized at 0, 1 and 3 months with DNA plasmid expressing gp160 of HIV-1 subtypes A, B and C; revB; p17/p24 gag A and B and RTmut B. At nine months they were boosted with a heterologous MVA expressing HIV-1 env, gag, pol of CRF01_AE. Approximately 3 years after the first HIV-MVA vaccination, 24 volunteers were given a second HIV-MVA boost and the last 17 vaccinees were tested by FC-LPA.

Methods: Lymphoproliferative responses to AT-2 inactivated HIV-1 antigen (donated by Jeffrey Lifson, NCI, USA), were monitored by FC-LPA. We used a Flow-Cytometric Lymphoproliferation Assay (FC-LPA) to monitor HIV-1-vaccine-induced responses. Healthy volunteers were immunized at 0, 1 and 3 months with DNA plasmid expressing gp160 of HIV-1 subtypes A, B and C; revB; p17/p24 gag A and B and RTmut B. At nine months they were boosted with a heterologous MVA expressing HIV-1 env, gag, pol of CRF01_AE. Approximately 3 years after the first HIV-MVA vaccination, 24 volunteers were given a second HIV-MVA boost and the last 17 vaccinees were tested by FC-LPA.

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Vaccinia Ankara (MVA) Vaccines

**Construction and Characterization of HIV-1 Mosaic Modified Vaccinia Ankara (MVA) Vaccines**

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**Background:** Several Modified Vaccinia Ankara (MVA) vaccines containing natural HIV-1 sequences are currently showing promise in clinical trials. Recently, multivalent HIV-1 mosaic immunogens have demonstrated increased breadth and depth of cellular immune responses in primates. Here, we describe construction and preliminary characterization of HIV-1 mosaic MVA vaccines.

**Methods:** Two double recombinant MVA viruses were constructed, each containing the previously described M1 and M2 mosaic env, gag, and pol sequences, via successive rounds of plaque purification and amplification. Balb/c mice were immunized with 10^7 pfu of Mosaic MVA viruses at 0 and 4 weeks. Intracellular cytokine staining (ICS) and pentamer staining using KETI and KDTI gag peptides were performed 1 week after the 2nd immunization. Binding antibody titers to subtype A/E, C, D and A gp140 envelopes were evaluated 2 weeks after the 2nd immunization by ELISA.

**Results:** Two MVA viruses containing mosaic HIV-1 env/gag/pol inserts were constructed and expression of proteins was confirmed by immunostaining and Western blot. Stability of viruses was confirmed by PCR, sequencing and immunostaining after 10 rounds of serial passage. Mosaic MVAs generated positive CD8 T cell responses in mice to KETI and KDTI gag peptides by pentamer staining and also by production of IFN-gamma by ICS. Immunogenicity was also demonstrated by pooled peptide ELISPOT responses in mice. Binding antibodies to envelopes of diverse subtypes were generated at titers comparable to previously constructed MVA vaccines.

**Conclusion:** We demonstrate that HIV-1 mosaic MVA vaccines are comparable to previously constructed MVA vaccines in terms of titer, stability, expression and immunogenicity in mice. HIV-1 Mosaic MVA vaccines, in combination with HIV-1 Ad26 Mosaic vaccines, are currently undergoing evaluation in non human primate studies where evaluations of breadth and depth of the cellular immune response will be fully characterized.

P14.06

**Persistence of Vaccine-Induced Antibodies Following HIV-1 DNA Prime MVA Boost Vaccination Among Healthy Tanzanian Volunteers**


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**Background:** A phase I/II HIV vaccine trial (HIVIS03) using a multiclade, multigene HIV-1 DNA prime with two heterologous HIV-1 MVA boosts has been completed among healthy adults in Dar es Salaam, Tanzania.

**Methods:** Sixty HIV-uninfected volunteers randomized to three groups of 20, received DNA plasmids expressing HIV-1 gp160 subtypes A,B,C,revB/p17/p24gagA,B and Rtmut at 1 mg intradermally or 3.8 mg intramuscularly or placebo at months 0, 1 and 3 using a needle-free injection device (Biojector). Volunteers were boosted intramuscularly with 10^6 plaque-forming units of recombinant MVA expressing HIV-1 env, gag, pol of CRF01_AE or placebo at months 9 and 21. The volunteers were followed up 17–22 months after the second HIV-MVA boost and the presence of vaccine-induced antibodies was analyzed. Diagnostic HIV serological testing was performed using Murex (Abbott Murex, UK) and Integral (Siemens, Germany) HIV antigen/antibody ELISAs and Inno-Lia immunoblot (Inno-genetics, Belgium) assays.

**Results:** None of the vaccinees or placebo recipients was positive in the diagnostic HIV serological assays after the three HIV-DNA immunizations or after the first HIV-MVA boost. Four weeks after the second HIV-MVA boost, all 30 vaccinees (100%) were positive in all three diagnostic HIV assays. On the immunoblot assay, all 30 showed reactivity to both Gag (p24) and Env (gp120 and/or gp41). Twenty-nine out of 30 (97%) vaccinees were still reactive in both Murex and Integral HIV antigen/antibody ELISAs 17 to 22 months after the second HIV-MVA boost. On the Immunoblot assay, 21 out of 30 (70%) vaccinees fulfilled the diagnostic criteria for seropositivity and 9 were indeterminate. All of 30 (100%) vaccinees reacted against Gag and 21 out of 30 (70%) reacted against Env (gp120 or gp41). Testing by the Roche HIV-1 DNA PCR assay excluded HIV-1 infection in all these volunteers.

**Conclusion:** This study showed the durability of HIV-DNA/MVA vaccine-induced antibody responses in Tanzanian volunteers.
P14.07 Characterization of ANRS HIV LIPO-5 Vaccine in Healthy Volunteers Combining Cytokine Multiplex and Transcriptomic Analyses

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Background: ANRS Vac 18 study evaluated the immunogenicity of HIV-LIPO-5 vaccine (Gag17–35, 253–284, Pol325–355, Nef66–97 and 116–145, coupled to a palmytoil tail) administered at weeks 0, 4, and 12 in healthy volunteers. 62–69% of vaccinees developed HIV-specific CD8+ (12-days PBMC cultured IFN-γ ELispot using optimal peptides). Here, we measured cytokine and gene expression profiles associated with vaccine responses.

Methods: PBMCs from 12 vaccinees (W0 and 14) were stimulated with either LIPO-5 vaccine, or a pool of 15-mers Gag peptides. Transcriptomic (Illumina) and cytokine multiplex (Millipore) analyses were performed at 6/24H and D11, respectively. Statistical analyses (Wilcoxon for paired samples) were performed on concentration and raw fluorescence. Normalization and filtering were performed to select detected transcripts, and various conditions were compared using a false discovery rate set at 0.1.

Results: Gag Specific responses detected at W14 as compared to W0 were characterized by an increase of IFN-γ, TNF-a, IL-5, IL-10, IL-13 cytokines (all P values < .01). An adjuvant effect of Lipopeptide tail was characterized at W0 by an induction of IFN-γ and IL-10 in the supernatants of PBMC stimulated with LIPO-5. Consistently, a significant variation of gene transcripts after 6 hours (n = 160 probes) and 24 hours (n = 8 probes) stimulation was observed in LIPO-5 stimulation at W0. However, following vaccination, 3305 and 1821 probes were characterized by CXCL10, IL2RA, TNFAIP6, CCL3L1, IL-6 increased significantly (fold change > 2). Among these genes, IFN-γ, CXCL9, CXCL10, IL2RA, TNFAIP6, CCL3L1, IL-6 increased significantly (fold change > 2).

Conclusion: Specific responses to LIPO-5 vaccination are characterized by Th1 and Th2 profiles. The signature profile of LIPO-5 stimulation before vaccination could provide information about the adjuvant effect of lipid tail. Consistently with cytokine responses, vaccination is specifically associated with a large number of gene expression. This approach might help to identify new signatures associated with HIV vaccine responses.

P14.08 Lipopeptide Based HIV Vaccine Efficiently Primes Recombinant MVA (HIV-Clade B Gag/Pol/Nef) Elicited HIV-Specific T Cell Responses

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Background: HIV-lipopeptide candidate vaccine (Lipo-5) developed by the ANRS has been shown to induce HIV specific precursor T cells in more than 70% of healthy volunteers. The objective was to assess, in nonhuman primates (NHP), rMVA-HIV/lipo-5 and Lipo-5/rMVA-HIV prime/boost combinations.

Methods: Five groups of four cynomolgus macaques were injected 1) group 1 (L/L): with Lipo-5 alone (250 µg by the intramuscular route) on weeks 0, 8, 16 and 21, 2) group 2 (rM/L) with Lipo-5 (weeks 16 and 21) after a prime (weeks 0 and 8) with the ANRS rMVA (5x107 pfu by the subcutaneous route) 3) group 3 (L/rM): with rMVA (weeks 16 and 21) after priming with Lipo-5 (weeks 0 and 8). Groups 4 and 5 are control groups with wt MVA. Lipo-5 vaccine contains Gag17–35, Gag253–284, Pol325–355, Nef66–97 and Nef116–145. Immune response was assessed using PBMC stimulated ex vivo with pools of Gag, Pol and Nef peptides and by analyzing the frequency of IFN-g and IL-2 producing cells by ELISpots, cytokine secretion in culture supernatants and by intracellular staining (ICS).

Results: In L/L group no ELISpot IFN-g response was detectable. In rM/L maximal T cell responses were elicited in 4/4 NHP after two rMVA primes (mean +/- SD: 599 +/- 204 SFC/106 cells). In the group L/rM 0/4 NHP responded after the prime and 4/4 after a single injection of rMVA (mean +/- SD: 671 +/- 329 SFC/106 cells). In control groups wt MVA did not elicit any HIV specific response.

Conclusion: Sequence of prime/boost combining ANRS Lipo-5 and rMVA differently impacts HIV specific T cell responses. Lipo-5 primes efficiently for vector-induced HIV specific responses. This study provides a rationale for future clinical trials in human volunteers.

P14.09 Phase I Trial of a Prophylactic HIV-1 DNA gag-pol-env-nef Vaccine in St. Petersburg, Russia

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Background: A phase I trial was conducted to assess the safety and immunogenicity of an HIV-1clade A FSU DNA vaccine in healthy HIV-1 uninfected adults. Recombinant DNA vaccine candidate (DNA-4) consists of four plasmids encoding consensus sequences of the nef, gag, pol (rt) and env (gp140) HIV-1 subtype A FSU genes.

Methods: 21 participants were randomly divided into three groups (7 per group). Each group received 0.25, 0.5 or 1.0 mg of DNA-4, respectively. Vaccine was administrated four times intramuscularly at days 0, 6, 10 and 14. Immunogenicity was assessed by ex vivo IFN-γ ELISPOT, IFN-γ/IL-2/TNFα ICS, CFSE LPA and ELISA at days 14, 25, 43 and 60 after the first vaccination. The samples, which were taken before immunization, served as negative controls.

Results: The vaccine regimen was found to be safe and well tolerated in this sample. 4 vaccinees from the first group (57%), 4 - from the second group (57%), and 2 – from the third group (29%)
were IFN-γ ELISpot reactive. In the ICS assays 7/7 (100%) subjects, who received 0.25 mg of DNA-4, 2/7 (29%) subjects, who received 0.5 mg of DNA-4, and 6/7 (86%) subjects, who received 1 mg of DNA-4, had CD3+ CD8+ T-cell responses. CD3+ CD8+ T-cell responses were detected in 6/7 (86%), 5/7 (71%), 4/7 (57%) subjects from first, second and third groups respectively. 4/7 (57%), 4/6 (67%), 3/7 (43%) patients had positive CD8+ LPA responses; and 5/7 (71%), 5/6 (83%), 3/7 (43%) demonstrated CD8- LPA responses. Altogether 17/21 (81%) subjects revealed vaccine-induced seropositivity, 3 to Nef, 1 to Gag, and 1 to Gp140.

Conclusion: DNA-4 vaccine is well tolerated. Vaccination with Gag, and 1 to Gp140. vaccines exhibited vaccine-induced seropositivity, 3 to Nef, 1 to

The HIV Vaccine Candidate MVA-B Triggers Robust, Polyfunctional and Memory T Cell Responses to HIV-1 Antigens in a Phase I Clinical Trial in Spain

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Background: There is general consensus that for an HIV/AIDS vaccine to be effective it must trigger broad, polyfunctional and durable antigen-specific B and T cell immune responses. Attenuated poxvirus vectors expressing HIV-1 antigens are considered promising HIV/AIDS vaccine candidates. However, the modest efficacy observed in the RV144 trial point to the development of improved pox vectors.

Methods: Here we described the T cell immune responses induced in healthy volunteers participating in a phase I clinical trial in Spain (RISVAC02) after intramuscular administration of three doses of the recombinant MVA-B expressing monomeric gp120 and the fused Gag-Pol-Nef (GPN) polyprotein of clade B.

Results: The majority (92.3%) of the volunteers immunized had a positive specific T cell response at any time post-vaccination as detected by IFN-γ ICS assay. The CD4+ T cell responses were predominantly Env directed, whereas the CD8+ T cell responses were similarly distributed against Env, Gag and GPN. The proportion of responders after two doses of MVA-B was similar to that obtained after the third dose of MVA-B vaccination and the responses were sustained (84.6% at week 48). Vaccine-induced CD8+ T cells were polyfunctional and mainly distributed within the TEM and TEMRA cell populations. Anti-vector T cell responses were mostly induced by CD8+ T cells, highly polyfunctional and of TEMRA phenotype.

Conclusion: These findings demonstrate that the poxvirus MVA-B vaccine candidate given alone is highly immunogenic, inducing broad, polyfunctional and long-lasting CD4 and CD8 T cell responses. Thus, MVA-B can be considered as promising candidate HIV/AIDS vaccine vector for further clinical trials.
Methods: Several cross-sectional studies and one follow-up cohort study were analyzed to determine HIV prevalence, incidence, and subtyping; risk factors; circumcision; behavioral factors; and willingness to participate in vaccine trials among MSM.

Results: The estimated HIV prevalence has been 11–14% during the past ten years (800–1000 volunteers/year). HIV incidence, both in the cohort study and in cross sectional studies (Detune) was 4–6% persons-year. Phylogenetic analysis revealed that subtype B was the most common subtype present in MSM; yet, a higher frequency of non-B-subtypes (BF recombinants) was found in the last study conducted by the team as compared to previous studies (30.4% vs.10.5%, p < 0.001). The one-year follow-up cohort study showed a retention rate of 91%. Studies also showed that about 50% of MSM have unprotected intercourse, 12.5% are circumcised, and 53% of men who attended the NGO for testing are repeated testers. More than 60% of MSM expressed willingness to participate in vaccine trials.

Conclusion: The MSM population in Buenos Aires is very well characterized regarding HIV prevalence, incidence, molecular epidemiology, sexual risk behavior, and related parameters. Excellent retention rates were achieved in the past. Participants indicated strong commitment to participate in HIV-related studies, including vaccine clinical trials. These data clearly indicate that MSM from Argentina constitute a suitable group for HIV vaccine clinical trials.

P15.02
Circumcision and HIV Acquisition Risk Among Men Who Have Sex with Men (MSM) in Buenos Aires, Argentina

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Background: Several studies have demonstrated that circumcision reduces HIV acquisition among heterosexual men by approximately 60%. However, this may not be the case among MSM engaged in receptive anal intercourse (RAI). In Argentina, no information has been available about frequency of circumcision among MSM or its potential benefits. That was the focus of the present study.

Methods: 500 MSM recruited through RDS (respondent-driven sampling) for an HIV-prevalence study were asked if they were circumcised and, if not, whether they would be interested in circumcision if it could protect them against HIV. Inclusion criteria included age at least 18 years, resident of Buenos Aires, reporting sex with men at least 10 times in their lives and at least once in the past six months. All statistics have been weighted based on the participants’ self-reported network size.

Results: A total of 66 (14%) MSM reported being circumcised. No statistical differences were observed on age, condom-use, or sexual role (receptive or insertive with a man) between circumcised and uncircumcised. HIV prevalence was 17%. Being circumcised was not significantly associated with HIV prevalence (p = 0.211). Stratifying the group according to their sexual role, 36% reported being engaged in RAI. Among those who do not practice RAI, those who were circumcised (N = 37) had no cases of HIV infection, whereas among those who were uncircumcised, 15% (40 of 265) were HIV positive (p = 0.007). No difference in HIV prevalence was observed between circumcised and uncircumcised among those who practice RAI. Among those who were not circumcised, 75% said that they would not be willing to be circumcised even if it could be beneficial for HIV infection risk reduction.

Conclusion: Further exploration is required on the potentially protective role that circumcision may have for MSM who refrain from RAI. Attention should be paid to the lack of motivation of MSM to be circumcised for HIV-prevention.

P15.03
Community Awareness About AIDS Vaccine in Georgia

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Background: Community education for HIV/AIDS vaccine development and research and community preparedness for vaccine trials are fundamentally linked. Vaccines have already been proven to be effective and efficient means of preventing a number of other serious viral diseases. Knowledge and empowerment, through education, are the tools by which people and communities gain mastery over issues of concern. In Georgia, PLWHA has lack of information on AIDS vaccine.

Methods: Aim of the study was to assess community knowledge on HIV vaccine development. A study was carried out in four main cities of Georgia: Tbilisi, Kutaisi, Batumi and Zugdidi. The data were collected through administration of an anonymous questionnaire. In total 315 interviews were carried out. The interview addressed understanding of AIDS vaccine, opinions, views, fillings and target community approach.

Results: Results of this survey demonstrate informational gaps in community’s knowledge of HIV vaccine and emphasize the need for training on HIV Vaccine and its development. Only 37% had heard about an AIDS vaccine, while 63% did not have any information. Only 18% of respondents had a good understanding regarding AIDS vaccine. The main primary source regarding AIDS vaccines were 28% media and public 43%. The respondents had numerous questions regarding HIV vaccine (questions about testing, distribution, etc.) Some participants believed an HIV vaccine would be available in the future. Others believed it would not.

Conclusion: The present findings suggest the importance of carefully planning and developing strategies designed to reach communities at elevated risk for acquiring HIV/AIDS in order to increase HIV vaccine knowledge, acceptability and trust and to dispel misinformation and undue fears in regard to future HIV vaccine.

P15.04
Griffithsin, Cyanovirin-N and Scytovirin Inhibit HIV-1 Binding and Transfer via the DC-SIGN Receptor

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Background: The lectins Griffithsin (GRFT), Cyanovirin-N (CV-N) and Scytovirin (SVN) are potential microbicides that inhibit
HIV-1 infection by binding to mannose-rich glycans on the envelope. Since the DC-SIGN receptor plays a key role in the sexual transmission of HIV-1, we investigated whether these lectins could inhibit HIV-1 transfer and infection via this receptor.

**Methods:** Five subtype C (COT6.15, COT9.6, Du156.12, Du151.2 and CAP63.A9J) and three subtype B (QH0692.42, PVO.4 and CAAN5342.A2) viruses were incubated with each lectin then captured with the DC-SIGN receptor on Raji/DC-SIGN cells. HIV-1 capture was measured by p24 ELISA. For studies of inhibition of HIV-1 transfer and infection, the virus was either incubated with the lectin after capture by Raji/DC-SIGN cells but prior to transfer to JC53bl-13 cells. GRFT and CV-N were at least 10 times more potent in this format. A similar trend was noted for SVN although it was less potent. Lastly, the three lectins inhibited DC-SIGN binding and transfer of subtype B and C with similar potency.

**Results:** The 3 lectins inhibited HIV-1 capture via DC-SIGN of all 8 viruses albeit at modest levels: the average for CV-N, SVN and GRFT was 52, 38 and 26%, respectively. However, all three lectins potently inhibited the DC-SIGN mediated transfer of HIV-1 to JC53bl-13 cells and PBMCs. This was particularly evident when HIV-1 was incubated with the lectins after capture by Raji/DC-SIGN cells but prior to transfer to JC53bl-13 cells. GRFT and CV-N was noted for SVN although it was less potent. Lastly, the three lectins inhibited DC-SIGN binding and transfer of subtype B and C with similar potency.

**Conclusion:** Our study suggests that GRFT, CV-N and SVN in-hibit DC-SIGN-mediated HIV-1 infection and, therefore, sup-ports further studies of these compounds for HIV-1 prevention via mucosal routes.

P15.05 Women Fisher Folk: Recruitment, Retention and Participation in HIV Prevention Research


**Background:** In sub-Saharan Africa women constitute approximately 60% of people living with HIV/AIDS. Factors that contribute to this include lack of access to essential information and reproductive health services, economic disempowerment, sexual violence and partner risk behaviours. There is an urgent need to involve women in HIV prevention research. This study explores recruitment, retention and participation in HIV prevention research among women in fishing communities in Uganda.

**Methods:** With funding from EDCTP, Uganda Virus Research Institute and its partners conducted an 18 months prospective cohort study in 5 fishing communities along the shores of Lake Victoria in preparation for future HIV prevention research. 2,074 volunteers (men and women) aged 13-49 years were screened using demographic, medical history and risk behavior assessment questionnaires. 1,000 high risk HIV negative volunteers were enrolled and followed up every 6 months for 18 months.

**Results:** Women constituted 51% of those screened, and 46% of those enrolled with a median age of 28 years. Approximately 64% of women had primary education, 73% were married. Majority of Women (70%) were engaged in fish processing, 10% bar attend-ants and 20% were unemployed. One third of the women (33%) were engaged in transactional sex. Pregnancy rates at 18 months’ follow up were high (22%). 80% and 73% of the women and men respectively completed all the follow up visits. Overall 23% never reached 18 months follow up, of whom 40% were women. The overall HIV incidence was 5/100PY, 4.6/100PY among women and 5.3/100PY among men.

**Conclusion:** Approximately 30% were either unemployed or in vulnerable employment exposing them to economic disempowerment and high HIV risk exposure, in urgent need of interventions. These women were willing to participate in HIV prevention research with encouraging retention rates at 18 months.

P15.06 Prevention of HIV-1 Sexual Transmission Using Novel Small-Molecule CCR5 Antagonists Formulated into Temperature-Sensitive Acidic Microbicide


**Background:** The promising finding of a nucleotide analog reverse-transcriptase inhibitor tenofovir gel in protecting women from HIV infection makes microbicide an exciting preventive intervention against HIV sexual transmission in addition to vaccine. Here we report two novel small-molecule CCR5 inhibitors, TD-0232 and TD-0680, study their antiviral activity and mechanism against HIV-1 infection, and investigate the potential of TD-0232 as a microbicide.

**Methods:** We measured the antiviral activity of TD-0232 and TD-0680 by using both HIV pseudovirus and clinical isolate-based neutralization assays. We also determined the capability of TD-0232 and TD-0680 to inhibit Env-mediated cell-to-cell transmission. The combination effect of TD-0232 and TD-0680 with other classes of HIV-1 inhibitors was analyzed. Site-directed mutagenesis, combination assay and molecular docking were adopted to reveal the binding mode of TD-0232 and TD-0680 on CCR5. The stability profile and inhibitory activity of TD-0232 in a formulation of temperature-sensitive acidic gel were also investigated.

**Results:** TD-0232 and TD-0680 specifically inhibited R5-tropic HIV-1 and SIV strains and TD-0680 consistently had a higher potency. They also blocked cell-associated virus fusion and clinical isolate-based neutralization assays. We also determined the capability of TD-0232 and TD-0680 to inhibit Env-mediated cell-to-cell transmission. The combination effect of TD-0232 and TD-0680 with other classes of HIV-1 inhibitors was analyzed. Site-directed mutagenesis, combination assay and molecular docking were adopted to reveal the binding mode of TD-0232 and TD-0680 on CCR5. The stability profile and inhibitory activity of TD-0232 in a formulation of temperature-sensitive acidic gel were also investigated.

**Conclusion:** TD-0232 and TD-0680 are potent inhibitors against R5-tropic HIV-1 infectivity but with distinct binding profiles on CCR5. TD-0232 is also a promising candidate for preventive microbicide.
P15.07
Recruitment and Retention of HIV Serodiscordant Couples in a Pilot Daily and Intermittent Pre-Exposure Prophylaxis Study in Masaka, Uganda

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Background: Pre-Exposure Prophylaxis (PrEP) using antiretroviral drugs is one of the HIV prevention technologies currently being explored. High volunteer recruitment and retention rates are critical for the conduct of HIV Pre-Exposure prophylaxis trials. We describe screening, recruitment and retention of volunteers in HIV sero-discordant couple relationships participating in a pilot study of daily and intermittent PrEP in Masaka, Uganda.

Methods: Volunteers were recruited from an ongoing HIV serodiscordant couples’ HIV incidence cohort study. Enrolled volunteers were randomized to daily FTC/TDF or placebo, or intermittent FTC/TDF or placebo in a 2:1:2 ratio. Volunteers were reviewed weekly for the first two weeks and again 2 weeks later. Thereafter, monthly visits were conducted for 4 months and then a final post-trial drug visit 2 months after the end of the intervention period. Procedures conducted at every monthly study visit included: HIV and pregnancy tests, HIV (pre- and post-test), family planning, study medication adherence counseling, and clinical and laboratory safety assessments.

Results: A total of 133 HIV serodiscordant couples were screened between September 2009 and March 2010. Of these, 72 (50% F-M+) were enrolled, 45 (34%) screened out while 16 (12%) met the eligibility criteria but were not enrolled because study accrual had been achieved. The main reasons for screen failure were: low creatinine clearance (57.8%), abnormal urinalysis results (15.6%), breastfeeding (4.4%) and receipt of ART by HIV-infected partners (4.4%). All of the remaining 8 volunteers (17.8%) had a different individual reason for screen failure. Sixty eight (94%) volunteers completed the study while 4 dropped out due to pregnancy (3) and previously undiagnosed Hepatitis (1).

Conclusion: We successfully recruited and retained heterosexual HIV discordant couples in this pilot daily and intermittent PrEP study. Laboratory abnormalities were responsible for most screen failures while drop-outs were mostly due to pregnancy.

P15.08
Project VOGUE: HIV Vaccine Education in an Underserved Racial/Ethnic MSM Population

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Background: African-American and Latino men who have sex with men (MSM) are currently the most at risk for becoming infected with HIV among all racial sub-populations of MSM. The House/Ball community is a subset of this population and at particularly high risk related to unprotected anal sex and substance abuse within their closed social network. Rochester/Buffalo area has approximately 15 houses, with 10–12 members in each, age range 15–45. The House/Ball community has not been targeted for HIV prevention interventions/programs including interventions regarding participation in HIV vaccine clinical trials and other bio-medical research.

Methods: Project VOGUE, a 2-year initiative funded by the Legacy Project, consisted of community based participatory research (CBPR) principles; as well as education about HIV prevention and vaccine trials to the House/Ball community. Qualitative interviews and focus groups were conducted. A train the trainer session was held with 13 key informants that resulted in a final curriculum. A five session group level intervention resulted, consisting of—substance abuse, HIV prevention/transmission, partner violence, HIV vaccine clinical trials, and community resources. Knowledge; skill building exercises; a community change project by the attendees; and final session to evaluate the process through a post-questionnaire/knowledge assessment was conducted.

Results: The leaders of each house joined to form the “Council of Houses” (COH) as expert informants. The COH members have been engaged in the process and recognize the need for HIV prevention/vaccine research. The COH also developed governance structures that frame, inform, and influence the behavioral social norms of the local House Ball community.

Conclusion: Expert informants and CBPR methods proved effective in developing a project to educate MSM involved in the House Ball community about HIV vaccine research. This model can be piloted for reaching other hard to reach populations. Future research to measure the sustainability and effectiveness of such interventions need to be
Conclusion: In moving forward, the Alliance will build on Canadian strengths in HIV vaccine research and development. The renewed CHVI will enable Canada to be a leading contributor to global efforts in developing a safe, effective, affordable and globally accessible HIV vaccine, and will help cement Canada’s place in the world as a leader in HIV vaccine research.

P15.10 Evaluating Predictors of HIV Infection Among MSM in Nigeria Towards Participation in HIV Prevention Vaccine Trial

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Background: Men who have sex with men (MSM) have a high risk of HIV acquisition worldwide. Effective prevention strategies are needed among this group. They are hard-to-reach population and are often neglected in evidence-based prevention programs. Importantly, they can benefit from HIV vaccines and could participate in HIV prevention trials. The paper evaluates the predictors of HIV infection among MSM in Nigeria.

Methods: Secondary data analysis of a cross-sectional survey among MSM in six Nigerian states was done. Questionnaires were administered to 1545 MSM from a respondent-driven sampling with age range 18 – 49 years. Multivariate logistic regression models were used to evaluate risk factors to HIV infection among the MSM.

Results: HIV prevalence among MSM was 17.2% with state HIV prevalence being 5.7% for Cross rivers state; 9.6% Oyo; 11.4% Kano; 23.1% Kaduna; 27.1% Lagos and 44.4% in FCT. The mean age was 25.4 ± 6.0 years; 71.9% were currently married; median anal sex partner in the last 6 months was 3; those that were tested and received their HIV result in the last 12 months was 31.5%; condom use at last anal sex: 52.1% and 41.9% had sex with girlfriends. The significant predictors of HIV were being a paid partner OR 1.4 (95% CI 1.1 – 2.3), anal sex without condom OR 1.9 (95% CI 1.3 – 2.9); having ≥3 partners OR 1.5 (95% CI 1.1 – 2.6) while tertiary education was protective with OR 0.6 (95% CI 0.4 – 0.9).

Conclusion: Low condom uptake and paying for anal sex have serious implications in HIV prevention among MSM. Similarly, coordinated and effective prevention interventions are highly needed among them because their HIV prevalence is much higher than that of the national prevalence of 3.6% in Nigeria. These prevention efforts include partner reduction, uptake of HIV testing and promotion of condom use in any form of sexual relationships.

P15.11 Attitudes and Intent to Use PrEP Among Current Phase II Preventive HIV-1 Vaccine Trial Participants

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Background: In November 2010, the iPrEx study reported that pre-exposure prophylaxis (PrEP) with daily tenofovir/emtricitabine reduced HIV infections by 44% among men who have sex with men (MSM) and transgender (TG) women. To inform future HIV vaccine trial design, MSM and TG phase II vaccine trial participants were asked about perceived significance of these results, intent to use and access to PrEP, and impact on trial recruitment and retention.

Methods: HVTN 505 is a Phase II, randomized, placebo-controlled HIV vaccine trial enrolling 1350 healthy, at risk HIV-uninfected MSM and TG women at 21 sites in the United States. From January-March 2011, HVTN 505 participants completed an optional, anonymous web-survey during their regularly scheduled study visits.

Results: Of the 487 participants who had a visit during the survey time period, 354 completed the survey; 82% were white, and 73% had health insurance. Overall, 31% thought it moderately or very likely they would take PrEP in the next year, while 39% stated they weren’t likely to take it at all; 54% and 53% would be very likely to take PrEP if made available through a clinical trial or covered by their health insurance, respectively; 68% were not likely at all or slightly likely to take PrEP at their own expense. Most (90%) believed taking PrEP would not change their willingness to stay in HVTN 505, while 44% thought it may affect whether others would enroll. Overall, responses to open-ended questions revealed positive perceptions of iPrEx results, concerns about access and affordability, and continued commitment to vaccine trial participation.

Conclusion: Intent to use PrEP was modest among survey respondents enrolled in HVTN 505. While concerns about access may limit PrEP use, commitment to continued participation in 505 in the setting of PrEP is high. Enrolled trial participants can provide important formative input about emerging prevention technologies.

P16.01 HIV Vaccine Acceptability and Behavioural Risk Compensation Among Men Who Have Sex with Men in a Resource-Poor Setting in Africa

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Background: The Thailand phase-3 HIV vaccine trial two years ago raises hope of a future effective vaccine. But the effective roll-out and use of a future HIV vaccine depends largely on its acceptability. We assessed acceptability of HIV vaccines and behavioural risk compensation among men who have sex with men (MSMs) in a cosmopolitan high density sub Sahara African city, Lagos, Nigeria.

Methods: A structured questionnaire was designed based on formative research among some MSMs, HIV physicians and HIV preventive researchers in Nigeria. It was programmed on laptop computers and administered by trained interviewers. Participants were recruited using venue-based sampling from sex venues, public motor parks and garages, and inner-city club houses in Lagos. We assessed HIV vaccine acceptability using conjoint analysis and a factorial experimental design, and risk behaviour intentions in response to HIV vaccine use.
Results: Participants were 103 MSM; mean age = 30.4 (SD = 4.7) years. Only 9 (8.7%) engaged in paid sex. Had sex with average of 3.4 male partners in the last 6 months. More than half reported STI diagnosis in the last 1 year. HIV vaccine acceptability ranged from 87.9 (SD = 25.2) and 42.3 (SD = 36.5) on a 0-point scale. Vaccine induced seropositivity has the most impact on acceptability (27.4; \( p < 0.001 \)), followed by efficacy (22.1; \( p < 0.001 \)), side effects (11.5; \( p = 0.002 \)), duration (8.3; \( p = 0.002 \), and out-of-pocket cost (6.2; \( p = 0.002 \)). 60 (58.3%) reported intention to increase sexual risk behaviour after HIV vaccine.

Conclusion: Study confirms and support need for development of interventions to reduce impact of vaccine induced seropositivity, enhance acceptability of future vaccine, and improve understanding of possible side effects which may facilitate HIV vaccine uptake among MSM and other vulnerable groups in Nigeria and West African subregion. Finally behavioural interventions to reduce risk compensation remains a critical aspect of

P16.02
HIV and STI Prevalence Among Men Who Have Sex with Men Recruited Through Respondent Driven Sample in a Nigerian Oil-Rich Port Harcourt City, Nigeria

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Background: MSMs are one of the high risk populations for HIV and STI and a potential cohort for HIV vaccine trials. There is no prevalence study of MSMs in West Africa. Respondent driven sampling (RDS), a novel methodology designed to access hidden populations was used for the first time in West Africa to recruit MSM.

Methods: The recruitment for the study started with the selection of 10 first generation participants (seeds) with potential to track fellow MSMs through their networks. Criteria for recruitment included: residency of Port Harcourt; Nigeria; age \( \geq 18 \) years; self-report of having sex with men at least 8 times in their lives and at least once in the last six months. Participants were screened for HIV and STI. Data were analyzed with RDS Analysis Tool (RDSAT).

Results: 98 MSMs were recruited through RDS for the study. Prevalence of HIV, HBV, and HCV in the participants was 12.1%, 27.7%, and 10.3% respectively. 57 (55.9%) of the participants had HPV infection.

Conclusion: This is perhaps the first research study of MSMs in West Africa. Results show a high prevalence of HIV, HBV, HCV and HPV co-infections in MSM population. This lends support to MSMs as potential participants for HIV vaccine trials and also a target population for future use of the vaccine when it eventually becomes available. The study also revealed that RDS methodology helps recruit a diversity of MSMs, usually missed with other recruitment methods.

P16.03
Are Adults in Soweto Still Willing to Participate in Future Vaccine Trials? A Qualitative Study

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Background: South Africa bears the greatest burden of HIV/AIDS globally with a prevalence rate of 10.9%. The search for an effective preventative HIV vaccine is therefore ongoing. A potential candidate vaccine will be tested amongst high risk populations in phase II and III HIV vaccine trials. Large numbers of HIV-negative volunteers will be required to enroll and return regularly for assessment over a number of years. This study explored willingness of adults to participate in future preventative vaccine clinical trials.

Methods: Thirty HIV negative participants, aged 18-35 years, were recruited via the Voluntary Counselling and Testing site at the Perinatal HIV Research Unit in Soweto, Johannesburg. A qualitative methodology was used to collect data via in-depth interviews. Participants were allowed to use their language of choice. Interviews were recorded using digital recorders. Interviews were transcribed verbatim and translated to English. Data analysis was conducted using Maxqda, a qualitative software data management and analysis programme.

Results: The main themes were: knowledge about vaccines, enablers to participation in vaccine trials, vaccine knowledge was variable. Those with good knowledge had heard about trial participation from friends who had participated in clinical trials. Enablers included having enough time to make the decision to participate, treatment for side effects and partner and parent approval as well as support. Inhibitors were: being stigmatized as HIV positive, experiencing side effects, having misconceptions about vaccine trials, misconceptions about the intentions of the research unit, parent and partner disapproval, knowing friends who were HIV positive and knowing friends and family who had died of HIV/AIDS.

Conclusion: Providing the correct information about vaccine trials and involving significant others are important when involving volunteers to participate in future vaccine trials. This may translate to involving significant others in the information provision sessions prior to participation.

P16.04
Preparing for the Availability of a Partially Effective HIV Vaccine


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Background: A satellite symposium held at AIDS Vaccine 2010 updated thinking on “downstream” implementation issues for a partially effective HIV vaccine. The session provided the first comprehensive discussion since 2002 when WHO and CDC anticipated the results of the first Phase 3 HIV vaccine (VaxGen gp120) trial.

Methods: Presentations in 2010 included: 1) Results of mathematical models (some with health economic parameters) from different research groups applying a priori consensus RV144 trial results to Thailand, South Africa, U.S., and Australia and 2) Relevant lessons from implementation of hepatitis B vaccine (HBV), human papillomavirus (HPV) vaccine, antiretroviral pre-exposure prophylaxis (PrEP), annual influenza vaccine strain selection, and post-RV144 planning in Thailand. A moderated
discussion with audience members focused on developing and developed world considerations.

**Results:** Issues to emerge included: 1) All mathematical models projected a substantial number of HIV infections averted with the RV144 vaccine, especially if boosters turn out to be efficacious. 2) HBV vaccine introduction in almost all countries initially targeted high-risk populations given higher cost-effectiveness, however, acceptably higher uptake was attained only after change to universal infant vaccination policy combined with affordable vaccines. 3) Attempts to combine universal HPV vaccine introduction in adolescents in the US with school entry requirements was unsuccessful due to concerns about inadequate safety data. 4) Should future HIV epidemiology in the U.S. warrant recommendations for routine childhood HIV vaccination, the Vaccine For Children program provides a stable funding mechanism for those otherwise without health insurance. 5) Many of the challenges with annual influenza vaccine strain selection may be magnified with efforts to arrive at annual consensus HIV strains. 6) Globally, infant immunization programs are well-established while those for adolescents or adults are not. 7) Prioritization vs. competing priorities and access/affordability are also concerns.

**Conclusion:** This satellite provides a useful start on planning for complex HIV vaccine implementation issues.

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**P16.05**

**HIV Vaccine Knowledge, Concerns and Misconceptions Among Vulnerable Communities in Resource-Poor Settings of Two Adjoining Border Cities in Africa**

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**Background:** With the increased hope of availability of future effective HIV vaccine after the modest success of the Thai phase-3 HIV vaccine trial last year, it is important to start early preparation for effective distribution and uptake particularly among vulnerable communities in sub-Saharan Africa at highest risk for HIV. The study was to identify and assess knowledge, beliefs and concerns about future HIV vaccines among adults in vulnerable communities in resource-poor settings of Lagos and Cotonou in West Africa.

**Methods:** A cross-sectional survey (based on qualitative findings) was conducted among 615 adults (≥18 years) in two border communities of Lagos, Nigeria, and Cotonou, Benin, both in the West African subregion. They were recruited using multi-site venue based sampling. Median age was 26 years; 42% were females; and average annual income was <300USD per annum. The survey characteristics included HIV vaccine knowledge, beliefs and concerns, and the state/quality of (or access to) health services in their communities.

**Results:** Study revealed majority of participants (87%) never heard of HIV vaccines and almost three-quarters (74%) were concerned they might contract HIV from a vaccine. Concerns about HIV vaccine-related stigma and discriminations were shown by about 68%. Half reported concerns about confidentiality and most of the participants (97%) confirmed no access to quality health services in their community.

**Conclusion:** Lack of knowledge and concerns, misconceptions and mistrust among vulnerable communities in resource-poor settings present critical challenges to HIV vaccine distribution, and may hinder the effectiveness of vaccine in controlling AIDS epidemic. Culturally appropriate, empirically based individual- and community-level educational and social interventions and outreach may be vital to address HIV concerns and misconceptions so as to achieve successful dissemination.

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**P16.06**

**Sustaining Community Interest on the Road Towards an HIV Vaccine**

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**Background:** After years of conducting HIV Vaccine research, the hope of finding an HIV Vaccine has been rekindled by recent promising results on proof of concept and broad neutralizing antibodies. Community involvement is important for continued support of the research process. This abstract presents views of community members surrounding a research site in Entebbe Uganda on sustaining community interest for HIV Vaccine research collected in a qualitative study.

**Methods:** Focus group discussions and Key informant interviews were held with fourteen community members. Questions were based on a broad question; In the absence of an effective vaccine how can community interest be sustained? Participants were chosen purposively based on their involvement in community engagement and were gender balanced. Content and thematic analyses were conducted to get the final results.

**Results:** Community members appreciated scientific efforts to develop a vaccine. Most participants believed that a genuine partnership between researchers and communities was key to progress towards an HIV Vaccine. Key strategies were related to;

- Instill a sense of hope: Research teams should draw from the success of the polio and measles vaccines, and explain recent advances in order to demonstrate that there is renewed hope that an HIV vaccine is possible.
- Simplify communications: At all levels of conducting research information dissemination, priority should be given to simplifying scientific terms to contribute to sustaining community support.
- Community education: Innovative stakeholder education ensures ongoing support, increasing levels of study participation.

**Conclusion:** More than ever before, sustaining community interest in HIV Vaccine research requires instilling hope, communication and stakeholder support. This involves a number of innovative, adaptive, context driven strategies to ensure community support goes hand in hand with the research process. The recent promising results show that a vaccine is possible.

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**P16.07**

**Community Engagement in HIV Vaccine Research: A Multiple Embedded Case Study in Canada, India, South Africa and Thailand**

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Background: The need for community engagement in HIV vaccine research has become a mantra among populations at higher risk of HIV exposure, people living with HIV, advocacy groups, community-based, non-governmental and government organizations, clinical trialists and researchers across low-and-middle-income (LMIC) and high-income countries. Nevertheless, scant research has identified and assessed stakeholder roles and challenges for community engagement in diverse country settings.

Methods: We implemented an embedded exploratory case study with a multiple case design. We conducted in-depth, semi-structured interviews with persons from populations at higher risk of and living with HIV, community advocates, service providers and HIV experts in Ontario, Canada; Chennai, India; Johannesburg, South Africa; and Bangkok and Chiang Mai, Thailand, and community focus groups. The central questions were: What are appropriate roles of community stakeholders in HIV vaccine trials? What are the challenges and benefits of community engagement? Data were analyzed across and within multiple stakeholder groups and countries using narrative techniques and a constant comparative method.

Results: We conducted 92 interviews and 10 focus groups (n = 91) across 4 countries (N = 183): 55% men, 43% women, 2% transgender women. Cross-cutting themes included altruism; challenges in identification of “genuine” community representatives; trial literacy; mistrust of medical research; late invitation to engage in already-planned trials; lack of specificity on appropriate roles for community stakeholders; dealing with negative trial results; and dissemination of information. In LMIC sites, economic disparities between trial sites and sponsor nations, and sustaining support for community-based organizations beyond trial duration emerged as important challenges. Distinct “local vaccination cultures” arose in each country.

Conclusion: Meaningful community engagement challenges us to: identify appropriate roles for community stakeholders commensurate with time and expertise; engage early in the trial planning process; maintain transparency in information shared and bridge siloization of knowledge; and to support capacity-building and sustainable community infrastructure despite the episodic implementation of clinical trials.

Methods: To estimate annual investment in HIV vaccine R&D, data were collected from government agencies, nonprofit research organizations, foundations, and pharmaceutical/biotechnology companies on annual disbursements for product development, clinical trials and trial preparation, community education, and policy advocacy efforts.

Results: Preliminary estimates suggest funding for HIV vaccine research in 2010 continues a decline from its peak in 2007, with a slight decrease in funding by the U.S. government, by far the largest supporter of HIV vaccine research. European public sector funding for HIV vaccine research also continued its negative trend of the last two years. The Bill & Melinda Gates Foundation continued to provide the great majority of philanthropic funding for the HIV vaccine field, but at lower levels in 2010 due to funding cycles. 2010 also saw a renewed effort by a number of commercial entities in HIV vaccine research, primarily in the biotechnology industry, but their funding contributions remain quite small in comparison to public and philanthropic sector support.

Conclusion: Scientific momentum in the HIV vaccine R&D field continued in 2010, with the discovery of additional neutralizing antibodies, new work on mosaic gene vaccine design, crucial analysis of positive results stemming from the RV144 vaccine trial, and preparations for follow-up trials over the next five years. A continued downward or flat-funding trend at a time of public sector austerity could present difficult choices for the field, especially given the resource requirements for expensive clinical follow-up.

Background: The effectiveness of vaccines in reducing new HIV infections is contingent on their acceptability to end-users; yet limited investigations have addressed social- and structural-level factors that may influence acceptability. We explored social and structural factors associated with HIV vaccine acceptability in Thailand, a nation well-positioned to be among early adopters of a future vaccine.

Methods: From 2006–2009 we conducted a mixed methods investigation: Phase 1 (n = 39)—in-depth, semi-structured 1-hour interviews in four Thai cities among key populations at higher risk of HIV, community service providers and HIV experts recruited using purposive sampling. Interviews were digitally recorded, transcribed, translated into English, and analyzed using narrative techniques from grounded theory; Phase 2 (n = 326)—interviewer-administered survey programmed on laptop computers among participants recruited using venue-based sampling.

Results: Among 365 participants, 66% were men, 22% women, 12% transgender women; mean age = 28 years. Cross-cutting social and structural correlates of HIV vaccine acceptability included: social saturation; peer, familial and societal stigma; discrimination in healthcare; and vaccine cost. Population-specific challenges included: 1) gay men/MSM: anti-gay prejudice, being “outed”, and behavioral disinhibition; 2) transgender women: transphobia, being grouped with MSM, and challenges in condom negotiation with straight men; 3) male and female sex...
workers; primary partner’s mistrust; new challenges to enforcing condom use with male clients; and criminalization of sex work; and 4) injection drug users: lack of social and community support; criminalization of drug use, and lack of harm reduction approaches.

Conclusion: Population-specific and cross-cutting social and structural factors may place constraints on HIV vaccine uptake among key populations at higher risk for HIV in Thailand. Complementing HIV vaccine roll-out with social- and structural-level interventions that produce an enabling environment—encouraging social support for vaccination; combatting HIV and anti-gay stigma; subsidizing vaccine costs; and decriminalizing adult sex work and drug use—may optimize the effectiveness of vaccines in controlling HIV in Thailand.

P16.10
Community Conversations Around Good Participatory Practice Guidelines: The Need for Dialogue Between Communities, Governments and Researchers

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Background: A partnership between the Sao Paulo HVTU and the Brazilian NGO GIV has gathered Brazilian community members to discuss the Good Participatory Practice (GPP) Guidelines in a project funded by AVAC. The objective was to insist on the need to develop a national document to establish guidelines and regulations on participatory practices and create a favorable context to support the document and its implementation.

Methods: Brazilian community members strategic for HIV vaccine trials were invited to a one-day consultation meeting. Some have already participated in a previous consultation meeting held in 2008 and others were new to GPP, though engaged in LGBT and HIV/AIDS activism and/or AIDS programs at local, state and national levels. Presentation was developed on the results from the 2008 GPP consultation and its referrals, GPP Revision process and local consultations held in the South and Northeast of the country. Questions were raised to promote the debate around the steps needed for the implementation of a national document on GPP.

Results: Brazilian stakeholders recognized that national GPP guidelines for health research should be implemented. To allow implementation of such guidelines it was pointed that there should be:

- a link with the national ethical framework, though recognizing the need for more thorough mechanisms of monitor of daily trial activities by communities and register of volunteers’ experience, and problems they face;
- community education efforts developed in order to promote a minimum understanding on research processes and organize a campaign in health services to instruct people on what institutions should be seen if facing problems in trials;
- strategies to engender opportunities for dialogue between scientific community and civil society so that control does not depend only on external bodies.

Conclusion: To ensure implementation of Brazilian national GPP guidelines there is a need to strengthen dialogue strategies between community representatives, governmental stakeholders and researchers around participatory practices.

P17.01
Synergistic Upregulation of Systemic IL-10 and Functional Enhancement of CD4+ T Cells by IL-10 Receptor Blockade in HIV+/MTB Co-Infection

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Background: Due to the current dual-epidemic of HIV+/AIDS and TB, functional understanding of HIV+/MTB co-infection immunology is critical for furthering vaccine and therapeutic strategies against both pathogens. IL-10 is an anti-inflammatory cytokine, produced by lymphocytes and monocytes, that has been shown to down-regulate expression of anti-viral and anti-bacterial cytokines such as IFN-gamma, IL2 and TNF-alpha. Elevated IL-10 levels have been reported during chronic human HIV infection and ex vivo IL-10 receptor blockade in the same patients has been shown to increase HIV specific T cell function.

Methods: This study assessed plasma levels of IL-10 and other cytokines (TNFz, IFNy, IL-6, IL-2 and IL-13) in 119 subjects presenting different constellations of HIV and/or MTB mono-or co-infection. Cytokine concentrations were measured by high sensitivity bead array assay (Luminex) and correlated with infection status, parameters of HIV disease progression and state of MTB infection. Furthermore, the effect of IL-10 receptor blockade (IL-10Rβ) was assessed with regard to HIV and MTB specific CD4 T cell function in HIV+/LTBI co-infected subjects (as measured by Luminex).

Results: Elevated levels of IL-10 were observed in HIV+/TB active individuals as compared to Active TB mono-infected (p = 0.0295) and HIV mono-infected (p = 0.0450). Plasma IL-10 levels were found to correlate directly with CD4 count (p = 0.0002) and inversely with viral load (p = 0.0168) in HIV+/LTBI subjects but not in HIV+/TB active subjects. Ex vivo blockade of IL-10Rβ significantly enhanced MTB specific CD4 + T cell cytokine production as compared to isotype control in HIV+/MTB co-infected subjects: IFN-γ (p = 0.0078), TNF-α (p = 0.0078), IL-6 (p = 0.0013) and IL-2 (p = 0.0156).

Conclusion: This study concluded that there is a synergistic up-regulation of IL-10 in HIV+/TB active patients as compared to HIV and MTB mono-infection. Additionally, this study concludes that novel ex vivo enhancement of MTB and HIV specific CD4 T cell function is possible with IL-10Rβ blockade in HIV+/LTBI subjects.

P17.02
Frequency of CD4+ and CD8+ IL17-Secreting T Cells During Acute/Early HIV Infection and in Chronic Asymmetric Controllers

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Background: Understanding how immune homeostasis is perturbed during HIV infection is highly relevant. IL-17 is a...
protective cytokine against extracellular pathogens and helps maintain mucosal barrier. Objective: To evaluate IL-17-secreting T cell frequency during the first year following HIV.

**Methods:** Frozen PBMCs from 20 HIV+ subjects enrolled during seroconversion (by the Argentinean Group of Seroconverters), 12 HIV+ elite controllers (EC) and 9 healthy donors (HD) were used. Samples from seroconverters were obtained at enrollment (baseline), 3, 6, 9 and 12 months post-infection. Seroconverters were classified as “Progressors” if CD4 count dropped below 350 cells/microl or experienced AIDS-related B/C events within 12 months post-infection. IFN-gamma- and IL-17-secreting T cells were evaluated by flow cytometry upon stimulation with anti-CD3/anti-CD28 antibodies or

**Results:** CD3+ CD4+: HIV+ subjects showed lower Th17 cell frequency compared to HD but higher Th17/Th1 ratio, indicating that IL-17-producing cells were enriched in the CD4+ subset. No difference between “Progressor” and “Non-Progressor” seroconverters was observed at baseline samples. However, “Progressors” showed higher Th17 cell frequency and higher Th17/Th1 ratio than “Non-Progressors” (p = 0.0049 and p = 0.00485, respectively) at set-point. Neither Th17 cell frequency nor Th17/Th1 ratio changed significantly over time in both group of seroconverters. No significant difference was observed between seroconverters and EC. CD3+ CD8+: Upon strong PBMC stimulation (PMA/Iono), EC showed higher frequencies of Tc17 cells, double-positive IL-17+ IFN-γ+ cells and higher Tc17/Th1 ratio than seroconverters (even at set-point samples) and HD (p < 0.05 in all cases). Among seroconverters, no differences were observed between “Non-Progressor” and “Progressor”, independently of stimulation and time-point. However, Tc17 cell frequency significantly diminished between baseline and set-point samples, regardless of progression status and stimulation.

**Conclusion:** Results indicate that IL-17 produced by both CD4+ and CD8+ T cells might be an important mediator involved in host defense mechanisms against HIV-mediated immunopathogenesis during acute/early infection (CD4 and CD8) and aviremic chronic infection (CD8).

**P17.03 Preventing HIV-1 Infection, More Epitope Is Not Better: Analysis of Gag Epitope of 2 HLA Alleles Associated with Different Outcome of HIV Infection**

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**Background:** Current HIV vaccine candidates have been based on the conventional views of viral infection and attempt to induce broad T cell responses to HIV-1. Until now, the candidate vaccines based on such approach either failed to provide protection or produced modest effect that is not satisfactory for an effective vaccine. Since these vaccine candidates were not based on the correlates of protection against HIV-1 infection, improving such understanding could be critical for successful vaccine development.

**Methods:** A subset of women enrolled in the Pumwani Sex-worker Cohort remain uninfected by HIV-1 despite repeated exposures through sex work. This resistance to HIV-1 infection is associated with several alleles of Human Leukocyte Antigens (HLAs) and specific CD8+ and CD4+ T cell responses. In this study we systematically analyzed HIV-1 clade A and D Gag epitope profiles of two HLA class I alleles associated with different outcomes of HIV-1 infection, A*0101 is significantly associated with slower seroconversion while B*0702 is associated with rapid seroconversion. We screened a Gag peptide library with iTopia Epitope Discovery System to compare the peptide binding capacity of these two alleles. The identified peptides were characterized by affinity and off-rate assays and confirmed by interferon gamma ELISPOT assays using patient peripheral blood mononuclear cells.

**Results:** A*0101, an allele associated with protection from HIV-1 infection, only binds to 3 epitopes in Gag. Whereas, B*0702, an allele associated with rapid disease progression, has 30 Gag epitopes. There is no significant difference in peptide binding affinity, off-rate, ELISPOT SFU values and epitope specific Tem/Tcm frequencies.

**Conclusion:** In contrast to the broad peptide binding spectrum of B*0702, A*0101’s epitopes are narrowly directed. Observations of this study question the current approach for HIV-1 vaccine development and propose a different vaccine development strategy.

**P17.04 Epitope Mapping of HIV Clade A1-Specific CD8 T Cell Responses by Multiple Immunological Readouts Reveals Distinct Specificities Defined by Function**

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**Background:** The limited success of HIV vaccine candidates to date highlights our need to better characterize protective cell-mediated immunity. While HIV-specific CD8+ T cell responses have been largely defined by measuring IFN-γ, these responses are not always protective, and it is unclear whether the same epitopes would predominate if other functional parameters were examined.

**Methods:** Previously, we measured CD8+ T cell responses to clade A1 HIV p24, in a Kenyan commensal sex worker cohort, using an unbiased epitope mapping approach and identified 50 functionally specific-epitopes. Here we extended our findings and further characterize HIV-specific CD8+ T cell responses measuring seven CD8+ T cell functions (IFN-γ, CD107a, MIP-1α, MIP-1β, TNF-α, IL-2 and proliferative capacity) in 80 chronically HIV-infected individuals to eleven identified epitopes.

**Results:** Epitope mapping revealed that most epitope-specific responses were IFN-γ negative (50/69). Many responses had multifunctional (33%) and proliferative (19%) components. An inverse association between IL-2 and proliferation responses was also observed, contrary to what has been described. Characterization of the eleven epitopes of interest was consistent with our previous findings. Most epitope-specific responses were IFN-γ negative (64%) and many responses were multifunctional (38%). Two of the eleven epitopes were recognized at significantly higher frequencies (p < 0.02) and we identified epitopes that preferentially elicited specific cytokines (p < 0.02). Preliminary data suggests that some of these epitopes are significantly more likely to elicit a multifunctional response.

**Conclusion:** Together, these data suggest that the specificity of CD8+ T cell responses differs depending on immunologic
readout, with a 3.5-fold increase in breadth detected by including multiple parameters. Furthermore, identification of epitopes that elicit polyfunctional responses reinforces the need for the comprehensive evaluation of HIV vaccine candidates, and may represent novel targets for CMI-based vaccines.

**P17.05**

**Characterization of CD8+ T Cell Epitopes Recognizing HIV-1 Epitope Variants**

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**Background:** One major challenge in the creation of an HIV-1 vaccine is the extreme genetic diversity of the virus. It is thought that a more cross-reactive T-cell response would be beneficial in circumventing this issue. Despite this, little is known about the characteristics of these responses in nature, and many aspects of the variant-epitope CD8+ T-cell response remain poorly defined. Here, we characterize CD8+ T-cells specific to an immunodominant HIV-1 epitope, IW9, and 2 of its variants, to better understand the level of cross-reactivity between them.

**Methods:** Using samples from the Pumwani commercial sex-worker cohort in Nairobi, Kenya, individuals positive for either HLA-B*4201 or HLA-B*0702 were screened for binding to tetramers specific to the IW9 epitope and 2 variants. Analysis of epitope specific cytokine expression and proliferation was performed to determine the level of functional cross-reactivity of the variant-specific T-cell pools. Additionally, heteroduplex mobility assays (HDMA) were performed to determine differences in TCR usage among the T-cells responding to each variant.

**Results:** Tetramer co-staining experiments indicate that there is some cross-reactivity among these variants, shown by strong double-positive populations in flow cytometry. However, in some cases, separate cell populations are being recognized by each variant. Proliferation assays show that T-cells stimulated by one variant can be recognized by tetramers specific to another variant. HDMA revealed that there are differences in TCR usage among variant-specific CD8+ T-cells both at the family and clonotype level. This may suggest a combination of public and private clonotype usage, which could explain the differences in cross-reactivity and functionality.

**Conclusion:** In order to create a T-cell vaccine that can target and produce protective immune responses, it will be necessary to fully understand the nature of cross-reactive responses. This study has shown evidence that cross reactivity exists within the IW9 epitope, and that there are several factors that may affect this.

**P17.06**

**Cryptic Epitopes from Alternative Reading Frames Restricted by HLAs Associated with a Good Prognosis Are Frequently Recognized in HIV Infection**

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**Background:** Cryptic epitopes (CE) are products of translation of alternate reading frames (ARF, 2 sense and 3 antisense) that are commonly targeted during HIV/SIV infection. To understand the full extent of contributions of HIV specific CE in HIV-1 pathogenesis, we performed mapping of the CE derived from ARFs of nine HIV-1 encoded proteins.

**Methods:** For a comprehensive approach, we designed overlapping peptides (OLPs; 12–18 mers) for the 5 ARFs of clade B gag and pol (PeptGen program, Los Alamos). We also predicted (using clade B sequences/EpiRed program) 9–11mer peptides that bind HLA-B*27, B*57 or B*5801 and span the ARFs of the nine HIV-1 encoded proteins. PBMC from seronegative donors (n = 42) and chronically (CHI, n = 93) HIV clade B infected patients were used to evaluate CE specific T-cell responses in an IFN-g ELISpot assay. Among CHI, 80% patients had either B*27, B*57 and/or B*5801 alleles.

**Results:** We predicted 520 (B*27), 955 (B*57) and 1027 (B*5801) potential CE in each of the five ARFs of the nine HIV-1 encoded proteins. The antisense frames encoded a majority of these CE. Predicted peptides with >50% probability of being an epitope were synthesized; B*27 (N = 30), B*57 (N = 39) and B*58 (N = 90). Overall, 26% and 2% of CHI patients and seronegative donors respectively had CE responses (p = 0.0006, Fischer’s exact). The overall responder frequency for HLA specific CE response was 22% (range 6%–43%) with the highest recognition frequency noted for HLA-B*5801/B*57 patients (p = 0.005, Fischer’s exact). In our comprehensive mapping analysis, 17% patients targeted CE from gag/pol ARFs and all responders were either HLA-B*27, B*57 or B*5801. Among these, the most frequently targeted were pol 2 (31%) followed by gag 2 and 5 (25%) frames.

**Conclusion:** These data underscore the importance of CE targeting especially those that are presented by the so-called “protective alleles” in HIV-1 infection and hence have implications for vaccine design.

**P17.07**

**Differences in Magnitude, Breadth and Targets of HIV-Specific Response in Progressive Versus Non-Progressive Acute/Early HIV Infection**

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**Background:** Characterizing HIV-specific immune response during acute infection and how it is modulated over time is highly relevant at HIV-vaccine design setting. Objective: To study HIV-specific T cell responses in Argentinean subjects (enrolled by the Argentinean Group of Seroconverters), during HIV seroconversion and up to the first year of infection.

**Methods:** Frozen PBMCs from 21 HIV+ seroconverters and 10 HIV+ elite controllers (EC) were used. Samples from seroconverters were obtained at enrollment (baseline), 3, 6 and 12 months post-infection. Seroconverters were classified as “Progressors” if CD4 count dropped below 350 cells/microl or
experienced AIDS-related B/C events within the first year post-infection. HIV-specific T cell responses were evaluated by IFN-gamma ELISPOT. Magnitude, breadth and spot size (as indicator of released IFN-gamma) were compared inter- and intra-groups at baseline and follow-up samples, using parametric and non-parametric statistics.

Results: Magnitude: Among seroconverters, Gag was preferentially targeted in “Non-Progressors” (NP) and Nef in “Progressors” (P). In EC, responses against Gag were predominantly found (Gag vs Nef and Env $p < 0.005$). Within Gag, p24-specific cells dominated in NP and EC while p17 and p2-p7-p1-p6-specific cells dominated in P. Both at baseline and set-point samples, Gag-specific responses versus viral load and Nef-specific responses versus CD4 counts correlated significantly (inversely and directly, respectively). Breadth: Both at baseline ($p = 0.0115$) and set-point samples ($p = 0.0428$), P recognized fewer viral regions than EC. Spot size: NP and EC produced larger spots than P both when stimulating cells with HIV-specific peptides ($p = 0.0017$ and $p = 0.0002$, respectively) or CEF (CMV, Epstein Barr, Influenza)-specific peptides ($p = 0.02$ and $p = 0.0039$, respectively). This trend was maintained over time.

Conclusion: Early T cell responses (at baseline and set-point samples) preferentially targeting p24, higher breadth and larger spot size were associated with non-progressive acute/early infection indicating that these factors play an important role in early viral containment and should be considered for rational vaccine design.

P17.08
HIV-1-Specific Cytotoxic T Lymphocytes Cross-Recognizing an Escape Mutation at Early Phase of HIV-1 Infection

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Background: Previous studies showed that HIV-1-specific cytotoxic T lymphocytes (CTLs) recognizing both wild-type and escape-mutant epitopes (cross-reactive CTLs) exist in vivo. However, a role of cross-reactive CTLs in the control of HIV-1 replication remains unclear. We here studied cross-reactive CTLs specific to the HLA-A*2402-restricted Gag28–36 (KKYKHKIVV: WT) epitope and its escape mutant (3R).

Methods: We analyzed 11 HLA-A*2402+ individuals with primary HIV-1 infection who were monitored from early to chronic phases.

Results: Sequence analysis of plasma viruses at an early phase showed that three and eight patients were infected with WT and 3R virus, respectively. Analysis of bulk CTLs stimulated with the WT or 3R peptides showed that cross-reactive CTLs were elicited at an early phase in three (KI-092, -158, and -161) and one (KI-091) of the eight patients. WT and 3R-specific CTLs were also detected in two (KI-092 and -161) and four (KI-134, -136, -151, and -163) patients, respectively.

3R peptide-induced CTL clones established from KI-091 (KI-091 CTL clones), which recognized the WT and 3R peptides equally, lysed both WT and 3R virus-infected cells. In contrast, WT peptide-induced CTL clones established from KI-092 (KI-092 CTL clones), which recognized the WT peptide more effectively than 3R, lysed only WT virus-infected cells. However, in vitro replication suppression assay showed that the KI-091 CTL clones failed to suppress the replication of both viruses, whereas the KI-092 CTL clones effectively suppressed that of the WT virus.

Conclusion: These results show that cross-reactive CTLs are elicited at an early phase in some individuals infected with the WT virus before the selection of the 3R one or with the 3R virus and that their ability to suppress the replication of HIV-1 is much lower than that of WT-specific CTLs. The present study suggests that cross-reactive CTLs cannot effectively control the WT and 3R-escape-mutant viruses.

P17.09
Interdisciplinary Evaluation of Broadly- Reactive HLA Class II Restricted Epitopes Eliciting HIV-Specific CD4+ T Cell Responses

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Background: CD4+ T cells orchestrate immune protection by “helping” other cells of our immune system to clear viral infections. It is well known that the preferential infection and depletion of CD4+ T cells contributes to hampered systemic T cell help following HIV infection. However, the functional and immunodominant discrepancies of CD4+ T cell responses targeting promiscuous MHC II restricted HIV epitopes remains poorly defined. Thus, utilization of interdisciplinary approaches might aid revealing broadly-reactive peptides eliciting CD4+ T cell responses.

Methods: We utilized the novel bioinformatic prediction program NetMHCIIpan to select 64 optimized MHC II restricted epitopes located in the HIV Gag, Pol, Env, Nef and Tat regions. The epitopes were selected to cover the global diversity of the virus (multiple subtypes) and the human immune system (diverse MHC II types). Optimized polychromatic flow cytometry analysis, including the functional markers IFNγ, IL-2, IL-21, MIP-1β, and TNFα, revealed immunogenicity of the individual epitopes. The study subjects (n = 38) were of diverse ethnic background infected by different HIV subtypes. High resolution HLA typing and sequences of the HIV-Gag and Nef regions were obtained.

Results: The FACS analysis revealed immunogenicity against 73% of the epitopes. All subjects, except one, recognized at least one epitope. Interestingly, almost all epitopes located in Gag (15/15) and Nef (14/15) elicited responses, while epitopes in Pol (10/15) and Env (5/15) revealed restricted CD4+ T cell immunogenicity. This difference in immunogenicity between the regions was significant (One-way ANOVA: $p < 0.001$). Additionally, Gag and Nef epitopes generated greater polyfunctionality than Pol- and Env-specific CD4+ T cells. Importantly, we found that the use of optimized epitopes improved the polyfunctionality compared with overlapping HIV Gag (p35) peptides.

Conclusion: Using an unbiased approach where we have predicted peptides with same prerequisites, we demonstrate that HIV-specific CD4+ T cell immunodominance is heavily skewed, targeting particularly Gag and Nef.

P17.10
Virus Suppressive Activities of CD8+ T Cells Correlate with SIV-Specific CD8+ T Cell Responses

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Background: The immune correlates of the remarkable efficacy of protection induced by vaccination of rhesus macaques with SIVAnf remain elusive. We addressed the question whether CD8+ T-cells from SIVAnf-vaccinated animals could suppress SIV replication in autologous CD4+ T-cells and whether the virus suppressive activity correlated with SIV-specific immune responses.

Methods: CD4+ T cells from 5 uninfected, 13 WT-SIV-infected and 19 SIVAnf-vaccinated animals were infected with SIVmac239 and co-cultured with purified CD8+ T cells at a wide range of effector-to-target (E:T) ratios. Intracellular expression of p27 was measured and IC50-values denoting the E:T ratio providing 50% inhibition were determined. ELISPOT assays and intracellular cytokine assays were performed. Statistical analysis included Mann-Whitney test, One-Way ANOVA followed by Tukey test and Pearson correlation analysis.

Results: Determining IC50-values significantly improved the dynamic range as revealed by substantial differences in virus suppressive activity (38-fold) in either WT-SIV or SIVAnf-infected animals compared with uninfected animals (P < 0.0001). No differences in virus suppressive activity were observed between SIVAnf-vaccinated and WT-SIV-infected animals. The magnitude of immune responses as detected by IFN-$\gamma$ ELISPOT assays did not differ between WT-SIV- and SIVAnf-infected animals, nor did ELISPOT responses correlate with the potency of virus suppression by CD8+ T cells. Intracellular cytokine staining assays revealed similar levels of IFN-$\gamma$, TNF-$\alpha$, IL-2, MIP-1$\beta$ and CD107a responses following Gag- or Env stimulation in SIVAnf-vaccinated and WT-SIV-infected animals. However, we identified significant correlations of Gag-specific IFN-$\gamma$-, TNF-$\alpha$-, MIP-1$\beta$- and CD107a+CD8+ T cell responses with virus suppressive activity.

Conclusion: CD8+ T-cells from SIVAnf-vaccinated animals are able to mediate potent inhibition of SIV replication in autologous CD4+ T cells, with activity that was similar to that observed in WT-SIV-infected animals. The magnitude of virus suppression correlated with the magnitude of SIV-specific CD8+ T cell responses, suggesting that these properties are closely linked.

P17.12

Spontaneous HIV-1 Control Is Associated with Distinct Epitopes that Promiscuously Bind Multiple HLA Class II Alleles

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Background: Although HIV-specific CD4+ T-cells are preferentially infected, there is growing evidence that these cells play a pivotal role in control of viremia. However, little is known about the recognition and HLA-class II restriction of HIV-specific CD4+ T-cells in the setting of chronic or spontaneously controlled HIV-infection. Yet, this knowledge will be crucial for the induction of protective immunity in a prophylactic vaccine.

Methods: CD8+ depleted PBMCs from 94 HIV-infected subjects (controller, progression and ART-treated) were screened for IFN-$\gamma$-responses to 410 overlapping clade B peptides in a modified Elispot. HLA-class II restriction was defined by testing CD4+ T-cell lines against peptide-loaded L-cells transfected with a single HLA-DR.

Results: HIV-specific CD4+ T-cell responses were detected in all patient subgroup yet the breadth of these responses was significantly expanded in HIV-controllers (p = 0.029). Most HIV-specific CD4+ T-cells targeted epitopes within Gag, Nef and gp120. Strikingly, we observed significant differences in the immunodominance profile between patient subgroups that distinguished not only elite controllers from rapid progressors, but also from viremic controllers. While elite controllers dominantly targeted a tight cluster of conserved epitopes within p24, chronic progressors preferentially targeted epitopes within the C1/C3 domain of gp120. Moreover, the ratio of Env- and Gag-specific responses was a clear indicator of viral control. A multivariate bootstrap analysis identified four distinct Gag epitopes that were associated with spontaneous control (Probability = 0.60-0.85), while a single gp120 peptide in C3 was associated with high viremia (Probability = 0.82). Detailed characterization showed
that promiscuous binding to multiple HLA-DR alleles occurs frequently, and revealed that HLA-DRB1*0701 is associated with slow disease progression (Probability = 0.88).

**Conclusion:** Our data demonstrate that significant differences exist in protein targeting by HIV-specific CD4+ T-cells between controllers and progressors. We also identified distinct epitopes associated with viral control that are conserved and bind promiscuously to multiple HLA-class II alleles, which will be important for vaccine design.

**P17.13**

**Role of Hexon- and Fiber-Specific Antibodies in Ad5 Neutralization**

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**Background:** The immunogenicity of adenovirus serotype 5 (Ad5) vectors has been shown to be suppressed by neutralizing antibodies (NAbs) against the hexon hypervariable regions (HVRs). The role of NAbs directed against the fiber, however, remains unclear.

**Methods:** Chimeric recombinant Ad5 vectors containing the hexon HVRs of Ad48 (Ad5HVR48), the fiber knob of chimpanzee Ad5C68 (Ad5KC68), or both (Ad5HVR48KC68) were constructed. All vectors exhibited comparable growth and infectivity parameters. Human and murine sera were assessed for NAb responses against each vector. CS7BL/6 mice, either naïve or preimmunized twice with 10^10 vp of Ad5-Empty, were immunized with 10^7 viral particles (vp) of each vector expressing SIV Gag. Gag-specific immune responses were assessed by tetramer, ELISPOT, and ICS assays.

**Results:** Serology studies in both Ad5-preimmunized mice and 116 healthy Ad5-seropositive humans from sub-Saharan Africa demonstrated that Ad5-specific NAbs were directed primarily against the hexon HVRs. Residual non-hexon NAbs were largely directed against the fiber knob. Ad5, Ad5KC68, Ad5HVR48 and Ad5HVR48KC68 vectors expressing SIV Gag proved comparably immunogenic in naïve mice by tetramer, ELISPOT, and ICS assays. In the presence of Ad5-specific preexisting immunity, however, the immunogenicity of Ad5 and Ad5KC68 was abrogated, and only Ad5HVR48 and Ad5HVR48KC68 were immunogenic. Ad5HVR48KC68 evaded preexisting Ad5 immunity more effectively than did Ad5HVR48.

**Conclusion:** These data indicate that the hexon HVRs are the primary target of Ad5 NAbs following both Ad5 vaccination and natural Ad5 exposure. Residual non-hexon NAbs are directed largely against the fiber knob. Exchanging both the hexon HVRs and the fiber knob results in a vector that nearly completely evades Ad5-specific NAbs.

**P17.14**

**In Vitro CD8-Mediated Inhibition of SIV Correlates with In Vivo Virologic Control in Rhesus Macaques**

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**Background:** The in vitro viral inhibition assay was developed to measure CD8-mediated control of HIV-1 replication. However, the utility of the in vitro SIV inhibition assay remains to be determined. We therefore optimized the SIV inhibition assay in a cohort of vaccinated and SIV-infected rhesus macaques to explore correlates of virologic control.

**Methods:** PBMC were isolated from 10 rhesus macaques infected with SIVmac251 with viral loads ranging from 3.2 to 6.9 log_{10} SIV RNA. All animals had been previously vaccinated with MVA/Ad26, Ad26/MVA, or a sham vaccine. CD8-depleted PBMC were cultured with and without CD8 cells for 2 weeks, and p27 content was measured by ELISA on days 7 and 14. CD8-mediated inhibition was expressed as the log_{10} reduction in p27 content between cultures of mixed CD8 and CD8-depleted cells, compared to CD8-depleted cells alone.

**Results:** CD8-mediated inhibition on day 7 ranged from 0 to 2.1 log_{10} reduction in p27, with median inhibition of 0.7 (SD 0.57). In vitro inhibition was significantly inversely correlated with plasma SIV RNA (p = 0.0104) with a Pearson correlation coefficient of −0.76 (CI −0.94 to −0.25). Inhibition ranged from 0 to 2.8 on day 14, with median inhibition of 0.9 (SD 0.9). The correlation between in vitro inhibition and plasma SIV RNA trended towards significance (p = 0.0974) on day 14 but was weaker than on day 7.

**Conclusion:** CD8-mediated inhibition of SIV in vitro correlates with virologic control in vivo. The performance of this assay was optimal when measuring endogenous SIV replication after 7 days of culture. These data suggest that this viral inhibition assay will be useful for evaluating immune correlates of protection in non-human primates.

**P17.15**

**Accurate Recapitulation of Human HIV-Specific CD8+ T Cell Responses in BLT Humanized Mice During Acute Phase Infection**

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**Background:** The SIV-infected macaque has proven to be an extremely valuable model of HIV infection. However, differences in host genetics between humans and macaques, and substantial sequence differences between HIV and SIV, limit its ability to accurately recapitulate those observed in human infections.

**Methods:** Human liver, thymus and CD34+ hematopoietic stem cells derived from 6 distinct human donor tissues encoding unique HLA haplotypes were used to generate 38 humanized BLT mice. Genome-wide HIV-1 sequence evolution was used as an initial marker of early HIV-specific CD8+ T-cell responses. IFN-gamma ELISPOT and intracellular cytokine staining (ICS) assays were employed to identify antigen specific CD8 responses.

**Results:** Following HIV infection, peak viral loads and set points above 1.0x10^5 copies/ml were observed. Mice expressing HLA-A01 and Cw03 exhibited reproducible and rapid viral escape within two CD8 epitopes in Env and Nef, respectively. Two other groups of mice expressing either A01 or Cw03 revealed similar evolution. These epitopes are immunodominantly targeted during natural acute HIV infection of humans, and IFN-gamma ELISPOT and ICS assays confirmed the presence of CD8 responses to these epitopes. CD8 responses against the normally acute...
phase HLA-B57 restricted TW10, IWI9 and KFI11 epitopes in Gag were detected in mice expressing HLA-B57 at ex-vivo frequencies similar to those seen in humans. The presence of the HLA-B57 allele was also associated with a statistically significant reduction in viral loads between 6–12 weeks post infection.

**Conclusion:** The specificity, magnitude and immunodominance patterns of HIV-specific CD8 responses in humanized BLT mice appear to accurately reflect those of HIV-infected subjects, supporting the potential of this small animal model to explore the efficacy of natural and vaccine-elicited cellular immune responses.

**P17.16**
The CD8+ T Cell Antiviral Inhibitory Activity Predicts the Rate of CD4+ Cell Decline in Early HIV-1 Infection and Is Independent of Viral Subtype

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**Background:** The lack of a reliable marker of protective immunity and the genetic diversity of HIV-1 are major obstacles to the development of an effective HIV-1 vaccine. Rare individuals who control HIV-1 without treatment show potent CD8+ T cell-mediated viral inhibition in vitro, indicative of effective immune control. We investigated the relationship between CD8+ T cell antiviral activity and the rate of CD4+ cell loss in early HIV-1 infection.

**Methods:** We studied individuals with documented recent infection to determine the capacity of ex vivo CD8+ T cells to suppress replication of an exogenous HIV-1 isolate. We quantified HIV-1BaL super-infected CD4+ T cells by intracellular p24 staining, after culture in the presence or absence of autologous CD8+ T cells. We studied prospectively the interaction between CD8+ T cell antiviral activity and the rate of CD4+ cell loss in early HIV-1 infection.

**Results:** We showed that the expression of CD160 on CD8+ T cells was significantly up-regulated during HIV-1 infections. The increased percentage of CD160+ CD8+ T cells in TEM/TEMRA cells during HIV infection, which was significantly higher in LTNP than in SP, was associated positively with CD4 counts but inversely with HIV-1 viral loads. CD160+ CTLs exhibited a dominant PD-1-CD38-CCR7-CD62L- phenotype. The intracellular staining data of non-stimulated PBMC showed that the proportions of CD160+ Granzyme B+ or Perforin+ CTL in HIV-1 infected subjects were significantly higher than CD160- Granzyme B+ or Perforin+ CTL. Furthermore, we observed that stimulation with anti-CD160 antibody resulted in increased HIV-gag specific Granzyme B but not IFN-gamma ELISPOT responses. We also found that HIV-gag/CEF-specific degranulation ability of CD160+ CTL was stronger than that of CD160- CTL, addition of anti-CD160 antibody led to significantly enhanced degranulation capacity of CD160high CTL subsets.

**Conclusion:** Our data indicates that engagement of CD160 may trigger a co-stimulatory signal pathway for cytotoxic function of CD8+ T cells and thereby play an immune protective role in HIV-1 infection.

**P17.17**
Prevalence, Incidence, Risk Factors and Willingness to Participate in HIV Vaccine Trials Among Gay and Bisexual Men and Transgender Persons Seeking HI


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**Background:** The role of CD160 in CD4 T and NK cells has been intensively explored, whereas its role in CD8+ T cells remains poorly understood.

**Methods:** We used multi-color flow cytometry to characterized the co-expression of CD160 with PD-1, CD38, CD45RA and CD62L on circulating CD8+ T cells derived from cohorts of HIV-1 sero-negative and HIV-1 infected subjects including slow progressors (SP) and long term non-progressors (LTNPs) who maintained stable CD4 counts over 400 and low HIV-1 viral loads in the absence of ART for more than 10 years. Furthermore we have investigated the ex vivo function of HIV/CEF-specific CD160+CTL by multiple assays including IFN-gamma/Granzyme B based ELISPOT, intracellular Granzyme B/Perforin staining and CD107a degranulation etc.

**Results:** We showed that the expression of CD160 on CD8+ T cells was significantly up-regulated during HIV-1 infections. The increased percentage of CD160+ CD8+ T cells in TEM/TEMRA cells during HIV infection, which was significantly higher in LTNP than in SP, was associated positively with CD4 counts but inversely with HIV-1 viral loads. CD160+ CTLs exhibited a dominant PD-1-CD38-CCR7-CD62L- phenotype. The intracellular staining data of non-stimulated PBMC showed that the proportions of CD160+ Granzyme B+ or Perforin+ CTL in HIV-1 LTNPs are significantly higher than CD160- Granzyme B+ or Perforin+ CTL. Furthermore, we observed that stimulation with anti-CD160 antibody resulted in increased HIV-gag specific Granzyme B but not IFN-gamma ELISPOT responses. We also found that HIV-gag/CEF-specific degranulation ability of CD160+ CTL was stronger than that of CD160- CTL, addition of anti-CD160 antibody led to significantly enhanced degranulation capacity of CD160high CTL subsets.

**Conclusion:** Our data indicates that engagement of CD160 may trigger a co-stimulatory signal pathway for cytotoxic function of CD8+ T cells and thereby play an immune protective role in HIV-1 infection.

**P17.18**
Up-Regulation of LAG-3 Expression on T Cells in HIV-1 Infection Is Correlated with Disease Progression


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**Background:** Lymphocyte activation gene-3 (LAG-3), expressed on activated CD4+ and CD8+ T cells, is known to negatively regulates T-cell responses, but its role in HIV-1 infection in vivo remains unclear.

**Methods:** In the present study, we analyzed LAG-3 expression differences between HIV-uninfected and HIV-infected individuals using microarrays, quantitative PCR and flow cytometry.

**Results:** We found that LAG-3 expression in PBMC cells was higher in HIV-1 infected individuals than HIV sero-negative persons. LAG-3 expression was correlated with disease progression.

LAG3 expression was up-regulated on both CD4 and CD8 T cells from HIV-1 infected individuals, which was also correlated positively with viral load and inversely with CD4 count. We further characterized Lag-3+ CD4 and Lag-3+ CD8 T cells. The coexpression of LAG-3 and immune activation marker was
P17.19
Low Level of Regulatory T Cells and Maintenance of Balance Between Regulatory T Cells and TH17 Cells in HIV-1+ Elite Controllers

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Background: A subset of T cells with immunosuppressive properties is the regulatory T cells (Treg), implicated in microbial infections. However, the role of Tregs in HIV-1 infection is controversial and two main hypotheses consider Tregs as 1) beneficial – Tregs prevent chronic immune activation or 2) harmful – Tregs suppress anti-HIV-1 immune responses. Also, T helper 17 (TH17) effector cells have been implicated in chronic inflammatory disease, but studies of TH17 cells in HIV-1 infection are few and conflicting. Furthermore, the balance between Tregs and TH17 cells appears to be important for disease outcome. To gain more information on the role of Tregs and TH17 cells in chronic HIV-1 infection, we evaluated their frequency and relationship in chronically infected HIV-1 patients with different control of infection.

Methods: PBMC from 17 viremic individuals, 19 HAART patients, 13 Elite Controllers and 10 uninfected individuals were analyzed. Treg identification: PBMCs were subjected to staining and flow cytometry using antibodies against: CD3, CD4, CD25, CD127, FOXP3 and Live/Dead Fixable Dead Cell Stain. Identification of IL17A producing TH17 cells: PBMCs were stimulated with PMA/ionomycin in the presence of Brefeldin A and subjected to staining and flow cytometry using antibodies against: CD3, CD4, INFg, IL17A and Live/Dead Fixable Dead Cell Stain.

Results: We demonstrate that Elite Controllers had lower levels of Tregs compared with HIV-1-infected viremic individuals but that the low Treg level did not differ between individuals with HIV-1 control, whether natural or therapy-induced. We also show that TH17/Treg ratio was similar in Elite Controllers and uninfected controls, whereas in viremic and treated HIV-1-infected individuals the TH17/Treg ratio was lower compared with uninfected controls.

Conclusion: We demonstrate that Elite Controllers have low level of Tregs compared with viremic individuals and that one characteristic of spontaneous HIV-1 control is a maintained balance between Tregs and TH17 cells.

P17.21
Influence of HAART on Alternative Reading Frame Immune Responses over the Course of HIV-1 Infection

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Background: Translational errors can result in bypassing of the main viral protein reading frames and the production of alternate reading frames (ARF). Within HIV-1, there are many such ARFs
in both sense and the antisense directions of transcription. These ARFs have the potential to generate immunogenic peptides called cryptic epitopes (CE).

Therapy and immune response exert a mutational pressure on HIV-1. Immune pressure exerted by ARF CD8+ T-cells on the virus has already been observed in vitro. HAART has also been described to select HIV-1 variants for drug escape mutations. Since the mutational pressure exerted on one location of the HIV-1 genome can potentially affect the 3 reading frames, we hypothesized that ARF responses would be affected by this drug pressure in vivo.

**Methods:** In this study, we identified new CE derived from sense and antisense transcription of HIV-1 using an interferon-gamma ELISpot assay. To evaluate the influence of HAART on HIV-1, ARF T-cell responses over the course of HIV-1 infection were tested in acutely infected patients enrolled in a structured HAART interruption program and in chronically infected patients before and after HAART introduction.

**Results:** We measured T cell responses to 199 HIV-1 CE encoded within 13 sense and 34 antisense HIV-1 alternative reading frames. We were able to observe that these ARF responses are more frequent and stronger in patients chronically infected compared to acutely infected, and that HAART increased the breadth of ARF responses.

**Conclusion:** We confirmed that translation of ARF is an important source of T cell epitopes in HIV-1 infection. These ARF responses are present both in the acute and the chronic phase of HIV-1 infection, but are stronger in chronically infected individuals. We observed that HAART modified the dynamics of ARF T-cell responses, suggesting that ARF T-cells may play a role in the emergence of virus mutation escape.

**P17.23**

**In Vitro Derived Dendritic Cells from CD34+ Precursors Share Features of Dendritic Cells from Several Tissues that Favour T Cell Immunity**

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**Background:** Dendritic cells (DCs) are heterogeneous cells spreading the body with a unique ability to prime naïve T cells against invading antigens. In vitro derived DCs can be therefore considered for vaccination against HIV. Here, we assessed the resemblance between in vitro derived DCs and antigen-presenting cells (APCs) from several tissues, and we demonstrated its ability to induce anti-Gag T cell responses in cynomolgus macaques.

**Methods:** Phenotype of DC subsets from cynomolgus macaque bone marrow (BM), blood, spleen, lymph nodes (LN) and epidermis as well as DCs derived in vitro from BM CD34+ progenitors (CD34-DCs) were studied by flow cytometry. CD34-DCs were transfected with mRNA encoding HIV-Gag and injected into macaques by subcutaneous and intradermal routes. Immune response was studied by using ex vivo stimulated PBMC with pools of Gag peptides and by analyzing cytokine secretion in culture supernatants, the frequency of IFN-γ and IL-2 producing cells in ELISpot and the polyfunctional T cells by intra-cellular cytokine staining.

**Results:** Three main APCs subsets reside in all tissues with the exception of the epidermis, which included only cells with Langerhans phenotype. CD34-DC cultures produced immature CD34-DCs sharing similarities with CD14+ APCs, and mature CD34-DCs resembling CD11c+ myeloid DCs. These CD34-DCs efficiently stimulate T cells in vitro and vaccination of macaques with Gag-expressing CD34-DCs induced Gag specific CD4+ and CD8+ T cells producing IFN-γ, TNF-α, MIP-1β and IL2-2. Interestingly, the number of boost injections increased the frequency of polyfunctional CD8+ T cells and memory CD45RA- T cells.

**Conclusion:** In vitro derived CD34-DCs shared features from both CD11c+ and CD14+ APC subsets. These characteristics might favour their high efficiency at inducing T cell immunity when used as vaccine vector.
P17.24
Delayed-Type Hypersensitivity (DTH) Elicited by Defined Cytotoxic T Lymphocyte (CTL) Epitopes as a Potential Immune Read-out for Vaccine Trials

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Background: Assays that measure physiologically relevant effector function(s) of vaccine induced immune responses are a prerequisite for effective HIV vaccine development. Immune responses to some vaccines are commonly tested by measuring cutaneous reactions to intradermal antigen injections. In most cases, entire protein antigens are used for this purpose. However, even short peptides can induce intra-dermal infiltration of CD4+ and CD8+ T-cells, suggesting that optimally-defined CTL epitopes could possibly elicit such delayed type hypersensitivity (DTH) responses on their own.

Methods: In order to assess the potential to induce DTH reactions, 2 HLA-A*0201 restricted epitopes derived from HIV (SLYNTVATL, “SL9”) and Influenza (GILGFVFTL, “GL9”), respectively were used in 10 HIV infected (8 HLA-A*0201 +) and 10 HIV uninfected (7 HLA-A*0201+) individuals. Local immune response (erythema and induration) were determined after 72h. Blood samples were obtained before injection and after 72h.

Results: No reactions were observed to SL9 injection while 6 of the 7 HIV negative and 5 of the 8 HIV positive HLA-A*0201 expressing individuals showed reaction to GL9 injection (induration diameter 3–15mm). HLA-A*0201 negative individuals did not show any reactivity. Among the HIV infected group, GL9 reactivity was not associated with CD4 counts or viral loads. Phenotypic analyses of epitope specific populations, including homing markers (CD103, CLA, CCR7, CXCR1 and LTBR) are currently being conducted to identify markers that could explain the differential migration potential of these epitope-specific CTL populations.

Conclusion: Our data demonstrates that DTH reactions can be elicited by short CTL epitopes alone, in a HLA-dependent manner. The inability of HIV-specific cells induced upon natural infection to elicit a DTH reaction may reflect their impaired functionality in vivo. These data could help establish CTL-DTH as a simple, cheap and sensitive immune monitoring tool for large-scale vaccine trials for HIV and other pathogens.

P17.25
Conserved HIV-1 p24 epitopes elicit cellular immune responses that impact disease outcome

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Background: Breadth of HIV-1-specific cellular immune response and their impact on the control of viral replication have already been addressed by a number of studies. However, reported data have proven controversial. Here, we hypothesized that nature of targeted epitopes, rather than simply the total breadth or magnitude of HIV-1-specific responses, could clearer associate with disease outcome in infected patients.

Methods: To address this issue, we investigated the possible presence of patterns of Gag p24 recognition among 27 HIV-infected patients. We then attempted to explore epitope features that could define such patterns and how these patterns distinctly associate with disease progression. By ELISPOT-IFN-g assays, we screened mononuclear cells from HIV-1-infected subjects against Gag p24 15mer peptides encompassing the whole protein.

Results: Obtained data was used to carry out a clustering analysis that unveiled the presence of two groups of patients with distinct patterns of Gag p24 recognition. Of note, despite targeted Gag p24 peptides were completely different between two groups, breadth and magnitude of the responses were not significantly different. Interestingly, viral control, measured as RNA copies in plasma, and preservation of CD4 + T cells were increased in one of the two groups. Additionally, we compared genetic conservation in the amino acid sequences of the peptides recognized by our patients, as well as the HLA-I-restricted epitopes within them. Markedly, subjects presenting higher control of HIV-1 replication were the ones targeting the more conserved epitopes. Furthermore, such higher genetic variation was present mainly in anchor residues for HLA-I molecules.

Conclusion: Despite being already a concept among HIV vaccinologists, we show here, for the first time, experimental evidence from cases of the AIDS virus infection in humans that cellular immune responses targeting conserved HLA-I-restricted epitopes do associate to a better control of viral replication and maintenance of CD4 + T cells.

P17.26
Enhancement of Gag-Specific but Reduction of Env- and Pol-Specific CD8+ T Cell Responses by SIV Regulatory and Accessory Proteins in Mice

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Background: Accessory and regulatory proteins (nonstructural proteins) have received increasing attention as components in novel HIV/SIV vaccine design. However, the complicated interactions between nonstructural proteins (Nef, Vif, Vpr, Vpx, Tat and Rev) and structural proteins(Gag, Pol and Env) remain poorly understood, especially their effects on immunogenicity.

Methods: In this study, the immunogenicity of structural proteins with and without nonstructural proteins was compared. First, a series of recombinant plasmids and adenoviral vectors carrying various SIVmac239 nonstructural and structural genes were constructed. Then mice were primed with DNA plasmids and boosted with corresponding Ad5 vectors, and the resulting immune responses were measured.

Results: Our results demonstrated that when the individual Gag, Pol or Env gene products were co-immunized with the whole repertoire of nonstructural proteins, the Gag-specific CD8+ T response was enhanced, while the Env and Pol-specific CD8+ T responses were significantly reduced. The same pattern was not observed in CD4+ T cell responses. Antibody responses against both the Gag and Env proteins were elicited more effectively when these structural antigens were immunized together with nonstructural antigens.
Conclusion: These findings may provide useful guidance for designing novel HIV/SIV vaccine regimens that include non-structural proteins as a component.

P17.27
HIV Infection Induces the Alteration of Cytolytic Effector Molecule Expression Diversity in CD8\(^+\) T Cell Population

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Background: Although many studies reported that cytolytic function of HIV-specific CD8\(^+\) T cells are often impaired during the course of HIV infection, it remains unclear whether this functional failure is selective for HIV-specific population or an overall phenomenon. An analysis of a bulk CD8\(^+\) T cell population can improve our understanding of a neglected area of CD8\(^+\) T cell impairment that stretches far beyond HIV-specific CD8\(^+\) T cell population.

Methods: In this study, we determined the diversity of CD8\(^+\) T cells in term of cytolytic molecule expression including perforin (Pr), granzyme A (GrA) and B (GrB) by using intracellular staining and flow cytometry technique. The results were compared between healthy individuals and untreated HIV infected patients. Correlations with markers of disease progression including the percentage of CD4\(^+\) T cells, absolute CD4 count and viral load were also determined in this study.

Results: We demonstrated the presence of three different subsets of CD8\(^+\) T cells that expressed different combinations of cytolytic effector molecules including triple (Pr\(^+\)GrA\(^+\)GrB\(^+\)), double (Pr\(^+\)GrA\(^+\)GrB\(^+\)) and single (Pr\(^+\)GrA\(^+\)GrB\(^-\)) positive subsets. Results showed significant increase in the frequencies of triple and double positive subsets whereas a decrease in single positive subset was observed in HIV infected patients when compared to healthy individuals. Furthermore, a positive correlation between the frequency of triple positive subset and viral load was observed whereas a negative correlation between the frequency of single positive subset and viral load was identified.

Conclusion: We demonstrated that the diversity of CD8\(^+\) T cell subsets based on co-expression of cytolytic effector molecules changed during HIV infection and this alteration correlated with stages of disease progression. However, the mechanism that initiates this subset alteration remains unclear.

P18.01
Optimizing Delivery of the HIVconsv Immunogen in Rhesus Macaques

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Background: A safe and effective vaccine remains the best hope for halting the HIV-1 pandemic. Here we follow-on from a previous macaque trial to further define the responses in which HIVconsv, an immunogen which encodes the most conserved regions of clades A-D, was administered as SLP and shown to increase the breadth of T-cell responses to HIVconsv in three Mamu-A*01\(^+\) rhesus macaques.

Methods: Three groups of five rhesus macaques were vaccinated with HIVconsv. HIVconsv was vectored in plasmid DNA for electroporation(D), chimpanzee adenovirus(C), modified vaccinia virus Ankara(M), and in synthetic long peptides(S). Each group received either SSSCMS, DSSCMS, or DDDCMS regimen and were followed for qualitative and quantitative T-cell responses. The DDDCMS group was further vaccinated with HIVconsv vectored in Bacillus Calmette-Guérin mycoplasma(B).

Results: SLP vaccination did not induce robust responses against HIVconsv even after prior administration of plasmid DNA however, electroporation did spare the amount of DNA needed. The DDDCM regimen mimicked a DDDAM(A = Human adenovirus HAdV5) regimen previously shown to be highly immunogenic in macaques and also being trialled in humans. Next, the CD4 T-cell compartment was determined to be the major responder to SLP/HIVconsv vaccination increasing in T-cell breadth and proliferative response. Multiple cytokine production from both central memory and effector memory T-cell subsets were also augmented by SLP vaccination. Finally, boosting with HIVconsv vectored in BCG further enhanced the polyfunctionality of both central and effector memory T-cell responses more than the other vectors.

Conclusion: Our macaque trial clearly demonstrates the potency of SLP to generate broad CD4 T-cell responses with a polyfunctional profile against conserved regions of HIV-1 in rhesus macaques when administered in a strict regimen. Because SLP vaccination, in this regimen, greatly expands the CD4 T-cell compartment responding to HIVconsv, it facilitates the possibility of amalgamating an antibody vaccine into an effective CTL vaccine.

P18.02
Immune Selection of T Helper Epitopes in Healthy Volunteers who Received a Multiepitope Candidate HIV Vaccine

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Background: Previously, we detected helper T lymphocyte (HTL) responses in 27/39 individuals two weeks after the 4th vaccination with a recombinant protein HIV vaccine comprised of 18 HTL epitopes in a Phase I clinical trial, HVTN064. In current study, we examined the individual epitope-specific responses and correlated these responses with predicted protein structure.

Methods: PBMC from the 27 responders were stimulated for 20–22 hours by individual peptide epitopes, and IFN\(_\gamma\), TNF\(_\alpha\), IL-2, IL-4, IL-5 and IL-10 were measured in culture supernatants with the Cytometric Bead Array (CBA) assay. We used PSIPRED to predict secondary structure for the entire HTL vaccine polypeptide, and examined the association between protein secondary structure and epitope selection.

Results: All 18 epitopes were recognized (> mean + 3SD of controls) in at least one of the 27 individuals, 14 epitopes were recognized in more than 30% of individuals, and 5 epitopes were recognized in more than 50% of individuals. Of the 9 epitopes that stimulated IL-2, IFN\(_\gamma\), and IL-5 responses in vaccinated mice, 8 stimulated secretion of these three cytokines in more than 20% of human vaccine recipients. Among 9 immunodominant epitopes
that stimulated a triple cytokine response in more than 30% of subjects, 6/9 sit in the regions of strand and/or coil, while 3/9 were in the helix regions. Additionally, 3 of the epitopes were positioned on either ends of the protein vaccine.

Conclusion: We found that half (9/18) of the predicted HIV HTL epitopes elicited strong immunity when tested as part of a vaccine, and protein structure analysis did not completely predict the immune response hierarchy. Thus, current methods of HIV HTL multiepitope vaccine design and preclinical testing in mice, while valuable, do not translate entirely into human studies, therefore improved methods for HTL vaccine design and testing are needed.

P18.03
DNA Boosting Elicited Broader T Cell Responses Against HIV-1 Tat-Rev-Integrase(C-half)-Vif-Nef Fusion Gene Vaccine than Recombinant Vaccinia Boosting

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Background: We previously compared the immunogenicities of DNA vaccines containing tat-rev-integrase(C-half)-vif-nef fusion genes derived from the top three of the most prevalent HIV-1 clades in China, including B', B'/C and AE recombinant subtype of HIV-1. And our data showed that Integrase(C-half) and Vif were consistently the most immunogenic components in three DNA vaccines. As both cellular immune breadth and depth are crucial for the efficacy of T cell based HIV-1 vaccine, we were thus curious about whether the T cell responses against Tat, Rev and Nef could be improved.

Methods: Three recombinant Tiantan vaccinia vectored vaccines were constructed respectively by using polynucleotides encompassing tat, rev, integrase, vif and nef genes(TRYV) of HIV-1cn54(B'/C), HIV-1rl42(B') and HIV-1ae2f(AE), which had been designed and synthesized previously. Female BALB/c mice were immunized in either DNA priming-DNA boosting or DNA priming-rTTV boosting regimen. IFN-γ Elispot assay was used to read out the specific T cell immunity.

Results: The regimen of mixed DNA priming-mixed DNA boosting seemed to be able to elicit comparable magnitude of specific T cell responses against HIV-1 consensus B peptides with regimen of mixed DNA priming-mixed rTTV boosting (500 ± 155 SFCs/106 splenocytes vs 430 ± 173 SFCs/106 splenocytes). Both were significantly higher than those induced by pSV-TRYV(r42) and pSV-TRYV(cn54). And more interestingly, our data showed the mixed DNA-mixed DNA regimen could enhance T cell responses against sub-dominant coments of the fusion gene vaccine, e.g. peptides of consensus B Tat and HIV-1 AE Nef. While, when boosting with mixed rTTV, specific T cell responses tended to be more concentrated upon immune dominant constituents, including Integrase(C-half) and Vif.

Conclusion: Our data suggested that boosted with mixed DNA vaccine could somehow elicit broader T cell responses against less immunogenic parts of tat-rev-integrase(C-half)-vif-nef fusion gene vaccine.

P18.04
Low Dose Immunization of Codon-Optimized Recombinant BCG Confers Long-Lasting SIV-Specific Memory T Cells After Recombinant Vaccinia Boost in Macaques

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Background: Mycobacterium bovis bacillus Calmette-Guérin (BCG) has been extensively studied as a primer of heterologous vaccination regimens because of its safety record, affordability and easy antigen delivery to the professional antigen presenting cells, and thereby to the T cells. As we reported, a prime-boost regimen combining recombinant BCG (rBCG)-SIVgag with a non-replicating vaccinia virus Ds (rDs-SIVgag) induced effective cellular immunity that was able to control a highly pathogenic SHIV after mucosal challenge in macaques.

Methods: rBCG harboring the codon-optimized SIV gag gene (rBCG-SIV-gag-opt) with a 10-fold higher expression than the native gag gene (rBCG-SIVgag) was constructed. Cynomolgus macaques were immunized with a low dose (10⁶ bacilli) of this construct or rBCG-SIVgag, respectively, and then boosted with 10⁷ plaque-forming-unit of rDs-SIVgag twice at 30 and 61 weeks post-priming. After 3 years of the second boost, the animals were challenged with high dose of SIVmac 239 intrarectally and plasma viral load and CD4⁺ cell counts in PBMC were monitored. SIV Gag-specific cellular responses were evaluated by interferon-gamma ELISPOT and flow cytometric analyses.

Results: After rDs-boost, rBCG-SIVgag-opt-primed macaques exhibited higher CD4⁺ and CD8⁺ T cell responses than those in rBCG-SIVgag-primed ones and showed significant Gag-specific CD8⁺ T cell recall responses even after 3-years interval. Two out of 3 animals in the rBCG-SIVgag-opt-primed group showed about 2 log reduction of plasma viral load at set-point in comparison with those in the control group.

Conclusion: A low dose of rBCG-SIVgag-opt induced optimal priming of Gag-specific T cells and prolonged the maintenance of memory T cell response after vaccinia Ds boost. These results imply that the quality of the priming vaccine is a critical factor for inducing a desirable immune response against immunodeficiency viruses.

P18.05
Boosting of Replication Competent NYVAC Primed HIV-1-Specific T-Cell Responses by HIV Synthetic Long Peptides (SLP)

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Background: To enhance the induction of insert specific immune responses, a new generation of replication competent poxvirus vectors was designed and evaluated against non-replicating poxvirus vectors in a HIV vaccine study in non human primates.

Methods: Rhesus macaques were immunized with either the non-replicating variant NYVAC-GagPolNef HIV-1 clade C or the replicating NYVAC-GagPolNef-C-KC, boosted with HIVGag-PolEnv-SLP and immune responses were monitored.

Results: Gag-specific T-cell responses were only detected in animals immunized with the replicating NYVAC-GagPolNef-C-KC variant. Further enhancement and broadening of the immune
response was studied by boosting the animals with novel T-cell immunogens HIVconsv synthetic long peptides (SLP), which direct vaccine-induced responses to the most conserved regions of HIV and contain both CD4 T-helper and CD8 CTL epitopes.

The adjuvanted (Montanide ISA-720) SLP divided into subpools and delivered into anatomically separate sites enhanced the Gag-specific T-cell responses in 4 out of 6 animals, to more than 1000 SFC/10^6 PBMC in some animals. Furthermore, the SLP immunization broadened the immune response in 4 out of 6 animals to multiple Pol epitopes. Even Env-specific responses, to which the animals had not been primed, were induced by SLP in 2 out of 6 animals.

**Conclusion:** This new immunization strategy of priming with replicating competent poxvirus NYVAC-HIVGagPolNef and boosting with HIVGagPolEnvSLP, induced strong and broad T-cell responses and provides a promising new HIV vaccine approach. This study was performed within the Poxvirus T-cell Vaccine Discovery Consortium (PTVDC) which is part of the CAVD program.

**P18.06**

**Characterization of Cellular Immune Responses Elicited by Ad35-GRIN/ENV and MVA Construct Prime-Boost Regimens**

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**Background:** We characterized prime-boost vaccine regimens using heterologous and homologous vector and gene inserts. Heterologous regimens offer a promising approach to focusing the cell-mediated immune response on the insert and away from vector-dominated responses.

**Methods:** Ad35-GRIN/ENV vaccine is comprised of two vectors containing sequences from HIV-1 subtype A (GRIN = gag, rt, int and nef and ENV = env). MVA-CMDR, MVA-KEA and MVA-TZC vaccines contain gag, env and pol genes from HIV-1 subtype CRF01 AE, subtype A and subtype C, respectively. Balb/c mice were immunized with different heterologous and homologous vector and insert prime-boost combinations. Mice were examined for HIV and vector-specific immune responses on days 3, 7, 14 and 28 post-boost. Gag-specific IFN-g ELISPOT, ICS (CD107a, IFN-g, TNFa and IL-2), pentamer staining, and T-cell memory markers were used to differentiate responses to homologous vs. heterologous inserts.

**Results:** Ad35-GRIN/ENV prime followed by boost with any of the MVA constructs induced Gag-specific responses superior to MVA prime-Ad35 boost or homologous Ad35-Ad35 or MVA-MVA prime-boost combinations. Notably, there was a dramatic shift from a vector focused response in homologous vector prime-boost regimens to an insert focused response in the heterologous vector prime-boost regimens (0.78% CD8 + IFN-g to Gag and 4.24% CD8 + IFN-g to MVAp581 in MVA-CMDR/MVA-CMDR vs. 5.74% CD8 + IFN-g to Gag and 0.61% CD8 + IFN-g to MVAp581 in Ad35-GRIN/ENV-MVA-CMDR). Gag-specific central and effector memory T cells were detected earlier and in greater frequency in the heterologous prime-boost regimens. IFN-g ELISPOT and ICS responses to other vaccine proteins showed similar response patterns.

**Conclusion:** These results suggest that heterologous prime-boost vaccination regimens can direct and enhance immunity by increasing the magnitude and polyfunctionality of an insert-specific cell-mediated immune response that is more rapidly detectable, compared to homologous vaccination regimens. This study supports the rationale and current plans for testing heterologous prime-boost regimens in clinical trials.

**P18.07**

**Optimization of the MVA Vaccine Potential After Deletion of a Viral Gene Coding for the IL-18 Binding Protein**

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**Background:** Modified Vaccinia Ankara (MVA) is an attenuated strain of Vaccinia virus (VV) currently employed in many clinical trials against HIV/AIDS. MVA still retains genes involved in host immune response evasion, enabling its optimization by removing some of them. The aim of this work was to evaluate cellular immune responses (CIR) induced by a MVA bearing an IL-18 binding protein gene deletion (MVAAIL-18bp).

**Methods:** Balb/c and C57Bl/6 mice were immunized with different doses of MVAAIL-18bp or MVA wild type (MVAwt), then CIR to VV epitopes were evaluated in spleen and draining lymph nodes at acute and memory phases (7 and 40 days post-immunization respectively). The proportion of IFN-gamma and IL-2 producing cells were measured by ELISPOT. The percentage of cytotoxic TCD8\(^+\) cells was analyzed by flow cytometry through CD107a/b surface marker. In vivo protection against an intranasal challenge with the replicative Western Reserve (WR) strain was evaluated during the memory phase, measuring the weight of individual Balb/c mice during two weeks. Finally, CIR against HIV antigens expressed from MVA vectors was analyzed by ELISPOT using DNA prime/MVA boost schemes.

**Results:** Compared with MVAwt, MVAAIL-18bp immunization induced a significant two or three-fold increase in TCD8\(^+\) cells (p < 0.01) and TCD4\(^+\) (p < 0.05) responses to different VV epitopes, and increased the percentage of cytotoxic TCD8\(^+\) cells (p < 0.05) in the acute phase. Potentiation of MVA's immunogenicity was also observed in the memory phase, in correlation with a higher protection against an intranasal challenge with WR. More importantly we also observed a significant increase in the CIR against HIV antigens such as Env, Gag, Pol and Nef from different subtypes expressed from MVAAIL-18bp in DNA prime/MVA boost vaccination regimens.

**Conclusion:** IL-18bp contributes to immune response evasion during MVA infection, as its deletion from the viral genome potentiated the MVA immunogenicity observed against vector antigens and more importantly to HIV antigens.
A-98

Poster Abstracts

A-98

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Background: In Argentina, the HIV epidemic is characterized by the co-circulation of subtype B and BF variants, mostly CRF12_BF. Nef is a highly variable protein among subtypes, making it a good tool to study the impact of HIV-1 inter-variant variability in the vaccine design setting (a difference between B and BF=24%)

We previously reported a highly specific response against NefBF with low cross-reactivity to NefB in mice. The aim of this work was to analyze the possibility to improve the immune response induced by the co-delivery of cytokotines during the priming doses.

Methods: Mice received an intramuscular prime: 3xDNAnefBF (Group I, GI), 3xDNAanefBF + DNA-IL-12 (GII), or 3xDNAanefBF + DNA-GM-CSF (GIII), or 3xDNAanefBF + DNA-IL-12 + DNA-GM-CSF (GIV). Afterwards, all the groups received MVNefBF as intraperitoneal boost. Nine days after the last immunization, the specific cellular immune response (CIR) was evaluated in the spleen using overlapping peptides representing NefBF or NefB by ELISPOT.

Results: The highest responses were detected in GII (p = 0.0317) and GIV. The observed increments vs. GI were (median [range]): GII 2.38 [1.21–2.71], GIV 1.535 [1.05–2.86].

After fine mapping the response, the peptides targeted were identified: all groups recognized two peptides, located on the N-terminal (BF) and the loop of the protein (BF and cross-reactivity against B). GII and GIV also recognized a peptide located on the central core region (BF). Even more, GII recognized the B peptide with a frequency higher than the other groups, showing an enhanced cross-reactivity.

We evaluated the cell avidity against the loop peptide (comparing sequences BF vs. B). We found that GII showed a higher avidity against the peptide B compared to GI (p = 0.0011), whereas in both groups the highest avidity was detected against the peptide BF (BF vs. B: p < 0.001).

Conclusion: The use of cytokines in DNA/MVA schemes improved the CIR, incrementing the magnitude, avidity and breadth (B cross-reactivity) of the response induced.

P18.09
Deletion of Genes B8R and A52L Enhances Vaccinia Virus Tiantan Immunogenicity and Attenuates Neurovirulence in Mice

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Background: Vaccinia virus Tiantan (vTT) is the vaccine that used to eradicate smallpox in China and is being developed as a recombinant vaccine for AIDS. Removal of specific immunomodulatory genes encoded by Tiantan may improve its potential as a vaccine. Protein A52 (referred as WR A41) has sequence similarity to the VACV chemokine-binding protein and associated with reduced inflammation during dermal infection. B8R gene encodes a secreted protein with homology to IFN-gamma receptor, which neutralizes the antiviral and immunological regulation activities of IFN-gamma.

Methods: The deletion mutants vTTA8BR/A52L was engineered from VTT containing B8R and A52L gene deletions. HIV genes (gag, pol and gp145) from HIV-1 B’/C 97CN54 were inserted into the TK region of vTTA8BR/A52L. To assess the pathogenicity of the deletion mutant, groups of female BALB/c mice were infected with vTTA8BR/A52L or vTT by the intranasal and intracephalic routes. A DNA prime/vaccinia boost regime was used to compare adaptive and memory HIV-1 specific immune responses induced by vTTA8BR/A52L-gpe and vTT-gpe.

Results: Double deletion of B8R and A52L greatly reduced the virus replication in the brain of mice, but did not affect the outcome of intranasal infection of BALB/c mice with dose of 5 x 10^4 or 5 x 10^5 PFU. Flow cytometry analysis and ELISPOT revealed that both vTTA8BR/A52L-gpe and vTT-gpe triggered HIV-1 specific CD8+ T cells, however, vTTA8BR/A52L-gpe enhanced the magnitude of HIV-1 memory responses. Especially, vTTA8BR/A52L-gpe reduced the vector-specific T cell response. Both vectors were capable of inducing similar levels of antibody against gp120.

Conclusion: Double deletion of B8R and A52L could attenuate the neurovirulence in mice. Both vTTA8BR/A52L-gpe and vTT-gpe induced robust T-cell response to HIV-1, but vTTA8BR/A52L-gpe showed more durable HIV-1 memory responses and weaker vector-specific T cell response.

P18.10
A Novel HIV-1 Vaccine Strategy Using Live Recombinant Vesicular Stomatitis Viruses Expressing HIV-1 Protease Cleavage Sites


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Background: Even though it has been more than twenty-five years since the discovery of HIV, an effective preventative vaccine remains elusive. The difficulty in developing an effective HIV vaccine using traditional approaches highlights the need for novel strategies. One novel vaccine strategy is to target the function of HIV-1 protease. Protease cleavage sites (PCS) of HIV-1 are highly conserved amongst the major subtypes. Proper cleavage of all 12 sites is vital in the generation of a viable virion. Directing immune responses against these cleavage sites could destroy the virus before it could establish itself in the host. Also, it would force the virus to accumulate mutations at the PCSs, thus eliminating the ability of the protease to generate infectious virions. In this study, we tested the feasibility of generating immune responses to the peptides corresponding to 12 PCSs of SIVmac239 using a vesicular stomatitis virus (VSV) vector.

Methods: Thirty nucleotides upstream and downstream of each SIVmac239 PCS was codon optimized and cloned into pATX-Stomavac. The feasibility of generating immune responses to the peptides corresponding to 12 PCSs of SIVmac239 using a vesicular stomatitis virus (VSV) vector.

Results: RT-PCR confirmed the expression of PCS mRNA by all 12 rVSVs, in vitro. Two and 4 weeks post-vaccination, peptide specific IgM was detected in all groups of mice immunized with the rVSVs, except mice vaccinated with rVSVs expressing PCS 7 epitope.

Conclusion: We successfully generated 12 rVSVs expressing peptides overlapping the 12 PCS. Eleven of the 12 peptides were
P18.11
Priming with a Mixture of Recombinant BCG Expressing HIV-1 Gag, RT and gp120 and Boosting with Recombinant MVA Induces an Effective Immune Response

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Background: Mycobacterium bovis BCG (BCG) has a number of characteristics that give it great potential to act as a vehicle for the delivery of recombinant vaccines. However, its success depends on overcoming the challenges of poor antigen expression levels and genetic instability. Our studies using an optimized mycobacterial shuttle vector which utilizes the Mycobacterium tuberculosis mtrA promoter, induced upon infection of macrophages, and the M. tuberculosis 19 kDa signal sequence may overcome these issues. We have used this system to generate recombinant BCG (rBCG) expressing HIV-1 subtype C full length Gag, RT and gp120. The V3 CTL epitope, recognised by BALB/c mice, was translationally fused to the HIV proteins for immunogenicity assays. Methods: BALB/c mice were vaccinated with each rBCG vaccine fused to the HIV proteins for immunogenicity assays. Immune responses were monitored using an IFN-γ ELISPOT assay. Results: Mice primed with a single, simultaneous inoculation of all three recombinants and boosted with SAAVI MVA-C gave a peak V3 CTL-specific response of 708 ± 73 spot forming units (SFU)/10^6 splenocytes. A high magnitude of RT-specific CD8^+ T cells and a low magnitude of Gag-specific CD8^+ and CD4^+ T cells were also detected. Higher peak responses were observed to Gag and RT peptides in mice primed with a single rBCG than to those primed with a mixture of all three recombinants. Conclusion: These results show that a mixture of three recombinant BCG strains expressing HIV-1 Gag, RT and gp120 can prime a robust T cell response in mice when used in combination with a MVA boost. Acknowledgments: This work was supported by NIH, USA (grant# R21/R33-A1073182-01A1)

P18.12
Assessment of the Feasibility of a Different HIV Vaccine Approach

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Background: The failure of Merck STEP and Phambili trials and the modest effect of RV144 trial emphasize the importance of understanding the correlates of protective immunity. Our study showed that the epitope recognition of HLA alleles associated with protection from HIV-1 infection is very narrow, thus vaccines focus on the key sites of HIV-1 might work better. Since the protease cleavage sites of HIV-1 are highly conserved among major subtypes, direct immune responses against these sites would yield two major advantages. First, the immune response could destroy the virus before it can establish permanently in the host. Second, the vaccine could force the virus to accumulate mutations eliminating the normal function of the HIV protease thus eliminating viable virions. For this vaccine strategy to work a given individual must have a HLA class I allele that can recognize one of the peptides overlapping one of the 12 protease cleavage sites of HIV-1. In this study we examined the population coverage of this vaccine approach using several approaches. Methods: The population coverage was predicted using computational algorithms, the Population Coverage Calculator (http://www.immuneepitope.org/) with the clade A and D peptides overlapping the protease cleavage sites (PCSs). The population coverage was also calculated based on the T cell epitopes that have already been identified at these sites. Furthermore, the peptides overlapping the 12 PCSs were screened with 8 HLA class I alleles using iTopia Epitope Discovery system and confirmed using IFN-γ ELISPOT assays with PBMCs. Results: Analysis using all three approaches showed that the percentage of populations in the world can recognize peptides overlapping at least one PCS is very high, including more than 90% population in Sub-Saharan Africa. iTopia epitope Discovery System screen showed that the eight common HLA alleles have epitopes in multiple PCSs (4 to 12). Conclusion: This vaccine approach has good population coverage.

P18.13
Immunogenicity of a DNA Vaccine Containing Multiple HLA-DR Binding, Conserved M Group HIV-1 Epitopes in BALB/c Mice


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Background: An ideal T-cell based vaccine should elicit CD4 as well as CD8 T cell responses. The HIV-1 genetic diversity is a major concern in HIV vaccine development. Our group showed that a DNA vaccine encoding "promiscuous" multiple HLA-DR binding, conserved B-subtype HIV-1 CD4 T cell epitopes HIVBr18 induced broad cellular immune responses in mice. To include other prevalent HIV subtypes and increase the breadth of the induced response, we designed a new DNA vaccine containing a higher number of promiscuous epitopes, based on the conserved regions from HIV-1 M group consensus sequence. Methods: Regions of the HIV-1 M-group consensus sequence predicted to bind to 18 or more HLA-DR molecules tested by the TEPITOPE algorithm were selected. Among those, sequences with at least 50% conservation among HIV subtype consensus sequences were further selected. We identified 27 conserved, promiscuous HIV-1 CD4 T cell epitopes from 7 HIV-1 proteins and inserted their sequences in pVAX1. BALB/c mice were immunized with pVAX1, HIVBr18 or HIVBr27, 3x every 2 weeks. Immune responses were assessed 2 weeks after the last dose. Results: HIVBr27 and HIVBr18 elicited IFN-γ production in ELISPOT assays against 11 and 7 vaccine-encoded epitopes, respectively; similar results were observed in CD4+ and CD8+ proliferation assays. The magnitude of cytokine responses elicited by HIVBr27 was significantly higher than that induced by HIVBr18. HIVBr27 induced significantly higher frequencies of polyfunctional CD4+ and CD8+ T cells, able to proliferate and produce IFN-γ and/or TNF-α, when compared to HIVBr18.
Conclusion: The strategy of including a large number of promiscuous conserved M group epitopes in a DNA vaccine may thus be able to elicit a broad immune response against widely diverse HIV-1 isolates around the world, circumventing HIV-1 genetic diversity.

P18.14
Using HLA Binding Prediction Algorithms for Epitope Mapping in HIV Vaccine Clinical Trials

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Background: A common endpoint in any T-cell based vaccine trial is measurements of vaccine-induced T-cell responses such as breadth and magnitude, measured using functional assays that measure the release of cytokines such as IFN-γ. The major limiting factor in these mapping studies is sample availability, as each test requires ~100K live cells. Current mapping strategies use a group-testing approach in which responses to an immunogen are measured using peptide pools that span a protein, and are then further refined using sets of mini-pools and a peptide matrix. Positive K-mers are then de-convoluted to identify the optimal epitope and restricting HLA allele.

Methods: We explore the use of HLA binding predictors to improve the efficiency of epitope mapping protocols in vaccine trials. Given information about participant’s HLA alleles, we predict vaccine induced T-cell responses at various levels of refinement, based on the current group-testing hierarchical mapping approach. We benchmark these methods on epitope mapping data from a cohort of 12 HIV acutely infected individuals from the Seattle Primary Infection Cohort.

Results: We find that incorporating HLA prediction methods provides significant improvements in mapping efficiency in each of the four stages of the current epitope mapping approach. For example, ranking all 9/10mer peptide-HLA pairs in a given reactive 15mer in order identify the optimal epitope, we find that 43/52 optimal epitopes were the highest ranked pair from a total of 66 potential pairs. We also find that by ranking the row and column mini-pools of a reactive peptide pool in order to identify the reactive 15mer, an average number of 9/20 mini-pools need to be tested, a savings of more than 50% of the number of tests.

Conclusion: HLA binding predictors provide significant improvements to current mapping protocols and novel protocols that make use of these predictors should be designed and implemented.

P18.15
Characterization of Cell-Mediated Immune Responses Conferred by a Recombinant Measles-HIV-1 Clade C Vaccine

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Background: Live attenuated measles virus (MV) is one of the most efficient and safest vaccines available making it an attractive candidate vector for an HIV-1 vaccine aimed at eliciting cell-mediated immune responses. We first assessed the immunogenicity of a recombinant measles-HIV-1 vaccine (MV1-F4), carrying inserts for Gag, RT and Nef from HIV-1 Clade C, in mice (CD46/IFNAR). The vaccine candidate induced potent T cell responses with cross-reactive activity against Clade B antigens.

Methods: We then characterized the immunogenicity of this vaccine candidate in cynomolgus macaques. Two groups of 8 MHC-matched macaques received intramuscular administration of Clade C MV1-F4 at days 0 and 84: group (A) received 10^4 CCID50 and group (B) 10^6 CCID50. Polyfunctional flow cytometry (CD3/CD4/CD8/CD40L/IL-2/IFNγ/TNFα) was performed to detect intracellular cytokine responses following stimulation with Clade C synthetic peptide pools covering p24, RT, Nef and p17 sequences.

Results: Significant HIV-insert-specific responses were detected in all individuals that received either the low dose or high dose of Clade C MV1-F4. CD4+ T cell responses to HIV-1 inserts were more frequent than CD8+ T cell responses. Boosting of insert-specific CD4+ T cell responses was observed after the second immunization in both groups, but was not observed for the CD8+ T cell responses. The intensity of the HIV-specific CD4+ T cell response was slightly increased when a high dose of the Clade C MV1-F4 vaccine was used as compared to low dose, but the levels of CD8+ T cells were comparable in both groups. The cross-clade reactivity of the T cell responses and the presence of HIV-specific T cell responses in secondary lymphoid organ tissues are being investigated.

Conclusion: Vaccination with Clade C MV1-F4 induces a robust and boostable cellular immune response in non-human primates, further supporting the exploration of the MV vector for HIV-1 vaccine strategies.

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P18.16
Determination of Cellular Immune Responses Induced by Consensus or Mosaic HIV-1 Env Vaccines in HLA-B7 Transgenic Mice

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Background: The genomic variability of HIV impedes development of globally relevant HIV vaccines. Developing vaccine platforms that induce strong T cell breadth and magnitude has been difficult. In the past DNA vaccines were poorly immune potent in humans and were studied in prime boost approaches. Recently, data from the PENNVAX™-B clinical trial (HVTN 080) showed that enhanced highly optimized DNA delivered by EP (E-DNA) drives strong CD4+ and CD8+ T cell responses that are equivalent/superior to viral & nonviral approaches. We build on these data by studying ways to further enhance the breadth and magnitude of E-DNA vaccines. Accordingly we compared two important epitope focusing strategies for their immune profiles, a novel optimized synthetic clade B env and a designed mosaic insert when both are enhanced for expression and delivered by EP.
**Methods:** An optimized clade B consensus envelope vaccine (pEY2E1-B) and an optimized mosaic gp160 envelope vaccine were developed (pMosEnv). Following immunization with electroporation, cellular immune responses were assayed by IFN-γ ELISPOT in human HLA-B7 (mouse MHC KO) transgenic mice. **Results:** pEY2E1-B induced stronger cross-reactive responses against consensus peptides from individual subtypes compared to pMosEnv. The Elispot responses induced by pEY2E1-B against four pools of consensus subtype A, B and C peptides were 238, 1150 and 372. While the responses induced by pMosEnv were 54, 415 and 168. The consensus immunogen was up to 3x more potent at driving subtype-specific responses that cross-recognized diverse clade antigens. When a PTE peptide set was used, pMosEnv was more robust compared to pEY2E1-B. We have also explored prime boost approaches using these constructs and observed further robust immune enhancement. **Conclusion:** These strategies have unique strengths and specific differences in their immune targeting abilities. The consensus immunogen exhibits improved focus on conserved regions while the mosaic immunogen targets more variable epitopes. Combination approaches look very promising.

P18.17
Design of Thermo-Stable Human/Simian Adenoviral T-Cell Based Vaccines Expressing Ubiquitin Fusions of Full or Fragmented HIV/SIV Genetic Components

**Methods:** Ad-OVA-GFP vectors (4x10^9 vg) in C57BL/6 mice and footpad-troporation, cellular immune responses were assayed by IFN-γ ELISPOT in human HLA-B7 (mouse MHC KO) transgenic mice. We subsequently constructed a cohort of adenoviral [human (Ad5/Ad11)/simian (Ad30/37)] vectors in which HIV-1 genetic components (gag/pol/env/rev) have been genetically engineered to stimulate broader CTL responses. As an example, full size HIVCN54 or SIVmac251 gag genes including an across clade conserved epitope were engineered, fused to mono- (+N-end rule) or tetra-ubiquitin (Ub) sequences and further tested on DC and non-DC cell lines by using plasmid, in vitro synthesised mRNA, or human adenoviral vectors. We subsequently constructed a cohort (n = 34) of rAd vectors carrying genetically fragmented ubiquitinated (4, 1, 0 Ub) fusions for either HIVCN54 (n = 10) or SIVmac251 (n = 7) gag genes attempting to reduce antigenic competition and/or alter epitope (sub)dominance. **Conclusion:** We have designed a cohort of human/simian adenoviral T-cell based vaccines expressing ubiquitin fusions of full or fragmented HIV/SIV genetic components. We are currently extending our studies using an in vitro CTL epitope-mapping system employing human monocyte-derived DCs from healthy and HIV-1 + individuals alongside mouse in vivo studies impacting the antigen ubiquitination and genetic fragmentation on the quality of the generated immune responses. SIV constructs are currently forward tested in non-human primate (NHP) models (cynomolgus macaques).

P18.18
DNA Vaccine Encoding HIV CD4 T Cell Epitopes Enhances CD8 T Cell Responses Induced by Immunization with DNA Vaccines Encoding Whole Gag (p55) and Vif


**Background:** Generation of competent and long-lived memory CD8 + T cells is highly dependent on CD4 + T cell help. A vaccine able to provide cognate help may efficiently prime CD8 + T cell responses to establish an appropriate immune control of HIV. Our group has identified a set of 18 conserved CD4 epitopes from 8 HIV-1 proteins. HIVBr18 DNA vaccine encoding such epitopes induced strong cellular immune responses in mice. Early Gag and Vif CD8 + T cell responses have been described as fundamental to control SIV replication. We aim test whether preimmunization with HIVBr18, encoding Vif and Gag CD4 epitopes, could boost Gag and Vif-specific CD8 + T cell responses induced by subsequent immunization with plasmids encoding whole Gag (p55) and Vif.

**Methods:** Synthetic genes encoding the 18 HIV-1 previously identified CD4 + T cell epitopes or HIV-1 Gag and Vif whole sequences were subcloned in the pVAX1 vector to obtain HIVBr18, pVAX-gag and pVAX-vif, respectively. BALB/c mice were immunized with the plasmids pVAX-gag and pVAX-vif or preimmunized with the multiepitopic plasmid, HIVBr18, followed by immunizations with pVAX-gag and pVAX-vif, in a regimen of 4 doses.

**Results:** Preimmunization with HIVBr18 elicited broader and stronger IFN-γ secretion as well as T cell proliferation against Gag and Vif peptides than immunization with pVAX-gag + pVAX-vif alone. These increases were also observed against Gag and Vif regions not shared with the HIV CD4 epitope vaccine.

**Conclusion:** Our results suggest that this vaccine may be useful as a source of cognate CD4 + T cell help for T cell responses, thereby increasing the efficacy of novel HIV-1 vaccine candidates.
P18.19
Conservation Across Time and Sequence: Validation of Immunogenic HLA-A2 “Achilles’ Heel” Epitopes for the GAIA HIV Vaccine

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Background: The genomic sequence variability of HIV has complicated efforts to generate an effective and globally relevant vaccine. Viral variation in response to humoral and cellular immunity, and the pressures exerted by the immune system, have made it difficult to develop an effective vaccine. The importance of conserved regions of the viral genome, which are less likely to change over time and in response to immune pressure, is becoming increasingly apparent. These segments, referred to as “Achilles’ Heel” epitopes, are of particular interest as they may represent regions of the viral genome that are less likely to change over time and in response to immune pressure.

Methods: In this study, we used an immunoinformatics tool to select highly conserved putative HLA-A2 epitopes. This analysis was performed in 2002 on 10,803 HIV-1 sequences and re-evaluated in 2009 on an expanded database of 43,822 sequences. Analysis tools were the EpiMatrix suite of immunoinformatic algorithms. Validation of these selected epitopes was performed in both Providence, RI, and Bamako, Mali.

Results: Thirty-five (92%) of the 38 selected peptides stimulated IFN-γ response in PBMC from HIV-infected patients in Providence, RI, and/or Bamako, Mali.

Conclusion: Validation of these selected HLA-A2 epitopes across time (2002-2009) and space (Providence and Mali), support the hypothesis that these epitopes would be appropriate candidates for inclusion in a globally relevant HIV vaccine.

P18.20
The GAIA HIV Vaccine Progress Report: Further Validation of Broadly Conserved Class I Epitopes in West Africa

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Background: The GAIA HIV Vaccine Program aims to develop an HIV vaccine that targets a broad range of evolutionary sequences of the virus. The importance of conserved regions of the viral genome, which are less likely to change over time and in response to immune pressure, is becoming increasingly apparent. These segments, referred to as “Achilles’ Heel” epitopes, are of particular interest as they may represent regions of the viral genome that are less likely to change over time and in response to immune pressure.

Methods: Here we employ this advanced combination of technologies to focus on improving the B cell response induced by DNA vaccine to HIV plasmid encoded antigens. Furthermore, we have compared this new DNA delivery to HIV envelope antigen vaccination as well as in a DNA prime-protein boost setting.
Results: We observe for the first time that the antibody responses induced by this DNA platform alone are equal to and in some cases surpass the antibody responses induced by recombinant HIV env protein antigen in side by side studies.

Furthermore in the prime boost setting both the quality and the magnitude of the antibodies are improved by up to several logs over protein immunization alone. We have also studied binding antibodies and observe that there is greater breadth of antibody binding induced by the DNA over that induced by protein, and this binding response is further enhanced in the prime boost setting. In addition the neutralization phenotype is expanded in the prime boost setting, over either immunogen platform alone to levels that mimic or surpass those induced by viral infection. Examination of T cell responses shows that the antibody enhancement does not negatively impact the high CTL responses generated by the DNA platform.

Conclusion: This study suggests that these improved DNA construct designs and improved delivery are important for further investigation and optimization and may represent a uniquely malleable and important platform for HIV.

P19.02
Design of a Dual Purpose Lentiviral Vector for Wild-Type CCR5 Phenotype Abolition, and CCR5 Delta-32/Delta-32 Genotype Transduction

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Background: Two chemokine receptors are known to participate in HIV entry: CCR5 and CXCR4. The CCR5 is coded by a gene located on chromosome 3, Locus 3p21.31, within the chemokine receptor gene cluster region. A naturally occurring CCR5 delta 32 mutant with a deletion in the second extracellular loop of the gene product results in impaired membrane expression of the receptor and leads to resistance to HIV-1 infection in homozygous individuals. There is evidence for the cure of HIV by transplantation of CCR5 delta 32/delta 32 stem cells. Stem cell transplantation is, however, not feasible for the cure of HIV world-wide. Affordable alternatives to CCR5 delta 32/delta 32 stem cell transplantation are required. Here, we advance a conceptual design of a single lentiviral vector for CCR5 delta 32/delta 32 genotype transduction.

Methods: Design: Theoretical modeling; materials: CCR5 wild-type gene, CCR5 mutant gene, lentiviral vector design, and RNA interference model; Interventions: Three models were theoretically conceived: (a) wild-type CCR5 phenotype abolition, (b) mutant CCR5 (delta-32/delta-32) transduction, and (c) dual CCR5 wild-type phenotype abolition plus CCR5 mutant phenotype transduction.

Results: We observed that- in CD4+ ve progenitor cell-lines: First, expression of wild-type CCR5 phenotype can be abolished using a lentiviral vector carrying small interfering Ribonucleic acids (siRNA) targeted to any 18-22 linear base sequences within this gene’s delta-32 transcript region. Second, effective and efficient mutant CCR5 (delta-32/delta-32) transduction can be combinatorially be achieved using a lentiviral vector carrying this trait’s gene sequence. Third, dual CCR5 wild-type phenotype abolition plus CCR5 delta-32/delta-32 phenotype transduction—may be effected using a single lentiviral dual-purpose vector carrying (1) siRNA targeted to 18-22 linear base sequences within messenger RNA transcript corresponding to the 32 base-pair deletion, and (2) the entire CCR5 delta-32/delta-32 gene sequence.

Conclusion: The dual-functional lentiviral vector modeled above offers a more affordable alternative for CCR5 delta-32/delta-32 genotype transplantation—transduction.

P19.03
Purification of Native Env Trimmers in Particulate and Soluble Forms and an Analysis of Their Stability and Antigenicity

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Background: Native Env trimers may be ideal neutralizing antibody immunogens if they can be separated from antigenically “promiscuous” forms of Env that promote non-neutralizing responses. We recently found that enzyme digests substantially deplete “junk” Env from HIV particle surfaces, leaving native Env trimers intact. Here, we sought to:

1) Improve enzyme digest efficiency to produce pure “trimer-VLPs”
2) Evaluate “trimer-VLP” antigenicity
3) Isolate native Env trimers in soluble form
4) Evaluate the stability and antigenicity of soluble native trimers

Methods: VLPs were glycosidase-protease digested and analyzed by blue native PAGE (BN-PAGE). Various digest conditions were modified to improve efficiency. The antigenicity of particulate trimers was assessed by VLP-ELISA and BN-PAGE. Soluble trimers were liberated from particles by detergent and their stability and antigenicity was assessed by BN-PAGE.

Results:

1) Studies involving various Env mutants, the use of various enzymes and doses, and modifying incubation times and physical digest conditions led to substantial improvements in VLP digests, such that pure “trimer VLPs” can be isolated routinely.
2) In VLP-ELISA, undigested Env-VLPs were recognized by various mAbs, regardless of neutralizing activity. 2F5 bound substantially to particles lacking Env, presumably due to cross-reactivity to membrane lipids. Digested wild-type-VLPs (“trimer-VLPs”) were recognized by neutralizing mAbs but not non-neutralizing mAbs.
3) Following VLP solubilization, “SOS” mutant trimers (disulfide linking gp120-gp41) could be isolated and remained stable.
4) Soluble SOS Env trimers were recognized only by neutralizing mAbs, including PG9 and PG16, suggesting the quaternary structure is intact. MPER mAbs bound more efficiently than they did to particulate trimers, perhaps due to a loss in membrane constraints.

Conclusion: These results will enable the evaluation of pure native trimer immunogens in particle and soluble forms. Rabbit studies are ongoing. Our antigens could also assist in the isolation of new neutralizing mAbs and in determining the structure of native Env trimers.

P19.04
ENVolution: Immunoselection of Recombinant Vesicular Stomatitis Virus Expressing HIV-1 Envelope Proteins by Broadly Neutralizing Antibodies

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Background: A formidable obstacle for human immunodeficiency virus (HIV) vaccine development is the design of an HIV envelope (Env) immunogen that elicits induction of broadly neutralizing antibodies (BnAbs), which block infectivity of several HIV strains. As with most RNA viruses, the vesicular stomatitis virus (VSV) RNA-dependent RNA polymerase lacks proof-reading function. Mutations accrue during serial passage for adaptation to specific environments. We have observed that recombinant VSV (rVSV) encoding a functional HIV Env in place of VSV G rapidly accumulated adaptive mutations in Env when propagated in the presence of BnAb b12 that enabled neutralization escape. This result suggests that selective pressure can be applied to rVSV-Env vectors to rapidly evolve novel HIV Env immunogens. BnAb b12 targets a discontinuous epitope near the CD4-binding site of gp120 subunit of HIV Env. The antigenicity of such epitopes may be altered by mutations that result in a conformational change of the overall trimeric complex; thus we currently are optimizing a system that employs VSV’s evolutionary potential to generate novel Env glycoproteins selected based on their b12 binding properties.

Methods: rVSV-GFP1-EnvG4 virus is captured by BnAb b12-Protein G beads. Ribonucleoprotein (RNP) complexes of captured virus are extracted using detergent and salt. Purified RNPs are transfected into CD4/CCR5(+) cells to select out non-binders to b12 and enrich the population with only those viruses that retain b12 binding.

Results: rVSV-GFP1-EnvG4 was immunoprecipitated by BnAb b12 as detected by Western Blot. Immunoprecipitated virus was successfully transfected into permissive cells after RNP extraction.

Conclusion: A system has been established to select out HIV Env proteins based on binding to a BnAb. We will perform several rounds of this immunoselection coupled with serial passaging to examine if novel immunogens may be developed by this technology. This system may be applied to epitope mapping, immunogen design and neutralization escape.

P19.05
Efficacy of DNA Vaccine Encoding Retrovirus-Based VLP to Induce Potent mucosal and Systemic Immune Responses and Its Use for HIV Vaccination

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Background: The key properties of viruses that are responsible for eliciting potent immune responses may be used as a framework for rational vaccine design. These important immunogenic properties of viruses include their size, geometry, surface molecule organization and an ability to induce innate immunity with appropriate conditioning of the adaptive immune responses. New-generation vaccines aim to harness these properties.

Methods: Consequently, we developed DNA-vaccines expressing recombinant retrovirus-based VLPs (plasmo-retroVLPs) pseudotyped with envelope glycoproteins expressed in their wild-type conformation. This strategy combines the efficiency of VLP-based vaccines with the simplicity and versatility of DNA-based vaccines.

Results: Here, we demonstrate that plasmo-retroVLPs induce significantly better epitope-specific CTL and envelope-specific neutralizing responses than standard plasmids expressing non-particulate antigens and thus constitute a new HIV vaccine candidate. We demonstrated in mice and macaques that plasmo-retroVLPs elicit HIV-specific cellular and antibody immune responses. Furthermore, our results show that the delivery vehicle and route of immunization are critical factors governing the vaccine efficacy. Remarkably, we demonstrated that potent HIV-specific mucosal and systemic immune responses are induced after mucosal immunization using formulated DNA vectors.

Conclusion: Taken together, DNA vaccines encoding HIV-pseudotyped retroVLPs represent efficient immunogens that can be used to induce systemic and/or local immunity. Supported by CUT'HIVAC (EU FP7 #241904) and ANRS.

P19.06
Inherent Capacity to Develop Primary CTL Against Autologous HIV Epitope Variants: Implications for Prophylactic and Therapeutic Vaccines

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Background: Dendritic cells (DC) have the unique ability to induce antiviral T cell immunity, making them ideal targets for HIV prophylactic and therapeutic vaccines. Here we use DC to demonstrate in vitro priming of HIV-specific CTL with effector function in HIV-negative donors. We then use this model to compare primary and memory T cell responses, using PBMC obtained before and after HIV infection, against autologous antigenic variants that accrued after infection.

Methods: Epitope variants were revealed through viral sequencing, were evaluated for MHC binding, and used with Th1 polarizing DC to optimally prime CTL from HIV negative subjects. This DC-peptide model was then used to stimulate autologous T cells from pre-infection, autologous PBMC of HIV infected subjects in the Multicenter AIDS Cohort Study. Recall responses were examined by IFN? ELISpot using PBMC from multiple post-infection time points of these same subjects.

Results: DC induced primary CTL against naturally evolving HIV A*0201 epitope variants in PBMC from HIV negative and HIV positive subjects, regardless of altered epitope binding to MHC. Despite loss of T cell recall responses during HIV infection to these variants, induction of primary CTL against these autologous epitopes demonstrated an inherent capacity to develop effector responses against naturally evolving virus variants.

Conclusion: The ability to induce primary CTLs in vitro against autologous variant HIV epitopes in PBMC prior to seroconversion suggests healthy DC and T cells are able to overcome the burden of virus variation. The progressive loss in vivo of memory T cells against these variants suggests T cell dysfunction, and not solely viral mutation, is contributing to the inability to mount effective immunity during untreated infection. We therefore believe the immune system can effectively respond to virus variants and can be exploited to implement therapeutic vaccinations once T cell function is restored in HIV infected persons on

P19.07
Refocusing the Antibody Response: The Use of an Antibody-Masking Technique to Elicit Broadly Neutralizing Antibodies

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Background: The main goal of HIV-1 vaccine development is the design of an immunogen capable of inducing antibodies that can broadly neutralize HIV-1. However, it has proved to be extremely difficult to elicit such antibodies through vaccination as the host immune system is usually distracted by immunodominant regions on the HIV Env protein, such as the hypervariable loops, and the antibodies generated are non-neutralizing. Here, we describe an approach which we aim to refocus the immune response towards key epitopes as defined by known BNAbs by utilizing non-neutralizing antibodies as a masking shield of the immunodominant regions on HIV Env protein.

Methods: Two immunogens have been designed aiming to encourage the elicitation of CD4bs-targeting and MPER-targeting antibodies in a two stage mice immunization schedule. All Env proteins are based on the Clade B HIV-1 JRFL. Female Balb/c mice (7 weeks old) are DNA primed followed by two protein boosts every three weeks. Sera were collected one week before and after DNA priming, and one week after each boost. IgG antibodies are purified from the pooled sera, and will be used to construct a masking shield in the second immunization.

Results: The two immunogens were tested and verified against different BNAbs. IgG against these immunogens were purified and digested into their Fab region. Future works involve the use of purified IgG Fab to construct a masking shield against and in vitro testing of the masking mechanism. Immunization of mice using the shielded materials will be carried out should the initial testing proved promising.

Conclusion: The masking of immunodominant regions on HIV Env protein by previously elicited non-neutralizing antibodies may prove to be a useful tool in refocusing the immune response towards key epitopes defined to date, and encourage the elicitation of broadly neutralizing antibodies.

P19.08 Cutaneous and Mucosal HIV Vaccination EU-FP7 Funded Large-Scale Program

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Background: The CUT’HIVAC project aims at assessing a new HIV vaccine strategy to prevent and control HIV infection based on transcutaneous and/or mucosal routes and immunogen delivery systems to preferentially promote and redirect immune responses towards high level of mucosal Abs and effector CD8 cell responses directed against various HIV antigens.

Methods: Taking advantage of new genetic and immunological information provided by scientific, industrial and academic partners, CUT’HIVAC will design, develop and validate innovative immunogens and delivery systems as well as immunization methods. Clinical trials will be implemented with the last cutting-edge generation of HIV DNA-GTU candidate applied by transcutaneous, intradermal routes and/or mucosal administration of HIV-envelope protein-based vaccine. Much work will be carried out on the new genetic design of HIV antigens and delivery systems for developed and developing countries. These new vaccines will be tested in innovative preclinical and innovative humanized mice approaches with a special highlight on routes of vaccination. The project also aims at rapidly translating preclinical approaches into prophylactic and therapeutic clinical trials in developed and developing countries that could help to prevent and eradicate HIV.

Results: The project will shed light on our understanding of the mechanisms of vaccine penetration in skin and mucosa, and will highlight their impact on the quality of immune responses. Based on strong scientific knowledge of HIV disease and new technical approaches in the field of vaccinology, CUT’HIVAC will redesign efficient vaccine candidates to provide the basis for the introduction of entirely novel vaccination systems into the clinic.

Conclusion: Through its integrative and multidisciplinary approach, CUT’HIVAC will therefore provide the basis for a novel approach in vaccination with a view to widening its application to other infectious diseases such as malaria and tuberculosis.

P19.09 Optimizing the Immune Response to MVA/HIV Vaccine Candidates in Mice

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Background: Recombinant MVA expressing HIV envelope and gagpol genes (MVA/HIV) are currently being tested in phase I and II vaccine trials. In attempts to make MVA/HIV candidates more immunogenic, we have reassessed the immune response of mice immunized 1) by different routes, and 2) with different configurations of the HIV envelope (secreted gp140, membrane-bound gp160, trimeric HIV/SIV gp140 chimera) in prime-boost experiments.

Methods: In one study, mice were immunized 2 times with MVA/HIV by intramuscular (IM), intradermal (ID), and skin scarification (SS) routes. In a separate study, mice were immunized by the SS route with viruses expressing HIV gp140, gp160, or trimeric HIV/SIV gp140 chimera and boosted with gp140 or gp140 chimera proteins. Vaccinia and HIV envelope CTL and binding antibody ELISAs, vaccinia and HIV neutralization assays (Clade B Tier 1 and Tier 2), and vaccinia challenge protection studies were performed.

Results: Higher CTL responses to vaccinia and HIV envelope were found by immunization using IM and SS routes. Superior vaccinia and HIV envelope binding ELISA and vaccinia neutralization responses were seen using ID and SS routes. The SS route afforded the best protection against weight loss by intranasal lethal vaccinia challenge. In prime-boost experiments with different HIV envelope constructs, all 3 groups induced similar HIV envelope CTLs and binding ELISA antibodies. The gp160-immunized group induced the highest level of neutralizing antibodies with the order of the three groups being gp160 > gp140 > gp140 chimera. No increased breadth of neutralizing antibodies was detected.

Conclusion: Taken together, our data suggest that MVA/HIV immunization by the SS route produces optimal immune response, and that membrane-bound HIV envelope is superior to secreted gp140 in induction of HIV neutralizing antibodies. The trimeric structure of the HIV/SIV gp140 chimera did not increase the breadth of neutralizing antibody produced.
P19.10
A Strategy to Circumvent Preexisting Adenovirus Neutralizing Antibodies Using Adenovirus-Infected Blood Cells: Proof-of-Concept in Rhesus Macaque

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Background: Adenoviral vectors have been extensively exploited for the development of prophylactic and therapeutic vaccines for a variety of infectious diseases and cancers. However, the practical application of Ad5 could be limited by the high prevalence of preexisting anti-Ad5 immunity, especially Ad5 neutralizing antibodies.

Methods: In the present study, we found that freshly isolated PBMCs, mostly CD14+ cells, from Ad5-seropositive primates (humans and rhesus macaques) can be infected with Ad5 vectors. Interestingly, these cells are infected more efficiently than those from Ad5-seronegative individuals. On the basis of this observation, a novel strategy to circumvent the attenuated delivery efficiency due to Ad5 neutralizing antibodies was explored.

Results: We demonstrated that the infuion of autologous PBMCs infected in vitro with Ad5-SIVenv can elicit robust immune responses in Ad5-seropositive rhesus macaques. Repeated infusion of Ad5-SIV vaccines carrying either homologous or heterologous SIV antigens using this strategy generated antigen-specific immune responses in those monkeys. Furthermore, the monkeys were challenged intravenously with highly pathogenic SIVmac239 virus, and this strategy resulted in a significant reduction of the viral load at both the peak time and at a setpoint period compared with control animals.

Conclusion: The results of this study warrant the further development of the adenoviral vector-infected PBMCs (AVIP) strategy as a simple but practically effective method for repeated delivery of Ad5-based vaccines.

P19.11
Novel Non-Integrative One-Cycle Lentiral Genomes Derived from a Naturally Attenuated Animal Lentivirus as HIV Vaccines

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Background: A safe and efficacious HIV vaccine is strongly needed to stop the continuing epidemics in humans. We developed lentiviral-based vectors as HIV DNA vaccines and have shown that our first generation of vectors was efficient alone to induce unique T cell responses and protection of vaccinated macaques from AIDS against pathogenic virus. We engineered a novel one-cycle replication, non-integrative (NONI-LV) lentivector driven by lentiviral LTRs that have constitutive promoters. NONI-LV was used in the NOD/SCID-hu mouse model to examine HIV-specific immune responses.

Methods: SIV LTRs were replaced with CAEV LTRs in the genome of SHIV-KU2 and the integrase gene was deleted. Resulting NONI-LV vaccine genome was transfected into HEK-293 T cells and expressed viral proteins were detected by RIPA analysis. Virus in the culture medium was examined for its capacity to induce a single cycle of infection in CEM-T4 cells. BALB/c and PBMC-humanized NOD/SCID beta2 mice were immunized with the NONI-LV DNA vaccine and humoral T cell immune responses were examined by ELISA and IFN-γ ELISPOT.

Results: RIPA analysis of viral proteins showed classical profiles in samples of cells transfected with NONI-LV and SHIV-KU2 DNAs. Infection of CEM-T4 cells showed a single round of infection of the culture medium from NONI-LV transfected cells, in contrast samples from SHIV-transfected cells produced virus that causes productive infections. NONI-LV and SHIV-KU2 DNAs but not D4SHIV-KU2 immunized mice induced HIV-specific antibody responses in sera of NOD/SCID-hu immunized mice. All NOD/SCID-hu mice immunized with the NONI-LV developed potent T cell IFN-γ ELISPOT responses that were nearly equivalent to those induced by SHIV-KU2 and much higher than those induced by D4SHIV-KU2 DNAs.

Conclusion: These data show a substantial increase of immunogenicity of the new vaccine compared to our former DNA vaccine. Ongoing experiments will help to characterize the induced responses and to examine the correlates of protection.

P19.12
A Novel Vaccine Design for Inducing Robust HIV-1 Specific Immunity

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Background: An ideal AIDS vaccine should induce broadly protective neutralizing antibody and cellular immune responses to cover all HIV-1 subtypes. To date, conventional vaccine strategies have failed to achieve this goal. It is, therefore, necessary to explore novel vaccine concepts for AIDS vaccine design. We hypothesized that a novel vaccine design might be targeting the test antigen to dendritic cells more effectively while inhibiting the negative effects of the PD-1/PD-L pathway on T cell function.

Methods: We investigated a novel DNA vaccine design by fusing a test antigen, human HIV-1 Gag p24, with the soluble functional domain of programmed death-1 (sPD-1). This sPD-1-p24-DNA and control vaccines (e.g. mutant-sPD-1-p24-DNA, lacking the ligand binding domain) were used to vaccinate groups of BALB/c mice via intramuscular electroporation (EP). Vaccinated animals were subsequently sacrificed for measurement of HIV-1 Gag-specific responses using ELISPOT, ICS, tetramer and ELISA assays. Vaccinated mice were also challenged with a vaccinia-Gag virus for efficacy evaluation.

Results: Purified sPD-1-p24 interacted specifically with cells expressing ligands PD-L1 and PD-L2, as well as with bone-marrow derived primary dendritic cells. sPD-1-p24-DNA induced significantly higher levels of Gag-specific IFN-γ and TNF-α, accounting for up to 27% and 4% of total CD8+ and CD4+ T cell populations, respectively. Moreover, up to 22% of total CD8+ T cells stained positive for HIV-1 Gag tetramer. Greater anti-Gag antibody titer was also observed with likely balanced IgG1 (Th2) and IgG2a (Th1) responses. Importantly, strong cell-mediated and antibody responses
RESULTS: Prime-boost immunization with plasmid and dotyped viruses/TZM-bl cells. CD40L (CD40Lm) in C57BL/6 mice. Then we analyzed cellular and humoral immune responses based on developing vaccination regimes that would efficiently induce both anti-HIV-1 humoral and cell-mediated immunities in humans particularly because of its very low neurovirulence. LC16m8A rendered mice 1000 fold more resistant to pathogenic VV WR strain than nonreplicating VV MVA did. It is our aim to investigate the utility of mucosally applied plasmid DNA as a means to initiate both systemic and mucosal humoral responses, which could then be greatly potentiated by protein boosting.

METHODS: Plasmid DNA was complexed to PEI and administered in a 15μl volume to either vaginal, sublingual or nasal mucosa of medroxyprogesterone treated BALB/c mice. Immunisations occurred fortnightly and mice were sampled (serum and vaginal wash) 7 days post each immunisation. Antigen-specific IgG and IgA production was assessed in the sera and mucosal lavage samples by quantitative ELISA and by splenocyte B cell ELISpot.

RESULTS: DNA topically applied to all mucosal surfaces tested primed gp140 antigen-specific humoral responses. Nasal and sublingual immunisation elicited antigen-specific IgG in serum and vaginal lavage however the latter was unable to generate mucosal IgA. Furthermore, both groups developed IgG and IgA spot forming units in splenocyte cultures. Vaginal immunisation generated low antigen-specific IgA antibody responses in serum and mucosal lavage in the absence of any IgG or B cell responses in the spleen. Finally, the nasal application of a heterologous DNA prime and gp140 protein boost revealed a single DNA prime to be sufficient to generate strong systemic and mucosal responses.

CONCLUSION: We have performed a series of linked studies in the murine model that aimed to enhance vaccination via the mucosal route and to determine the potential role of plasmid DNA in mucosal immunisation regimes. Here we conclude that plasmid DNA encoding a large transgene product can be complexed to PEI and applied to various mucosal surfaces in a small volume formulation and that ensuing humoral immune responses vary depending on the administration site.

P19.14
Mucosally Applied HIV gp140 DNA Prime/Protein Boost Strategies Generate Strong Serum and Mucosal Antigen-Specific Humoral Responses in Mice

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BACKGROUND: Mucosal surfaces are the first line of defence against most pathogens but there remains a distinct lack of mucosally applied vaccines capable of eliciting potent and effective mucosal immunity. We therefore aimed to investigate the utility of mucosally applied plasmid DNA as a means to initiate both systemic and mucosal humoral responses, which could then be greatly potentiated by protein boosting.

METHODS: Plasmid DNA was complexed to PEI and administered in a 15μl volume to either vaginal, sublingual or nasal mucosa of medroxyprogesterone treated BALB/c mice. Immunisations occurred fortnightly and mice were sampled (serum and vaginal wash) 7 days post each immunisation. Antigen-specific IgG and IgA production was assessed in the sera and mucosal lavage samples by quantitative ELISA and by splenocyte B cell ELISpot.

RESULTS: DNA topically applied to all mucosal surfaces tested primed gp140 antigen-specific humoral responses. Nasal and sublingual immunisation elicited antigen-specific IgG in serum and vaginal lavage however the latter was unable to generate mucosal IgA. Furthermore, both groups developed IgG and IgA spot forming units in splenocyte cultures. Vaginal immunisation generated low antigen-specific IgA antibody responses in serum and mucosal lavage in the absence of any IgG or B cell responses in the spleen. Finally, the nasal application of a heterologous DNA prime and gp140 protein boost revealed a single DNA prime to be sufficient to generate strong systemic and mucosal responses.

CONCLUSION: We have performed a series of linked studies in the murine model that aimed to enhance vaccination via the mucosal route and to determine the potential role of plasmid DNA in mucosal immunisation regimes. Here we conclude that plasmid DNA encoding a large transgene product can be complexed to PEI and applied to various mucosal surfaces in a small volume formulation and that ensuing humoral immune responses vary depending on the administration site.
P19.16 Priming with Synthetic Nanovaccine Followed by rMVA Boost Is the Best Combination to Elicit Strong and Long-Lasting Gag or Env Immune Responses

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**Background:** Heterologous prime boost vaccination is the best strategy to induce broad and potent immune responses. As the best combination implies the use of viral vectors, pre-existing immunity preclude their use on a repetitive basis. Recent advancement in HIV biodegradable nanovaccines technology permit to investigate innovative prime boost strategy by combining synthetic and viral vectors.

**Methods:** We designed Poly(Lactic Acid) (PLA) nanoparticles (200 nm) carrying HIV-1 or SIV antigens as synthetic vectors, and rMVA or Ad5 as viral vectors. Different groups of mice were primed by subcutaneous route with viral vectors and boost with nanoparticles or vice versa. Combination efficacy was assessed using p24 IgG, IgAs dosage and CTLs, Elispot assays. Best combination has been further evaluated in cynomolgus using PLA nanoparticles in presence of Poly I:C, followed by rMVA boost.

**Results:** When considering the broadest immune response, best combination was a double priming with PLAp24 nanoparticles followed by viral vector, either Ad5gag or rMVAgag. When using opposite order, cellular and humoral immune responses was lower. Interestingly enough, vaginal IgGs were also observed in mice with a PLA prime/rMVA boost, and vaginal or rectal IgAs could be significantly increased after intranasal delivery of such combination. In cynomolgus experiment, three prime of PLA nanoparticles carrying SIV p27 or HIVgp140 in presence of poly I:C, followed by one boost of rMVASIVgag or rMVA gp140 CladeC, was able to induce strong and long lasting humoral immune responses against both antigens in sera, up to one year. Analysis of mucosal and cellular immune responses is currently being performed.

**Conclusion:** We described for the first time a new heterologous prime/boost strategy using synthetic biodegradable vector as a prime and viral vector as a boost. Unexpectedly, priming with synthetic vector followed by viral boost, is the best strategy for inducing broad and long lasting HIV immune responses.

P19.17 Immunogenicity and Efficacy of a Heterologous Mucosal Prime Intramuscular Boost Vaccination Strategy Against SIVmac239 Challenging

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**Background:** Conventional vaccination strategies have failed to induce broadly neutralizing antibody and cellular immune responses to prevent HIV-1 infection. It is, therefore, critical to test novel strategies in an efficacy study.

**Methods:** We have generated a recombinant replication-competent modified vaccinia Tiantan (MVT), namely rMVTTSIVgpe, as a mucosal vaccine expressing SIVmac239 Gag, Pol and Env. The immunogenicity and efficacy of rMVTTSIVgpe was studied in combination with a rAd5-based vaccine rAd5SIVgpe in Chinese macaques (Macaca mulatta) without the protective MHC class I allele Mamu-A*01. rMVTTSIVgpe was given through intranasal and oral inoculations whereas rAd5SIVgpe was through intramuscular injection. Four macaques in each of the four study groups received following prime and boost vaccinations: rMVTTSIVgpe/rAd5SIVgpe, rMVTTSIVgpe/rAd5SIVgpe, rMVTTSIVgpe/rAd5SIVgpe, twice; rAd5SIVgpe/rAd5SIVgpe and placebo controls, respectively.

**Results:** The heterologous rMVTTSIVgpe/rAd5SIVgpe regimen elicited cellular immune responses with significantly enhanced magnitude, breadth, sustainability, and poly-functionality when compared with the homologous rAd5SIVgpe regimen. Higher levels of neutralizing antibody (Nab) responses were also induced by the rMVTTSIVgpe/rAd5SIVgpe regimen against the sensitive SIVmac1A11 strain. After intrarectal challenge with a pathogenic Chinese macaque-adapted SIVmac239 (5x105 TCID50 per animal), one of four monkeys vaccinated with the rMVTTSIVgpe/rAd5SIVgpe regimen was fully protected whereas the rest showed an 1.5 – 1.9 log and 1.8–2.2 log reduction of peak and set-point viral loads as compared with control animals, which was reproducible in a separate study using additional four macaques. The control of viremia was likely correlated to CMI against Gag and Pol. 586 days post viral challenge all four monkeys vaccinated with the rMVTTSIVgpe/rAd5SIVgpe regimen remain clinically healthy whereas 2/4–3/4 animals in other groups have died of SIDS.

**Conclusion:** These data demonstrated that one-time rMVTTSIVgpe/rAd5SIVgpe regimen induced durable immune control of a pathogenic, neutralization-resistant SIVmac239 challenge. Our findings have critical implications for the development of effective vaccination strategies against HIV-1 by engaging the mucosal immune system.

P19.18 A Candidate HIV/AIDS Vaccine (MVA-B) that Enhances the Magnitude and Polyfunctionality of Memory HIV-1-Specific T-Cell Responses

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**Background:** The poxvirus vector Modified Vaccinia Virus Ankara (MVA) expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B (MVA-B) is currently used as a HIV/AIDS vaccine candidate. A general strategy to try to improve the immunogenicity of poxvirus HIV-1 vaccine candidates is the deletion of known or suggested immunomodulatory vaccinia virus (VACV) genes.

**Methods:** We have generated and characterized the innate immune sensing and the immunogenicity profile of a new HIV-1 vaccine candidate, which contains a deletion in a VACV gene.

**Results:** We show that this VACV protein is expressed early during virus infection and localizes to the cytoplasm of infected cells. Deletion of this VACV gene from the MVA-B had no effect on virus growth kinetics; therefore this VACV protein is not essential for virus replication. The innate immune signals elicited...
by the MVA-B deletion mutant in human macrophages and monocyte-derived dendritic cells were characterized. In a DNA prime/MVA boost immunization protocol in mice, flow cytometry analysis revealed that the MVA-B deletion mutant enhanced the magnitude and polyfunctionality of the HIV-1-specific CD4+ and CD8+ T-cell memory immune responses, with most of the HIV-1 responses mediated by the CD8+ T-cell compartment with an effector phenotype. Significantly, while MVA-B induced preferentially Env- and Gag-specific CD8+ T-cell responses, the MVA-B deletion mutant induced more GPN-specific CD8+ T-cell responses. Furthermore, the MVA-B deletion mutant enhanced the levels of antibodies against Env in comparison with MVA-B.

Conclusion: These findings revealed that this new VACV protein can be considered as an immunomodulator and that deleting this gene in MVA-B confers an immunological benefit by inducing innate immune responses and increasing the magnitude and quality of the T-cell memory immune responses to HIV-1 antigens. Our observations are relevant for the improvement of MVA vectors as HIV-1 vaccines.

P19.19
HIV CN54gp140 + GLA Significantly Enhances Vaccine Antigen-Specific T and B Cell Immune Responses After Priming with DNA and MVA


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Background: Using a unique vaccine antigen matched and single Clade C approach we have assessed the immunogenicity of a DNA-poxvirus-protein regimen in mice, administering the MVA and protein either sequentially or simultaneously with a novel TLR4 adjuvant (GLA).

Methods: Groups of 10 BALB/c mice were primed with plasmid DNA encoding CN54 env/gag-pol-nef and then boosted with MVA-C (env-gag-pol-nef) and CN54gp140 protein with or without GLA (aqueous formulation). The MVA and protein were either given sequentially at 3 weekly intervals or simultaneously in different legs at 3 and 6 weeks. Mice were sampled (serum and vaginal wash) prior to each vaccination and three weeks after the final immunization. Absolute levels of antigen-specific IgG and IgA were measured in sera and mucosal lavage samples by quantitative ELISA. Splenocytes were harvested at necropsy and analysed for antigen-specific T cell responses using peptide pools spanning the Env and Gag proteins by IFN-gamma ELISpot assay.

Results: GLA-adjuvanted CN54gp140 substantially enhanced the antigen-specific antibody responses in animals primed with DNA and MVA or MVA alone. Administration of MVA and CN54gp140 protein at the same time did not significantly affect antigen-specific antibody responses. Importantly, however, such co-administration of MVA-C and adjuvanted CN54gp140 did significantly augment antigen-specific T cell responses to Gag peptide pools.

Conclusion: We have shown that GLA adjuvanted CN54gp140 is able to significantly boost vaccine antigen antibody responses and have further demonstrated that co-administration of the MVA and adjuvanted protein was equally effective to a sequential vaccination modality. This vaccine schedule shortens the duration of and simplifies the immunization regime, both central to long-term vaccine feasibility. In addition, a significant benefit of the combined inoculation was that T cell responses to proteins expressed by the MVA were potently enhanced, an effect that was likely due to the presence of systemic GLA.

P19.20
Impact of Pre-Existing Immunity on the Selection of Rare Adenovirus Vector Candidates: Implications for HIV Vaccine Development

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Background: Adenovirus serotype 5 (Ad5) phase IIb vaccine trial (STEP) was prematurely stopped due to a lack of efficacy and two-fold higher incidence of HIV infection among Ad5 seropositive vaccine recipients. We have recently demonstrated that Ad5 immune complexes (Ad5 ICs)-mediated activation of the dendritic cell (DC)-T cell axis was associated with the enhancement of HIV infection in vitro. Although the direct role of Ad5 neutralizing antibodies (NAbs) in the increase of HIV susceptibility during the STEP trial is still under debate, vector-specific NAbs remain a major hurdle for vector-based gene therapies or vaccine strategies. To surmount this obstacle, vectors based on “rare” Ad serotypes including Ad6, Ad26, Ad36 and Ad41 were engineered.

Methods: The present study aimed to determine whether Ad IC-mediated DC maturation could be circumvented using these Ad vector candidates.

Results: We found that all Ad vectors tested forming ICs with plasma containing serotype-specific NAbs had the capacity to 1) mature human DCs as monitored by the up-regulation of co-stimulatory molecules and the release of pro-inflammatory cytokines (TNF-a), via the stabilization of Ad capsid at endosomal but not lysosomal pH rendering Ad DNA/TLR9 interactions possible and 2) potentiate Ad-specific CD4 and CD8 T cell responses.

Conclusion: In conclusion, despite a conserved DC maturation potential, the low prevalence of serotype-specific NAbs renders rare Ad vectors attractive for vaccine strategies.

P19.21
Improving the Immunogenicity Against HIV-1 Subtype C Env/Gag-Pol-Nef Proteins Expressed from MVA by the Deletion of the Viral Anti-Apoptotic Gene F1L

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Background: Poxviruses are one of the best characterized and more commonly used viral models for vaccine purposes. Among them, the attenuated strain of vaccinia virus MVA provides a high level of exogenous gene expression and safety and
immunogenicity against heterologous antigens. Currently, MVA-based recombinants are being used in Phase I clinical trials for HIV/AIDS and a new clinical trial using this strain has recently been completed in Spain. However, efforts for the optimization of this vaccine candidate continue.

The aim of present work was the improvement of MVA as vaccine vector against HIV/AIDS by the deletion of the viral anti-apoptotic F1L gene which encodes a mitochondria-localized inhibitor of caspase-9 and pro-apoptotic members of the Bel 2-family (Bak and Bax). The deletion of this gene was motivated by previous observations in which dendritic cells that have phagocytosed infected apoptotic cells can present viral antigens to cytotoxic T cells and induce an immune response.

**Methods:** Using MVA-C as parental virus, which expresses from the TK locus gp120 and Gag-Pol-Nef proteins of HIV-1 (subtype C) under the transcriptional control of the synthetic early/late promoter, we have generated the recombinant MVA-C-deltaF1L in which F1L gene has been replaced by rsGFP gene.

**Results:** In this work we have characterized this vector analyzing several parameters both in vitro (viral growth, stability of HIV antigens, induction of apoptosis and secretion of pro-inflammatory cytokines in different murine and human cell lines) and in vivo. Studies on the immunogenicity of the recombinant virus in the mouse model show that F1L gene deletion quantitatively improved the immunogenicity against HIV antigens in a significant manner both during adaptive and memory phases of the immune response.

**Conclusion:** These observations suggest that deletion of the F1L gene could be a valid strategy for the optimization of MVA as vaccine vector.

**P19.22**

**Conserved Elements (CE) Vaccine**

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**Background:** We are developing an HIV-1.M group vaccine that focuses immune responses on conserved elements (CE) essential to viral function while precluding responses against immunodominant decoys - targets on the virus that can mutate while retaining function. We argue that we will need to target responses using these criteria to achieve high levels of protection.

**Methods:** We compare immune response of DNA vaccines derived from CE to complete gene products in mice, including HLA transgensics, human DC and T cells ex vivo, and macaques.

**Results:** Two “toggled” variants of CE DNA were made to address >99% of HIV-1.M variability. Expression of CEgag was maximized in secretion vectors. Proteolytic processing of p24 CE revealed 87% of the known optimal epitopes. Expression by DC elicited strong CD4 and CD8 responses in human T cells ex vivo. Balb/C and Black6 mice develop CEgag-specific CD4, CD8 and Ab. Macaques generated CD8 responses of the effector phenotype. p24 CE are highly immunogenic in HIV-1 infection and include >30 epitopes restricted by >40 HLA. Controllers had higher avidity (p = 0.01) and more cross-reactive responses than non-controllers (p = 0.01) to the entire p24 and CE regions. Responses of high functional avidity had a superior ability to recognize peptide variants than low avidity responses (p = 0.01). Database frequency was too crude a measure to determine the requirement for conservation of a given AA in p24, attributable in part to HLA imprinting over time.

**Conclusion:** p24 CEgag is immunogenic in HIV-1 infection. Immune responses can be focused on these elements by vaccination, to the exclusion of decoy epitopes.

**P19.23**

**Development of an In Vitro HIV Priming System for Evaluation of Potential Immunogens Prior to Clinical Testing**

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**Background:** An in vitro priming system has been developed for the evaluation of candidate vaccine inserts side by side with cells from the same seronegative individual. This system will allow the comparison of epitope specificities of induced T cells against different inserts and the ability of these T cells to control in vitro viral replication.

**Methods:** Monocyte-derived dendritic MDDC cells were generated by plastic adherence of PBMC and culture in GM-CSF and IL4. After 24 hours, MDDC were “matured” by the addition of IL-1β, IL-6, TNF-α and PGE2 and further incubated for 24 hours. CD45ROneg (naïve), CD8pos cells from the same donor were cultured with irradiated, peptide-pulsed MDDC, in media containing IL-21. Every 2–3 days, IL-2, IL-7 and IL-15 was added. Cells were re-stimulated with peptide-pulsed autologous irradiated PBMC and assessed for specificity by ELISpot.

**Results:** This method of generating MDDC (known as “fast DC”) produces MDDC of similar phenotype as the standard 7-day culture of adherent or CD14pos monocytes as measured by expression of HLA DR and CD11c. Addition of “maturation cocktail,” significantly increases expression of CD83. These MDDC are efficient at priming HIV-specific CD8 + T cells in vitro. An average of 5 to 30% of wells contained in vitro primed HIV RK-9 and SL-9 peptide-specific T cells after two rounds of stimulation as measured by IFN-γ ELISpot.

**Conclusion:** These data demonstrate that HIV-specific responses can be primed in vitro from HIV-seronegative donors. Further development of this in vitro priming system will allow us to define characteristics of immunogen inserts that are likely to be important for next generation HIV vaccine design and could be used as a first screen of candidate T cell based immunogens prior to moving forward into more costly human clinical trials.

**P19.24**

**Co-Delivery of Mucosal Chemokine Plasmids in a Systemically Administered DNA Vaccine Elicits Systemic and Mucosal Immune Responses in Rhesus Macaques**

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Background: The induction of mucosal immunity is a crucial goal for HIV vaccines, as the mucosa is the primary site of HIV transmission/viral replication. DNA vaccines which are non-live/non-proliferating have had limited success in this area. Mucosal immune cell homing is in part controlled by a subset of chemokines that include CCL27, CCL28 and CCL25. We hypothesized that mucosal immunity could be generated following chemokine co-delivery with antigens.

Methods: Using a rhesus macaque model, we created optimized rhesus CCL27, CCL25, and CCL28 plasmid adjuvants, and co-immunized with optimized/consensus macaque pol and sooty mangabey consensus gag/env antigens. Female macaques were immunized intramuscularly followed by electroporation (IM/EP) with antigenic plasmids plus/minus a single chemokine.

Results: Chemokine adjuvants boosted memory responses in an IFN-gamma ELISPOT over antigenic plasmids alone, and co-immunization elicited detectable and long-lived polyfunctional CD8+ T cells responses in the periphery as well as mucosal sites, including the intestine and vagina. Titers of antigen-specific IgA in sera and genital washes of chemokine-vaccinated macaques were observed with the CCL27 and CCL28 adjuvants leading to both neutralization and non-neutralizing B cell phenotypes. A repeated low dose intravaginal challenge with SmmE660 was carried out, and chemokine adjuvants were able to reduce viral set point vs. controls. Importantly, we have characterized the vaccine-specific immune responses to determine the functionality and phenotype of vaccine-induced T and B cells from the mucosal and systemic compartments.

Conclusion: The results of this study will be critical to the development of an effective vaccine against HIV. This is the first example of the use of mucosal chemokines to influence a DNA vaccine strategy, suggesting a novel approach for manipulation of vaccine-induced immune responses. This work is supported by funding through the NIH/NIAIDS (F32AI054152 to MA, HIV- RADP01A80739 to DBW).

P19.25 Efficacy of HIV Vaccine Candidate in Macaques Dependent on the Dose of SIVmac251 Challenge Exposure: Immune Correlates of Vaccine Efficacy

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Background: Mucosal transmission of HIV involves a limited number of viral variants, suggesting there may be a window of opportunity for an HIV vaccine. Thus, SIVmac251 infection of macaques, a widely used model in the assessment of relative efficacy of vaccine candidates for HIV, should recapitulate transmission of HIV to humans.

Methods: We investigated the impact of the mucosal SIVmac251 challenge dose on vaccine efficacy in twenty macaques that were immunized with a combination of DNA, ALVAC-SIV, and the gp120 envelope protein vaccines. The animals were exposed intrarectally to SIVmac251 either as a single dose of 6100 TCID50, or a weekly dose of 470 TCID50 of the same virus stock until all controls became infected.

Results: Vaccination did not protect from infection with a challenge dose of 6100 TCID50, but did result in a lower plasma virus in vaccinated versus control animals over the first three weeks of infection (p = 0.010). In contrast, two exposures of the remaining twenty-four macaques to 470 TCID50 of SIVmac251 resulted in infection of all control animals but only nine of the twelve vaccinated macaques (25% efficacy). The remaining vaccinated animals that became infected had a significant and durable reduction in plasma virus levels, were protected from CD4+ T-cell loss, and had a significant reduction in the number of transmitted virus variants (an average of 1). Vaccine-induced immune responses that correlated with protection from disease were: pre-challenge Gag and Envelope specific ELISPOT responses, Gag lymphoproliferative responses, and Gag specific CD8+ T cells producing TNF-α. A significant inverse correlation was found between virus levels and gp120 antibody avidity and Antibody Dependent Cellular Cytotoxicity.

Conclusion: Accurate modeling of SIV transmission in macaques is essential in the evaluation of vaccine efficacy. Durable vaccine protection from high levels of viral replication requires both B- and T-cell responses.

P19.26 Biologically Active Fusion Proteins of CD154 and SIVgp41 as Novel Vaccine Components

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Background: HIV gp41 includes conserved residues critical for the function of the viral spike, which suggests that these regions might be important targets for neutralizing antibodies; however, the poor immunogenicity of this region may be due to its lack of exposure on native virus. CD154 (CD40L) is a trimeric glycoprotein found on activated CD4 T-cells that binds to CD40 on APCs and leads to B-cell activation and differentiation to plasma cells. We aimed at improving immunogenicity of multigenic prime/boost vaccination approaches by designing a fusion protein of the CD40-binding domain of CD154 and gp41. This protein would assemble into trimers with the potential for inducing neutralizing antibodies to an exposed gp41 and stimulating activity on APC and B-cells.

Methods: CD154 and SIV gp41 are trimeric glycoproteins with opposing polarities. A flexible (FL), helical (HL), and random linker (NL) were used to join both protein domains. Stable cell lines were prepared that expressed each one of these fusion proteins, along with SIV Gag for production of VLPs that contained the fusion proteins. We also generated fusion proteins that contained SIV gp41 with shorted cytoplasmic tails (CT) and recombinant Vaccinia viruses (VV) that expressed these fusion proteins.

Results: Both the FL and NL allowed proper protein folding and biological activity. Surprisingly, VLPs containing the short gp41...
CT incorporated lower amount of fusion protein than the ones with full length gp41. Purified VLPs were able to induce cytokine expression from macaque PBMCs. VV expressing CD154/gp41 proteins also induced expression of cytokines associated with CD40 ligation from macaque PBMC.

Conclusion: Fusion proteins of CD154 and gp41 were made that assembled into trimers, incorporated into VLPs and activated immune cells. The ability of these fusion proteins to induce neutralizing antibodies will be tested in nonhuman primates.

P19.27 Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Is a Correlate of Protection by Live-Attenuated SIV

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Background: Live-attenuated strains of simian immunodeficiency virus (SIV) afford apparent sterilizing immunity against pathogenic SIV challenge with the greatest reliability achieved thus far in non-human primate models. Identification of the mechanisms responsible may provide insights important to the design of a safe and effective HIV-1 vaccine.

Methods: We measured the capacity of antibodies elicited by nef-deleted strains of live-attenuated SIV to direct ADCC against SIV-infected cells. Protection afforded by SIV Δ nef was studied in two SIVmac251 challenge experiments. One was designed to investigate temporal associations with protection, and the second addressed the effect of sequence similarity in Env with the vaccine strain on the extent of protection.

Results: Env-specific ADCC titers against SIV-infected cells emerged early and increased over time, whereas neutralization was largely undetectable. The development of these antibodies depended upon vaccine persistence and was proportional to the extent of SIV Δ nef replication. ADCC titers were higher against SIV strains matched in Env with the vaccine strain than against Env-mismatched strains. In both SIVmac251 challenge experiments, the macaques vaccinated with SIV Δ nef that remained uninfected had significantly higher ADCC titers against SIVmac251-infected cells than those that became infected. Higher ADCC titers were also associated with lower peak viral loads in the infected animals.

Conclusion: These results identify ADCC as a correlate of protection by live-attenuated SIV, and suggest ADCC may be a useful activity to elicit in designing vaccines to prevent HIV-1 infection.

P19.28 Carbohydrate-Based HIV Vaccine, from Concept to Mannose-Specific Virion-Binding Antibodies

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Background: Several lines of evidence indicate that the glycan shield may be a rewarding HIV-1 vaccine target. Over half-dozen constructs targeting the glycan shield have been developed in attempts to induce 2G12-like neutralizing antibodies.

Methods: A triple mutant (TM) strain of Saccharomyces cerevisiae was engineered by deleting three genes in the N-glycan pathway. Glycan profiling was analyzed by MALDI-TOF, and 2G12-cross reactive proteins were detected by Western blots and identified by nano-LC-MS/MS. Rabbit antisera were raised with TM yeast cells or single 2G12-reactive yeast glycoprotein, and tested by ELISA, Western blots and glycan microarray. HIV-1 pseudoviruses were generated in 293T cells, and neutralization assay was performed using U87.CD4.CCR5 or TZM-bl cells. The binding of the antiserum to pseudovirions was tested by a viral capture assay.

Results: The TM yeast cell expresses almost exclusively Man8-GlcNAc2N-linked glycans on glycoproteins. Five endogenous yeast glycoproteins that efficiently bind to 2G12 were identified. Unlike human glycoproteins, these 2G12-reactive yeast glycoproteins contain a large number and high density of N-linked glycans, similar to gp120. Immunization of rabbits with whole TM yeast or single 2G12-reactive yeast glycoprotein elicited antibodies that specifically bound to the clusters of terminal Man7,2-Man7,2-Man oligosaccharides. More importantly, the mannose-specific antibodies were able to bind to a broad range of monomeric gp120 from different HIV subtypes and SIV. Notably, the antibodies could also bind to the pseudovirions and efficiently neutralize a genetically diverse panel of HIV-1 pseudoviruses when the viruses were produced in the presence of a mannosidase inhibitor kifunensine to force retention of high-mannose Man9GlcNAc2N-linked glycans.

Conclusion: Mannose-specific HIV-1 Env cross-reactive antibodies can be elicited with 2G12-reactive yeast glycoproteins or whole TM yeast. This genetically manipulated yeast strain is a powerful, feasible, and cost-effective means to produce glycoantigens to recapitulate the 2G12 epitope for the development of a carbohydrate-based HIV-1 vaccine.

P19.29 Focusing the Antibody Response to the CD4-Binding Site with Designer Immunogens

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Background: The HIV envelope glycoproteins gp120 and gp41 are the primary targets for neutralizing antibodies (NAb), and thus of considerable interest for vaccine design. Of particular interest is the CD4-binding site (CD4bs) on gp120 which is targeted by bNAbs such as b12, VRC01/02 and HJ16. Although the elicitation of CD4bs-specific NAbs has been reported upon experimental immunization, such antibodies are typically of low titre and transient. Approaches to improve the potency and durability of CD4bs-specific responses are needed.

Methods: In earlier work, a panel of so-called hyperglycosylated gp120 mutants were generated. These mutant gp120s are engineered to expose the b12-binding site while occluding the epitopes of numerous non-NAb. Here, we present data on the effects of two adjuvants, monophosphoryl lipid A (MPL) and QuilA, on improving CD4bs-specific responses when mixed with one of the hyperglycosylated mutants, ΔN2mCHO(Q105N), in comparison to wild-type gp120 (gp120wt). To dissect CD4bs-directed specificities, a truncated gp120 outer domain construct (XOD6) and a resurfaced core protein (RSC3) were utilized. RSC3 specifically
POSTER ABSTRACTS

P20.01
Non-Random Distribution of Cryptic Repeating Triplets of Purines and Pyrimidines (RNYn) and Recombination in gp120 of HIV-1

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Background: The tendency of HIV-1 to undergo sequence variation, particularly in gp120, is one of the major impediments to the development of a successful preventive HIV vaccine. We have previously shown that region V4 of gp120 is characterized by high levels of intra-host length polymorphism due to insertions/deletions (indels) of multiple sets of repeats. In this study we have characterized the extent of host variability in all regions of gp120.

Methods: Cloning and sequencing of a fragment of gp120 derived from plasma RNA of seven patients with early infection and naïve to therapy. Recombination analysis was performed using Splitstree and the RDP3 package. Statistical data were obtained using a randomized test using an in-house R script in the R environment.

Results: Major insertions/deletions (indels) were found in gp120 in V1, V2, V4, and V5, and to a lesser extent in C3, altering the glycan profile of the regions in which they occurred. Inserted/deleted fragment consisted of duplications and stretches of repeats of the trinucleotides RRY and RNY (R = purine, Y = pyrimidine, N = any nucleotide), suggesting a possible involvement of these trinucleotides in the genesis of indels. Statistical analysis showed a non-random distribution of strings of (RNY)n with longer strings significantly more frequent in variable than in constant regions. Recombination was detected in all patients. Due to recombination events, polymorphic constant and variable regions of gp120 were found to rearrange in individual clones of the quasispecies, so that each region of gp120 appeared to evolve independently.

Conclusion: Cryptic repeats of purines and pyrimidines appear to play an important role in the generation of intra-host genetic diversity of gp120. Mutations due to length polymorphism spread through the quasispecies via recombination. This study provides evidence of the dramatic molecular complexity that characterizes the intra-patient variability of gp120 in vivo.

P20.02
HIV Integrase Polymorphisms in Treatment-Naïve and Treatment-Experienced HIV-Infected Patients in Thailand Where HIV Subtype A/E Predominates

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Background: Integrase inhibitor (INI) is a novel antiretroviral drug recommended for both treatment-naïve and treatment-experienced HIV-1-infected patients. Limited data are available on INI resistance in Thailand, where HIV-1 subtype A/E predominates. We aimed to investigate INI resistance-associated mutations (RAMs) among treatment-naïve patients and patients who experienced treatment failure with nonnucleoside reverse transcriptase inhibitor (NNRTI)-based or protease inhibitor (PI)-based antiretroviral therapy (ART) in Thailand.

Methods: One hundred and eight plasma samples of 58 treatment-naive and 50 treatment-experienced HIV-1-infected individuals were collected. HIV-1 integrase coding region was sequenced. Polymorphisms were compared between subtype A/E and B circulating in Thailand and between treatment-naïve and treatment-experienced groups. Resulting amino acids were interpreted for drug resistance according to Stanford algorithms.

Results: Ninety-seven samples were HIV-1 subtype A/E; 10 were subtype B and one was subtype C. Age, gender, and CD4 cell counts were similar between treatment-naïve and treatment-experienced groups. Major INI-RAM was not found in this study, but some minor INI-RAMs, such as V54I, L68I, L74M, T97A and S230N, were found. Comparing INI-RAMs between subtype A/E and B, prevalence of V51I and V72I was higher in subtype B than subtype A/E, while V201I was found in all sequences of subtype A/E. In subtype A/E, integrase polymorphisms were not different between treatment-naïve and treatment-experienced groups. However, number of amino acid substitutions were significantly higher in treatment-experienced group (P = 0.009). One NNRTI-based ART treated patient was found to have potential low-level INI-RAMs.

Conclusion: INI-RAMs are rare in both treatment-naïve and treatment-experienced patients in Thailand. This suggested that INI should be active in patients who are naïve to INI in Thailand.
and circulating viruses. In Brazil the B subtype is predominant followed by subtype F1 and B/F1 recombinants. The aim of the present study is to compare T cell responses to Nef HIV-1 peptides based on consensus and viral isolate sequences from B and F1 subtypes and group M consensus.

Methods: Fifty clade B and 36 clade F1 Brazilian sequences were aligned with CLUSTALW, and the corresponding subtype B and F1 consensus sequences were retrieved using DAMBE v.5.0 program. Sequences from those subtypes B and F viral isolates presenting the minor genetic distance to the set of sequences were also selected. The group M consensus was obtained from Los Alamos database. Nef-specific T cell responses were evaluated with IFN-γ ELISpot assay to three overlaid regions (R). PBMCs from 18 and 9 subjects infected with HIV-1 subtypes B and F1, respectively, were analyzed.

Results: A high cross-reactivity among subtype B, subtype F1 and group M Nef consensus peptides was detected. More than 70% of subjects had a positive response against all consensus peptides to at least one region analyzed. The evaluation of frequency and magnitude of responses also demonstrated that peptides sets based on consensus and isolates were similarly powerful in detecting intra-clade T cell responses. However, the frequency of inter-clade T cell responses to isolates was smaller than the corresponding responses to consensus in R2 (subtype B and subtype F1-infected subjects) and R3 (subtype B-infected subjects).

Conclusion: Our results suggest that Nef peptide sequences based on HIV-1 clade B, F1 or group M consensus are more appropriate for the design of immunogens than peptide sequences based on single viral isolates from the Brazilian epidemic.

P20.04 Characterization of HIV-1 Subtype Distribution Among Thai MSM Using MHAbce, a High Throughput Approach for Molecular Epidemiology Studies

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Background: Understanding the local molecular epidemiology of HIV-1 subtypes may be important for the development of HIV vaccines. Here, we describe the HIV-1 subtype distribution in a cohort of MSM in Bangkok, followed from April 2006–September 2009. Typing was performed using MHAbce, an RTPCR-based assay using subtype-specific probes targeting 8 gene regions.

Methods: Blood samples from 278 seroprevalent and 99 seroincident cases were analyzed. Among seroincident cases, MHA was performed on the first available blood-specimen after seroconversion. Samples were “non-typeable” when hybridization occurred at <4 loci. In apparent cases of dual infection, cloning and targeted genomic sequencing was performed to verify MHA results. Among seroincident cases, non-typeable and apparent recombinant cases were verified by direct sequencing of MHA amplification products.

Results: In 16 apparent dual infections, sequence verification confirmed the presence of two subtypes in 5, single infection in 2, and highly diverse sequences in 9. Sequencing confirmed a recombinant genome in 8/10 seroincident recombinants. In 6 non-typeable cases, MHA and sequencing results were entirely concordant. After confirmation, the overall distribution of HIV-1 subtypes was: CRF01_AE, 81.6%; CRF01_AE/B recombinants, 11.0%; B, 4.9%; dual CRF01_AE and B, 1.5%; B/CRF01_AE recombinants, 0.6%; and B/C recombinants, 0.3%. The subtype distribution did not vary significantly by year or by serostatus (incident vs. prevalent).

Conclusion: In this cohort of HIV-1 infected Thai MSM, CRF01_AE was the most common and B was least common subtype. All remaining infections were caused by complex recombinant forms. The predominance of CRF01_AE is similar to that currently observed in HIV-1 infected Thai heterosexuals and IDU. The presence of complex recombinant forms is probably due to rapid sexual partner turnover among MSM.

While the predominance of CRF01_AE may seem to simplify the design of an HIV-1 vaccine for Thailand, the presence of multiple complex recombinant forms may pose new challenges.

P20.05 Evolution of Nigeria’s Two Predominant HIV-1 Subtypes

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Background: HIV-1 CRF02_AG and subtype G (HIV-1G) account for most HIV infections in Nigeria, but their evolutionary trends remains obscure.

Methods: CRF02_AG gag and env data sets consisting of 28 and 33 sequences respectively, dating from 1994 to 2007, and HIV-1G gag and env data sets consisting of 23 and 50 sequences respectively, dating from 1992 to 2007 was utilized. The data sets consisted of the only available dated Nigerian gag and env sequences at time of study. To determine the origin of Nigerian CRF02_AG and HIV-1G epidemics, Maximum likelihood phylogenetic analysis (MLPA) was carried out on the data sets and reference sequences from different geographic origins. Bayesian analysis was carried out on data sets with well-supported Nigerian clade(s) in the Maximum likelihood trees (MLT).

Results: MLPA suggested multiple independent entries of both subtypes into Nigeria. HIV-1G gag and env trees had one and two well-supported clades (bootstrap >70%), respectively; while CRF02_AG trees had none. The best fit models, relaxed Bayesian skyline plot (BSP) and relaxed constant population size models, estimated Nigerian HIV-1G’s most recent common ancestor to 1985 to early-1990s, and a slower post early-1990s growth). While the predominance of CRF01_AE may seem to simplify the design of an HIV-1 vaccine for Thailand, the presence of multiple complex recombinant forms may pose new challenges.
P21.01
Electrophoretic Fingerprint of HIV-1 Env Reveals Conformational Changes that Model Male to Female Transmission Events

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Background: Understanding of the biochemical nature of events that take place during HIV-1 transmission enables rational design of immunogens and understanding of the environment where vaccine responses must be efficacious. Electrophoretic mobility and binding kinetic measurements allow characterization of the surface charge and determination of affinity changes between Env and receptors. It is hypothesized that different surface chemistries can be maintained through chemical cross-linking which defines conditions for inducing more potent immune responses.

Methods: Electrophoretic mobility and affinity for the CD4 molecule can be maintained through chemical cross-linking which defines conditions for inducing more potent immune responses.

Results: Electron microscopy analyses across a range of pH. The gp140 conformations present at varying pH values were fixed with gluteraldehyde and their immunogenicity and ability to induce neutralizing antibodies was assessed in rabbits. Intramuscular prime and boosts were administered at 4 week intervals with or without gp140 stabilizing molecule DS003 and adjuvanted with LASTS. Serum was collected and analyzed for native and immunogen specific IgG and neutralization in TZM-bl assays.

Conclusion: Env trimers from two different clades demonstrated distinct profiles, whereby their mobility increases from pH 6.0 to 7.5. This coincided with decreased CD4 binding to gp140. Maximal binding occurred at pH 4.5, and the strongest affinity at pH 5.5. Immunization with protein fixed at pH 4.0 enhanced CN54 specific IgG induction, and neutralization titre increased against strain MW965.26. Other fixation conditions or the presence of DS003 did not change the neutralization profile.

P21.03
Human Immunodeficiency Virus Type 1 Variants Issued from Mother-Child Pairs Display a Wide Spectrum of Biological Properties

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Background: Mother-to-child transmission is the leading source of HIV infection in children. Several studies have demonstrated that early virus population present in infected infants usually is homogeneous when compared to viral population of mothers at delivery. It has been shown also that variants in infants are frequently resistant to neutralization by autologous maternal plasma. In this study, we analyzed biological functions of envelope genes from two mother-infant pairs.

Methods: We explored antigenic and functional properties of pseudotyped viruses expressing gp120 encoded by several env clones issued from two mother-infant pairs (0978 and 1021) infected by CRF01_AE. We compared their sensitivity to neutralization by autologous and heterologous sera and by neutralizing monoclonal antibody b12. We also analyzed cell-tropism, sensitivity to sCD4 and to the CCR5 antagonist TAK-779, and infectivity in TZM-bl cells.

Results: We obtained infectious viruses from three mother’s env clones and three infant’s env clones from pair 0978, and from one mother’s env clone and two infant’s env clones from pair 1021. All nine clones exhibited CCR5 tropism, had a high sensitivity to heterologous neutralization, were resistant to b12 and presented equivalent sensitivity to TAK-779. However, sensitivity to sCD4, and infectivity were highly heterogeneous within both pairs and cases. All clones were resistant to autologous neutralization, except a single clone issued from infant 0978 (0978-02) which presented a high sensitivity to maternal plasma. By site-directed mutagenesis we showed that a single mutation Valine/Alanine at position 68 in the C1 region governed this sensitivity to autologous neutralization and was also involved in sensitivity to sCD4 and infectivity.

Conclusion: This study showed the already heterogeneous functional properties of env genes from infected infants despite a homogeneous genetic virus population early after infection, and demonstrated the possible impact of a specific C1 residue in both

P21.02
Epidemiology of Adenovirus Type 5 Neutralizing Antibodies in Healthy People and AIDS Patients in Southern China

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Background: Recombinant adenoviruses, especially serotype 5 (Ad5), have been extensively explored as vectors for vaccine or gene therapy. However, one major obstacle to their clinical applications is the high prevalence of preexisting immunity against adenoviruses resulting from natural infection. It has been reported that there are geographic variations in the prevalence of natural adenovirus infection.

Methods: We investigated the seroprevalence of Ad5 in Guangzhou, southern China by measuring the preexisting Ad5 immunity by SEAP-based neutralizing assay.

Results: The seroprevalence was 77.34% in general healthy population. The older population (age 41–72) had the highest seropositivity (84.8%) and the highest percentage (54.4%) having high antibody titers (>1000), while the younger population (age 12 or less) had the lowest percentage (30.6%) having high antibody titers (>1000). The dynamics of Ad5 neutralizing antibodies were stable and persistent over the course of eight months. In light of concerns that high Ad5 antibodies may be associated with increased susceptibility to HIV infection, we also investigated the seroprevalence of Ad5 in the HIV-infected AIDS patients in southern China, and our data demonstrate that there was not a significant correlation between Ad5 neutralizing antibodies titers with HIV infection.

Conclusion: Our study provided useful insights for the future development of Ad5-based HIV vaccine and gene therapy.
sensitivity to autologous neutralization, sensitivity to sCD4 and viral infectivity.

P21.04
Drug Resistance Pattern of HIV-1 Among Therapy-Naives in North-Central Nigeria Suggests Regional Differences in Transmitted Drug Resistance Rates

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Background: In Nigeria, the country with the second largest number of HIV-1 infected people globally, antiretroviral therapy rollout is now widespread with an increasing number of individuals and communities benefiting. However, the drug resistance profile of patients initiating or failing on antiretroviral therapy as well as transmitted drug resistance (TDR) rates is not well characterized; especially in North-Central Nigeria (NCN). NCN is one of Nigeria’s six geopolitical zones and NCN’s HIV prevalence has remained the highest over time.

Methods: Molecular variability of the protease and reverse transcriptase region of isolates from therapy-naive pregnant women sampled in 2007 from NCN was studied by amplifying, cloning and sequencing DNA after reverse transcription of plasma RNA. Nucleotide sequences were translated to amino acid sequences and submitted to the Stanford HIV Drug Resistance Database to identify drug resistance mutations/polymorphisms.

Results: Of 28 samples amplified, 14, one, one and 11 were found to be concordantly CRF02_AG, subtypes C, F2, and G respectively, in both genes. Among various Protease mutations/polymorphisms, high prevalence of K20I/R (28/28), M36I (28/28) and V82I (all subtype G samples) was observed. Contrary to observation from other geopolitical zones, lesser frequency of reverse transcriptase mutations/polymorphisms was observed.

Conclusion: The interaction between HIV and HSV-2 appears complex, and precise longitudinal studies are required to dissect their exact temporal relationship.

P21.05
Lack of Association Between HSV-2 and HIV-1 Infection in a Cohort of HIV-1 Discordant and Concordant Couples in Dakar, Senegal

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Background: A synergy between HIV-1 and HSV-2 infections has been reported in numerous studies. However, recent clinical trials testing the efficacy of HSV-2 suppressive acyclovir therapy failed to show an effect on HIV-1 acquisition and transmission.

Methods: In this study, we reassessed the putative association between HSV-2 and HIV-1 infection in a cohort of HIV-negative, HIV-1 discordant and HIV-1 positive concordant couples in Dakar, Senegal.

Results: In agreement with previous studies, we observed a strong overall association between HSV-2 and HIV-1 serostatus (OR, 4.61; P < 0.001). However, this correlation appeared to be driven by a low HSV-2 prevalence in HIV-negative couples relative to HIV-discordant and discordant couples (23% vs 59% and 66% of couples with at least one HSV-2 positive partner, respectively; P < 0.001). HIV-discordant and discordant couples showed comparable frequencies in HSV-2 seroprevalence (59% vs 66%; P = 0.483), and differences in HSV-2 status among index (59% vs 62%, P = 1.000) and recipient partners (63% vs 41%, P = 0.131) between the two groups were absent or insufficiently large to suggest a causal relationship between HSV-2 and HIV-1.

Conclusion: The interaction between HIV and HSV-2 appears complex, and precise longitudinal studies are required to dissect their exact temporal relationship.
P21.07 Influence of the Menstrual Cycle on Levels of Innate Immune Proteins in the Genital Tract of Female Pigtail Macaques

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**Background:** Mucosal innate factors from the female reproductive tract (FRT) might be critical in modulating HIV-1 transmission. Most of the innate factors are known to be influenced by sex hormones, shown in humans but not extensively studied in pigtail macaques. Considering that pigtailed macaques, like humans, exhibit continuous lunar menstrual cycles, and are important models for HIV-1 transmission, we examined levels of various innate proteins in the context of their menstrual cycles. We hypothesized that certain immune proteins would be up-regulated in the follicular phase of the cycle compared to the luteal phase. The virus must breach physical barriers before it infects mucosal CD4+ T cells. Understanding the structural features that characterize early-transmitting gp120s may thereby aid in the design of novel vaccine immunogens.

**Methods:** Blood and cervico-vaginal lavages were collected weekly over twelve weeks from ten untreated, uninfected adult female pigtailed macaques. In addition to measuring plasma progesterone, innate immune factors [trappin-2, secretory leukocyte protease inhibitor (SLPI), thymic stromal lymphopoietin, surfactant protein-D, defensins], cytokines (IL-1β, IL-6, IL-8, TNF-α, G-CSF), and chemokines (RANTES, eotaxin, MCP-1) were quantified in vaginal fluids by ELISA or Luminex technology. For this analysis, the beginning of a menstrual cycle in each animal was defined as the time point following steepest decline of progesterone; statistical significance was calculated using Mann-Whitney nonparametric test.

**Results:** Four proteins showed significantly higher levels in the follicular phase, including TNF-α (p = 0.0079), G-CSF (p = 0.0205), MCP-1 (p = 0.0041), and Eotaxin (p = 0.0349). The levels of remaining proteins, including SLPI (p = 0.1918), did not exhibit significant differences based on the phase of the menstrual cycle. Four mucosal innate factors were up-regulated in the follicular phase which is also the phase of lowered susceptibility to infection. This indicates that pigtail macaques have similarly regulated mucosal immune factors in the FRT as observed in women during the menstrual cycle. This study highlights the suitability of female pigtailed macaques as a model for female sexual HIV-1 acquisition.

**Conclusion:** These results suggest that at an early stage following transmission there exists a requirement for the productive infection of a4b7+/CD4+ T cells. Understanding the structural features that characterize early-transmitting gp120s and the role of integrin a4b7 receptor in HIV transmission fitness, may thereby aid in the design of novel vaccine immunogens.

P21.08 The Removal of Transmission-linked Glycosylation Sites in HIV gp120 Promotes High a4b7 – Reactivity


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**Background:** Mucosal transmission of HIV is typically inefficient. The virus must breach physical barriers before it infects mucosal CD4+ T cells. Low-level viral replication occurs initially in mucosal CD4+ T cells, but within days high-level replication occurs in Peyer’s patches, mesenteric lymph nodes and the gut lamina propria. Early-replicating viruses in subtypes A and C mucosal transmission tend to encode gp120s with reduced numbers of N-linked glycosylation sites at specific positions throughout the V1-V4 domains, relative to typical chronically replicating isolates. The selective advantage gained by the absence of these N-linked glycosylation sites is unknown.

**Methods:** Using primary a4b7+/CD4+ T cells and a flow-cytometry based steady-state binding assay we show that the removal of N-linked glycosylation sites associated with early stages of infection in chronic subtype A and C gp120s results in large increases in the specific reactivity of gp120 for integrin-a4b7.

**Results:** Next we followed infection longitudinally in individual patients and found that high-affinity for integrin a4b7-reactivity, although not found in only some chronic gp120s, was observed in the early-transmitting gp120s that we analyzed. Early-transmitting gp120s were further distinguished by their dependence on avidity-effects to interact with CD4, suggesting that gp120s with high affinity for a4b7+/CD4+ T cells bear unusual structural features not present in typical chronic gp120s.

**Conclusion:** These results suggest that at an early stage following transmission there exists a requirement for the productive infection of a4b7+/CD4+ T cells. Understanding the structural features that characterize early-transmitting gp120s and the role of integrin a4b7 receptor in HIV transmission fitness, may thereby aid in the design of novel vaccine immunogens.
Late Breaker Poster Abstracts

P03.02 LB
HIV Envelop Proteins Immunization Effect in Hu-SCID Mice Model

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Background: Immunizations and challenging of the immune deficient SCID mice capable for engraftment with human immunocompetent cells (Hu-SCID-mouse) as an animal model for the evaluation of the effectiveness of an HIV vaccine are described

Methods: Envelop proteins gp120-gp160 were isolated from 1.5–2 litres HIV-1 subtype A U455 and PokA-79 strains MT-4- or PBMC-6-weeks in vitro cultivation (“playbacks”) culture and identified with MS-MS. Immunocompetent cells were tailored and isolated in/from L. tarentolae expression system. Hu-SCID mice engrafted with single donor PBMC (peripheral blood mononuclear cells) and with DC (dendrite cells) immunocompetent cells were immunized sub-cutaneously with recombinant gp120-gp160 cocktails and challenged intraperitoneally with U455 and PokA-79 strains and their “playbacks”. HIV laboratory strains and PBMC-DC- “playbacks” challenging viral load as well as vaccination efficiency were measured in blood serum by Re-AllTime PCR. HIV-specific immune response in blood serum was evaluated by p120-p160

Results: U455 and PokA-79 HIV laboratory strains multiplied successfully in Hu-SCID mice engrafted with lymphocyte MT-4 or monocyte U937 cell culture backgrounds with titres 104–108 copies/ml but were unable to provide bloodstream viral load in immune-competent Hu-SCID mice engrafted with PBMC or DC background. In vitro controls showed HIV RNA 1010–1013 c/ml for U937-MT-4-cultivated HIV laboratory strains but negative dynamics with RNA 104–105 c/ml for DC- or first passages PBMC-cultivated strains. TCID50 data for in vitro controls followed the same ratio. However after 5–6 weeks of PBMC-background in vitro cultivation “playbacks” RNA c/ml titre and TCID50 data rise, show positive multiplication dynamics and gp120 sequence variability widening. “Playback” strains demonstrated positive bloodstream titres in Hu-SCID-PBMC mice. Recombinant gp120-gp160 cocktails tailored on base of “playbacks” envelope mapping used for Hu-SCID-PBMC immunizations blocked HIV challenging in these.

Conclusion: In vitro controls suggest HIV gp120-gp160 infection-responsible variability is determined by background human cell’s receptors diversity crucial for virus recognition, invasion and challenging.

P03.03 LB
Neutralization of Plasma vs. Antibodies: The HJ16, HGN194 and HK20 Comparisons

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Background: Several new human antibodies with a neutralizing potential across different subtypes have recently been described. Three mAbs, HJ16, HGN194 and HK20, were obtained from cells from three Sub-Saharan African women within the HIV-1 cohort of the Institute of Tropical Medicine.

Methods: Polyclonal plasma from these HIV-1 infected patients was compared to mAbs obtained from their memory B-cells. PBMC based neutralization assays with varying incubation (1h vs 24h), adsorption (1–2 h vs 24 h) and culture phases (7 or 14 days) were performed. We compared these PBMC assays with cell line based assays using TZMbl and GHOST cells. The role of primary replicating virus versus non-replicating pseudovirus was considered using a panel of tier 1 and 2 strains.

Results: The results clearly demonstrate that patient selection was highly dependent on the neutralization assay. Although the cross-neutralizing properties of the isolated Abs showed considerable variation with the neutralization assay format, all assays indicate that neutralizing Abs to other epitopes of the HIV-1 envelope than those targeted by the obtained mAbs are present in the plasma.

Conclusion: The present study indicates that different neutralization assays yield different results and it is still unclear which one is most predictive of in vivo neutralizing activity. Moreover, the strong profiles in the patients’ plasma were not solely due to antibodies represented by the newly isolated mAbs. This better understanding of in vitro neutralization characterizations of patient plasma and Abs will hopefully lead to more effective ways of discovering new Abs that ultimately can be used for HIV-1 immunogen design and subsequent vaccine development.

P04.36 LB
Structures of HIV-1 Quaternary-Structure-Preferring Antibodies, CH04 And PG9, Show Conserved Structural Elements Within A Generally Flexible CDR H3

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Background: Broadly neutralizing quaternary-structure-preferring antibodies, such as PG9, PG16, and more recently CH01-CH04, have been isolated from HIV-infected patients. These antibodies bind to a glycosylated epitope comprised of the second and third variable loops. They are highly affinity-matured and have long heavy chain 3rd complementarity-determining regions (CDR H3) of 24 to 28 amino acids in length. Although they neutralize a broad range of viruses, they can only bind monomeric gp120s from select strains, indicating that they preferentially recognize the oligomeric conformation of the HIV-1 spike.

Methods: To investigate paratope elements of recognition for the quaternary-structure-preferring antibodies, we obtained crystals of CH04, CH04H-light (CH04 heavy chain but a different light chain), and PG9 antigen-binding fragments (Fab) that diffracted X-rays to 1.9 Å, 1.8 Å and chain), and PG9 antigen-binding fragments (Fab) that diffracted X-rays to 1.9 Å, 1.8 Å and ~3 Å, respectively.

Results: Here we show that the CDR H3 of CH04, CH04-light and PG9 is highly flexible and can adopt different conformations depending on the crystal lattice. However, in each structure where the CDR H3 is ordered, a two-stranded antiparallel β-sheet structural element is conserved towards the tip of the CDR H3. This structural element was also observed in the PG16 Fab structure.

Conclusion: The unusually long CDR H3 of the quaternary-structure-preferring antibodies appears to be highly flexible and might adopt different conformations upon binding to the quaternary-structure-preferring epitope. Nevertheless, a conserved β-sheet structural element appears to be required to bind to the epitope.

P04.38 LB
Identification of Minimal Antigenic Domains In HIV-1 Envelope Glycoprotein Recognized by Broadly Neutralizing Monoclonal Antibodies VRC01 And PG9

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Background: VRC01 and PG9, identified by far as the two most potent human broadly neutralizing monoclonal antibodies (bnmAb) against HIV-1, are able to neutralize large assay of primary viruses of diverse genetic and geographic origins. Identification and characterization of the antigenic domains recognized by the two bnmAbs and their germline precursors may therefore provide valuable insights into better understanding of the mechanism of neutralization and the rational design of vaccines.

Methods: Here, we report the development of an efficient mapping technique for minimal antigenic domains based on the combinatorial antigen library of HIV-1 envelope glycoprotein displayed on the surface of the yeast Saccharomyces cerevisiae. In this technique, positive yeast clones recognized by the two bnmAbs were identified and obtained by fluorescence-activated cell sorting (FACS) followed by sequencing and structural analysis.

Results: Using this technique, we have identified several protein fragments reactive to VRC01 and PG9 that are variable in length and overlapping in nature. Although each protein fragment-antibody complex demonstrates a distinct profile of fluorescence intensity, those recognized by VRC01 invariably contain the V3, V4 and V5 regions while those of PG9 contain the V1 and V2 regions of gp120. These protein fragments very likely resemble the minimal antigenic domains recognized, as they contain neither the V1 and V2 regions shown to interact with VRC01 nor the V3 region with PG9 previously reported in structural and mutagenesis studies. Our results suggest that the major energetic binding residues for VRC01 or PG9 must be confined within the selected fragments, although detailed mapping of these residues requires further investigation.

Conclusion: We believe the novel mapping technique and minimal antigenic domains identified here are unprecedented. Application of this technique to study other bnmAb and their germline precursors will improve our understanding of protective immunity and guide rational design of vaccines.
Isolation of CD4-Binding Site and V2/V3 Conformational (Quaternary) Broadly Neutralizing Antibodies from the Same HIV-1 Infected African Subject


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Background: Recent studies have demonstrated the production of broadly neutralizing antibodies (bNAbs) in ~20% of chronically infected subjects and remarkable bNAb magnitude and breadth in the top 1–3% (level 3 neutralizers). Epitope mapping of plasma antibodies in some level 3 neutralizers has identified more than one neutralizing antibody specificity. A fundamental question is whether one or multiple specificities are responsible for the breadth of neutralization in broadly neutralizing plasma.

Methods: We used near clonal memory B-cell cultures, EBV transformation and antigen-specific memory B-cell sorting to isolate two clonal lineages of bNAbs from a single level 3 neutralizer (CH0219) infected with a subtype A virus.

Results: Plasma from CH0219 neutralized 93% of clade A, B, C, AG, AE, G and D HIV-1 strains tested. Four VH3-20/Vk3-20 mAbs that were in the same clonal lineage (CH01-CH04) were isolated from CH0219 memory B-cells and neutralized 45% of HIV-1 strains. CH01-CH04 mAbs were neutralization sensitive to N160, F159, K169, K171 and 181 gp120 mutations, and cross-blocked mAb PG9. Thus, CH01-04 mAbs were identified as V2/V3 conformational antibodies that bound to an epitope similar to that of mAb PG9. Another clonal lineage (VH1-2/Vk1-33) of five antibodies (VRC-CH30-34) was isolated from CH0219 by resurfaced core 3 antigen-specific memory B-cell sorting. These antibodies were found to target the CD4-binding site and to neutralize 84% of HIV-1 strains. Two additional neutralizing mAbs were isolated from CH0219 against the V3 and gp41 regions that did not have significant breadth. Mixture of the CH01 and VRC-CH31 mAbs neutralized 93% of HIV-1 strains, including all those neutralized by the plasma sample.

Conclusion: The CH0219 African donor made both V2/V3 conformational and CD4-binding site bNAbs and the combination of these two specificities recapitulated the plasma neutralization breadth. This is the first documented case of two bNAbs targeting distinct HIV-1 envelope epitopes isolated from a single individual.

Crystal Structures of Human Anti-V2 mAbs 697-30D and 8.9D and What We Can Learn From Their Antigen-Binding Sites


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Background: VRC01-like antibodies achieve neutralization of over 90% of circulating HIV-1 strains by partial mimicry of CD4 in their heavy chain. While this mode allows for precise recognition by heavy chain of the initial site of CD4 attachment, the light chain cannot be fully accommodated and its recognition extends outside of the initial site of CD4 attachment into the variable regions – including loop D and V5 – that surround this site.

Methods: How do VRC01-like antibodies accommodate variable regions on HIV-1 gp120 to achieve broad recognition and neutralization? To answer this question, we crystallized and studied the structural details of protein complexes between HIV-1 gp120 with diverse loop D and V5 regions and VRC01-like antibodies with different light chains. In particular, we analyzed VRC01-like antibodies with light chains from the kappa 3 family (VRC01 and VRC-PG04) as well as a VRC01-like antibody with a light chain from the kappa 1 family (VRC-CH31).

Results: We found that the third complementarity determining regions (CDR) L3 of the light chains from both kappa 1 and kappa 3 families had very similar conformations, with a conserved glutamate and hydrophobic patch interacting with conserved regions of loop D and V5. To accommodate variable residues and glycosylation on these loops, the CDR L1 of kappa 3 origin used a loop shortened by a two-amino-acids deletion to avoid steric clashes. In contrast, CDR L1 of kappa 1 origin had no deletion in sequence, but adopted a conformation which circumvented potential clashes with loop D of gp120.

Conclusion: In addition to using light chains of different family, VRC01-like antibodies utilize a conserved CDR L3 interaction as an anchor. The entire light chain pivots around the CDR L3 interaction, and does not recognize the loop D or V5 regions as much as avoid clashes, either through intrinsic flexibility or by affinity-maturation-evolved deletion.

Structural Basis for VRC01-Like-Antibody Recognition of Variable Loops that Surround the Site of CD4 Attachment on HIV-1 Gp120


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Background: The immunogenic V2 region of HIV-1 gp120 has been largely overlooked as a target for AIDS vaccine discovery. However, recent studies of sera from vaccinees in RV144 trial suggested that anti-V2 Abs were elicited and possibly contributed
to protection. Structural understanding of human anti-V2 mAbs and their epitopes can facilitate design of immunogens.

**Methods:** We determined crystal structures of Fab fragments of two human anti-V2 mAbs 697-30D and 8.9D, both at a resolution of 2.5 Å, and analyzed their antigen-binding sites (ABS) and possible modes of interactions with V1V2 of gp120.

**Results:** MAb 697-30D, from a subtype B virus infected subject and encoded by VH1-69 gene, is a broadly cross-reactive anti-V2 mAb able to neutralize Tier 1 pseudoviruses; its epitope was mapped to conserved residues in V2. Structural analysis of Fab 697-30D revealed that its ABS consists of two distinct regions: (1) A surface pocket is located at the center of CDR loops formed by large aromatic residues, and it can accommodate residues with large side chains in the epitope identified by functional studies. (2) A convex hydrophobic surface is comprised of a cluster of CDR H2/H3 residues. Comparison with structures of other VH1-69 mAbs suggests that mAb 697-30D likely binds to the region of a short helix or a relatively flat surface of V2. Autologous neutralizing mAb 8.9D was isolated from a subtype C infected subject, and its epitope was mapped to the stem of V1V2. Its ABS is split by the upward positioned Tyr100b of CDR H3 into a positively-charged side and a negatively-charged side. Surface pockets in these regions can bind side chains of charged residues of V1V2, facilitating escape by mutations.

**Conclusion:** Crystal structures of human anti-V2 mAbs provide structure-function insights of their epitopes. This information may contribute to rational design of immunogens targeting V2 region of gp120.

**P04.42 LB**

**454 Pyrosequencing of B Cells From HIV-1 Infected Donors With Quaternary-Structure-Preferences Antibodies**

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**Background:** Broadly neutralizing quaternary-structure-prefering antibodies such as PG9, PG16, and CH01-04 are found in selected HIV-1 infected donors. Some characteristics for these antibodies have been determined: they rarely bind monomeric gp120, they do not neutralize viruses grown in presence of kitutensine or with an N160K mutation. Sequences and structures of PG16 and other quaternary-structure-prefering antibodies indicated some similarities among all members of this class including long 3rd heavy chain complementary determining regions (CDR H3s) of over 24 residues, substantial affinity maturation (over 13% divergence in nucleotide sequence from germline), and VH3 gene family origin. Some members of the class, moreover, are tyrosine sulfated in their CDR H3 regions.

**Methods:** Five million PBMCs (~500,000 B Cells) of HIV-1 infected donors that display serum reactivities consistent with the “quaternary-structure-prefering” phenotype were used to obtain cDNAs, which were PCR amplified with VH family primers and antibodies sequences determined by 454 pyrosequencing.

**Results:** 200,000 to 1 million reads were analyzed based on sequences and structures signature for the quaternary-structure-prefering antibodies. Heavy chain sequences that had CDR H3s of 24 or more residues, displayed 13% or higher divergence from germline, and were predicted to be sulfated tyrosine were synthesized, paired with a light chain from a known quaternary-structure-prefering antibody, and the resulting reconstituted IgG tested for neutralization. Unfortunately, heavy/light chain chimeras between different quaternary-structure-prefering antibodies – especially between antibodies from different donors – did not display functional complementation, and we are currently overcoming this method by analyzing donors from which heavy/light antibody pairs have been previous identified.

**Conclusion:** 454 pyrosequencing is a powerful tool that can be used to understand B cell development based on explicit lineages involving thousands of antibody sequences. While difficulties with functional complementation complicate the identification of novel quaternary-structure-prefering antibodies, the technology should allow maturation lineages of known antibody pairs to be deciphered.

**P06.13 LB**

**Screening and Enrolment into a Phase I HIV Vaccine Trial In Kigali-Rwanda**

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**Background:** Pre-screening of potential phase I HIV vaccine volunteers reduces potential screen failures and work load during the screening phase. In a Phase I HIV Vaccine Trial, currently conducted at Projet San Francisco (PSF) in Kigali, Rwanda, we anticipated that by approaching low risk couples for enrollment into HIV vaccine trial and setting pre-screening criteria, we would remove potential barriers to volunteer enrollment and other common causes of enrolment failure. We present the common reasons for volunteer ineligibility despite doing pre-screening.

**Methods:** Between February-April 2011, 206 HIV concordant negative couples were enrolled in the Heterosexual (HT) study and 52 (104) couples were pre-screened out due to not using long term family planning methods (IUD or Implant), or having high HIV risk behaviors, abnormal hematolgy, chemistry and/or urinalysis, not showing enough willingness to participate, lactation, or had a clinically significant abnormal medical assessment. The remaining 154 couples where at least one partner was potentially eligible for screening underwent; informed consent procedures, medical assessment and screened for hepatitis B and C. Eligible volunteers were invited for enrolment. In an eligible couple, only one partner would be enrolled.

**Results:** Out of 154 couples, we screened 137 volunteers. Among these, 61 volunteers were eligible and 76 were ineligible. Of the 76, we had 23 (30%) Hepatitis (16 due to Hepatitis C, 5 due to Hepatitis B and 2 volunteers having co-infection of B and C), 11 (15%) Hepatomegaly/splenomegally, 17 (22%) other abnormal clinical condition 10 (13%) HIV risky behavior, other 15 reasons were; failure of assessment of understanding of informed consent, lactation, declined enrolment and abnormal urinalysis.

**Conclusion:** Our study showed relatively high prevalence of Hepatitis. Among the pre-screening parameters, very few showed up at screening. Therefore if prescreening is well planned and done, it can reduce the number of screen failures.
**P06.14 LB**

**Title:** Screening of Concordant Negative Couples during the Pre-screening Period for a Phase-One HIV Vaccine Trial in Lusaka, Zambia

**Methods:** ZEHRP partners with 15 government clinics where couples are invited to participate in CVCT. Cohabiting concordant negative couples using a long-term contraceptive method are invited to enroll in the HT study. Prior to formal screening for participation in the vaccine study, the researchers performed chart reviews for these individuals to assess study-specific inclusion/exclusion criteria and a positive attendance record.

**Results:** Of the 206 individuals pre-screened for inclusion in an HIV vaccine trial, 130 were ineligible (64 male; 66 female). The most common reasons for ineligibility were poor attendance record (55) during the HT study, high-risk behavior for HIV infection (36), unwilling/unavailable for duration of study (16), above age limit (16), absence of IUD/implant (9), and syphilis detected (8). Specific reasons for high-risk behavior for HIV infection were multiple sexual partners in the past year (14), partner having multiple sexual partners in the past year (14), frequent, excessive alcohol use (9), trichomonas positive (5), and partner trichomonas positive (5). One participant became infected with HIV during the pre-screening period.

**Conclusion:** Over one quarter (26.7%) of the individuals pre-screened demonstrated poor attendance during the prescreening period. Because poor attendance during clinical trials may impair data quality and distort conclusions, researchers should employ the pre-screening model whenever possible. Additionally, researchers should prioritize enrolling cohabiting, concordant negative couples. The low rate of seroconversion among our cohort, compared to previous trials that did not account for partner status, might be attributable to this strategy.

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**P06.15 LB**

**Title:** High Levels of Willingness to Participate in an HIV Vaccine Trial in Kigali Rwanda

**Background:** The concept of participating in clinical trials is still not well understood by African populations with low rates of literacy. In preparation for a phase I HIV vaccine trial, we held information seminars and then conducted an assessment of willingness to participate in the study in a cohort of HIV concordant negative couples in Kigali, Rwanda.

**Methods:** In February-March 2011, HIV negative couples who were enrolled in the Heterosexual Transmission Study were invited for information sessions on HIV vaccine trials. Information sessions included viewing the informed consent video, a group discussion and questions and answers session. After the session, couples were asked verbally if they were willing to participate in the trial, and those interested were given appointment to come back for consenting and screening procedures.

**Results:** A total of 206 HIV concordant negative couples were enrolled. Overall, the literacy level as assessed by the ability to read the local language was 67%. Most prospective volunteers spoke Kinyarwanda and only a very small proportion of study participants could understand French or English (6% of men vs 2% of women). Ninety per cent of the invited couples (186/206) attended an HIV vaccine trial seminar, and the willingness to participate in the study was very high: 179 men (96%) and 174 women (84%) stated that they were interested in participating into the study. At the time of screening, only two couples declared they were no longer interested in this study.

**Conclusion:** This study shows that, despite a low literacy level, when the information about HIV vaccines is provided, willingness to participate in an HIV vaccine trial is very high. This willingness, coupled with the strong reputation of our research center in the community, might contribute to high enrollment rates in HIV vaccine trials.
couples, both partners were ineligible. A total of 45 volunteers (23 men and 22 women) were enrolled in the trial.

**Conclusion:** This study shows that targeting concordant HIV negative couples is an effective strategy to recruit HIV low risk volunteers in phase I vaccine trials. The screening success rate was very high (58%), with nearly a 1:1 ratio of enrolled men and women. Concordant HIV negative couples are at low risk for HIV and should be considered for future safety and immunogenicity trials in high prevalence areas.

**P07.03 LB**

**IRF-1 Polymorphisms and Their Role in Resistance to HIV Infection in Highly Exposed Uninfected Kenyan Female Sex Workers**

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**Background:** Interferon Regulatory factor –1 (IRF-1) is a transcriptional activator of interferon genes and interferon inducible genes and plays a crucial role in host antiviral immunity and HIV replication. DNA sequence variations can cause phenotypic changes by multiple mechanisms, including mRNA splicing and turnover. Previous work has shown association of three polymorphisms in IRF-1 with decreased susceptibility to HIV-1 infection and a reduced likelihood of seroconversion. Peripheral blood mononuclear cells (PBMCs) from patients with protective IRF-1 genotypes exhibited significantly lower basal IRF-1 expression and reduced responsiveness to IFN-γ stimulation. This study will further characterize the effect of identified polymorphisms on IRF-1 expression and its effect on HIV-1 infection.

**Methods:** Alternative splicing and the functional impact of IRF-1 polymorphisms on expression of IRF-1 regulated genes was analysed using the Affymetrix Human Exon 1.0 ST microarray. Quantitative RT-PCR was used to investigate IRF-1 mRNA levels, as well as to confirm the microarray results. IRF-1 protein stability was analysed using Western Blot analysis. Exon splicing and transcript stability assay were performed in order to characterize the link between identified polymorphisms and altered IRF-1 protein levels.

**Results:** Data from this work shows an association of protective IRF-1 polymorphisms with increased expression of exon 7/8 and decreased IRF-1 protein stability. Resulting decrease in IRF-1 protein levels can prevent over-activation of the immune response and hinder HIV-1 replication. Further functional analysis of IRF-1 polymorphisms and HIV resistance is ongoing.

**Conclusion:** Individuals with protective IRF-1 genotypes are able to regulate the nature and strength of the immune response to HIV through altered IRF-1 protein stability. It is important to fully characterize the effect of IRF-1 polymorphisms as this will further the understanding of natural resistance to HIV infections and can contribute to the development of novel prophylactic or therapeutic modalities.

**P07.04 LB**

**Impact of HLA-B*35 Subtype Differences on HIV Outcome In Mexico**


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**Background:** Previous studies have consistently associated HLA-B*35 with rapid disease progression in the context of B clade HIV infection. HLA-B*35 subtypes of the PX group (such as HLA-B*3502/03/B*53) have particularly been associated with worse outcome. The mechanisms underlying these observations are not clear. This study focuses on the Mexican HIV epidemic where HLA-B*35 is expressed in approximately one-third of Mexicans.

**Methods:** The study cohort comprised 679 Mexican subjects with chronic HIV B clade infection. All subjects were HLA typed by sequence-based typing (Abbot). Viral load (VL) and CD4 + T cell counts were determined by real-time PCR (Abbot) and multiparametric flow cytometry (BD), respectively. Gag and pol genes were amplified from plasma virus as previously described to determine clade of infection.

**Results:** Consistent with previous studies of B clade infected Caucasian, HLA-B*57 and HLA-B*27 were the most protective alleles. In contrast with earlier work, no significant impact on VL setpoint or absolute CD4 + T cell count was observed between the HLA-B*35 PY versus PX groups (p = 0.9776 and p = 0.2141 respectively). Diverse ranking of HLA-B*35 alleles according to median viral setpoints was observed. HLA-B*3508 was 2nd of 45 HLA-B alleles, while B*3514 was 45th. HLA-B*3508 VL setpoint was significantly lower than HLA-B*3508 negatives (p = 0.0211) when the cohort was analyzed excluding previously determined protective alleles B*57 and B*27. HLA-B*3501, a PY allele not considered a risk allele, was significantly associated with higher VL and lower CD4 + T cell counts (p = 0.0254 and p = 0.0037 respectively).

**Conclusion:** These data suggest that certain HLA-B*35 alleles can be protective against HIV disease progression. We observe substantial differences in markers of HIV disease outcome associated with small differences between HLA-B*35 alleles. Defining the mechanisms underlying these differences will facilitate understanding of the mechanisms of immune control or lack of control of HIV, which is of relevance to HIV T-cell vaccine design.

**P07.05 LB**

**Specific Structural Interactions Between HLA-B and Epitopic Peptide Differentiate Alleles Associated With HIV Control and Progression**

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**Background:** HIV controllers maintain low viral loads in the absence of therapy. Recent analysis indicates that specific residues in the peptide binding groove of HLA-B, particularly 67, 70 and 97, predict disease outcome.

**Methods:** Modeller v9.9 software was used to perform homology modeling based on published crystal structures of the HLA-B/peptide complex, focusing initially on HLA B*5701/B*5801 (associated with control) and B*5802 (associated with progression), which differ at positions 94, 95 and 97. Ab initio peptide refinement was used to determine the mode of MHC docking. A mutant peptide library was constructed based on modeling results. We assessed MHC contact residues influencing HIV control and their binding to epitopes.
Results: Homology modeling revealed HIV control to be characterized by hydrogen bonds between the epitope and HLA-B*5701 and B*5801 position 97, and by conserved contact residues in the HIV epitope that are more commonly presented in HIV controllers. Furthermore, side chains of the epitope point towards position 97 in controllers, enhancing hydrogen bonding. This pattern is markedly different in the context of the B*5802 allele associated with HIV progression, where position 97 is unable to make the strong contacts observed in HIV controllers. Instead, epitope binding is characterized by non-polar van der Waals contacts between HLA-B and hydrophobic side chains.

Conclusion: Our data show that strong hydrogen bonding between HLA position 97 and epitope peptide is characteristic of HLA-B*57 and B*5801 alleles associated with HIV control, whereas weaker, van der Waals forces define B*5802, associated with HIV progression. The presence of significant patterns in MHC binding directly responsible for the strength of the epitope/HLA-B interaction indicates that a small number of conserved residues are critical in HIV control. Expansion of these studies to other alleles and epitopes is underway to further define HLA-peptide parameters associated with effective T cell mediated immune control.

P10.07 LB
Characterization of the Immune Response in the Gut Mucosa and Peripheral Blood in Acute HIV-Infected Thai Individuals


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Background: Mucosal surfaces are the major entry portal for HIV. The majority of CD4+ T cells are lost during acute HIV-infection, with mucosal compartments being most severely affected. Understanding the mucosal immune response in early HIV-infection is crucial for the development of an effective vaccine or treatment.

Methods: Thirty Thais with acute HIV-infection were enrolled and subsequently received antiretroviral therapy (ART). Prior to (baseline) and 6 months following ART initiation, mucosal mononuclear cells (MMC) isolated from sigmoid biopsies and peripheral blood mononuclear cells (PBMC) were analyzed for their immunological phenotype and HIV-specific immune response using multi-parameter flow cytometry.

Results: At baseline, the frequency of CD4+ T cells in MMC and PBMC was comparable to healthy individuals with a median of 48% and 54% respectively, with CD4+ central memory T cells in MMC (56%) and naïve CD4+ T cells in PBMC (53%) being the dominant population. There were no changes observed under ART. In contrast, the frequency of CD4+ CCR5+ T cells in the MMC at baseline was lower (median 38%) compared to published data for healthy individuals (60–80%) with a trend to increase under ART. At baseline, the frequency of activated CD8+ T cells (HLA-DR+ /CD38+) in MMC and PBMC was significantly increased at Fiebig III compared to Fiebig I (p = 0.01 and p = 0.03, respectively) while a significant reduction in the activation status was observed following 6 months of ART (MMC: p = 0.01; PBMC: p = 0.003). The HIV-specific immune response in PBMC was mainly CD8-mediated with 5/11 (45%) individuals responding at baseline against Gag, Env and/or Pol compared to 1/11 (10%) responding at baseline in the CD4 compartment.

Conclusion: In acute HIV-infection immunological changes in the mucosa include depletion of CD4+ CCR5+ T cells and activation of CD8 T cells occurring as early as Fiebig I to III with evidence for trend reversal with ART.

P10.08 LB
A Phase1 Clinical Trial to Evaluate the Safety, Mucosal and Innate Immunity of Adenovirus Type 26 HIV-1 Vaccine in Healthy, HIV-1 Uninfected Adults


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Background: HIV is largely acquired via mucosal contact. Characterizing mucosal immune responses is a priority. In addition, the STEP study has raised important questions regarding potential interactions of preexisting immunity to a recombinant Ad5 vectored HIV vaccine on risk of acquisition to HIV infection.

Methods: This is a placebo-controlled RCT to evaluate the safety and innate and mucosal immune responses of a single intramuscular dose of an HIV-1 recombinant Clade A env (Ad26.ENVA.01) vaccine at a dose of 5x 1010 VP in Ad26 seronegative (n = 16) and seropositive (n = 8) subjects. Blood samples for standard and innate immune assessments were collected on days 1, 3, 7, 14 and 28. Mucosal samples including rectal wicks and biopsies were collected at baseline and days 14 and 168. Blood and colorectal biopsy specimens were processed fresh within 2 hours of collection for multiparameter flow cytometry.

Results: 21/24 (88%) subjects have been enrolled (14/16 in Ad26 seronegative and 7/8 in Ad26 seropositive groups). 52% are <30 years old, 52% female, and 39% non-Caucasian and 12% Hispanic/Latino. No vaccine related SAEs have occurred, and no significant reactogenicity has been observed. Preliminary data from the first 12 Ad26 seronegative subjects showed EnvA-specific CD8+ T lymphocyte responses in PBMC and colorectal mucosa following vaccination. No increase in Ki67+ cellular activation, or in CCR5+ expression in total or vector specific CD4+ T lymphocytes were seen in PBMC and colorectal mucosa samples obtained before and after vaccination.

Conclusion: Ad26-EnvA vaccination is generally well tolerated and elicits both peripheral and mucosal EnvA-specific cellular immune responses. In Ad26 seronegative subjects, there is no evidence to date of increased total or vector-specific Ki67+ or CCR5+ CD4+ T lymphocytes in colorectal mucosa following Ad26-EnvA vaccination. Detailed characterization of mucosal antigen- and vector-specific immune responses in both Ad26 seronegative and seropositive subjects is ongoing.

P10.09 LB
Intramuscular Priming with Replicating Vaccinia Vector and Intranasal Boost With HIV Gp140 Trimer Elicits Strong Mucosal And Systematic Immune Response

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Background: Mucosal immunity at the portal of entry plays a crucial role in protection against HIV infection. However, there is no ideal approach to induce robust mucosal immune responses against HIV-1. Here we described a prime /boost immunization strategy which can induce effective mucosal and systemic immune response.

Methods: HIV-1 CN54 structural genes carrying replicating Tiantan vaccinia (rTV) and Listeria (Lm) and mammalian cell expressed gp140 trimers were used to immunize BALB/c mice systematically (im, sc and ip) or mucosally(in, ig). Three main prime boost strategies were investigated: rTV + gp140 trimmer; rTV + Lm + gp140 trimmer and rTV + Lm / gp140 trimmer. Oil in water, Cholera toxin B (CTB) and Flagellin were used as test adjuvant. Blood, spleen, saliva and vaginal lavage were collected and env-specific IgG and IgA antibody (ELISA) as well as T cell response (ELispot) were determined.

Results: Oil in water adjuvant is shown to induce better both serum and mucosal IgA than CTB or Flagellin. rTV is a better primer and gp140 or Lm/gp140 is a better boosting agents. rTV systemic, not mucosal, prime and gp140 or Lm/gp140 mucosal, not systematic boost, induce the highest serum and mucosal IgA and systematic responses than other immunization strategies. The mice immunized with rTV (im) + gp140 (in) induced very high and balanced HIV-1 env specific antibody titer both in serum (IgG > 150,000, IgA > 15,000) and at mucosal level (saliva IgA > 600, vaginal IgA > 500). rTV (im) + gp140 /Lm (in) immunization induced a slightly higher IgG in serum and detectable T cellular response but lower IgA antibodies in serum and at mucosal level.

Conclusion: Replicating viral vector systematic priming and mucosal protein boosts can induce strong and balanced mucosal and systematic antibody response to HIV-1. This immunization strategy is valuable in AIDS vaccine development.

Results: In non-adjuvanted naive animals serum anti-Gag p24 titers were detectable 2 weeks after priming, but only in the anti-DCIR-Gag p24 and anti-Langerin-Gag p24 groups. Antibody titers increased substantially after both the 1st (3.6 ± 0.6 and 3.8 ± 0.5 log titers for anti-DCIR-Gag and anti-Langerin-Gag groups, respectively) and 2nd boosts in the DC-targeting groups and were long-lasting, while low-minimal responses were detected in the control groups. Poly I:C increased the kinetics of the responses in the anti-DCIR-Gag p24 (4.7 ± 0.1) and human IgG4-Gag p24 groups (4.6 ± 0.1) evoked low responses in the Gag p24 group (3.2 ± 0.2), and had minimal impact on responses in anti-Langerin-Gag p24 group (4.0 ± 0.5). HIV Gag and H1N1 HA specific T cell responses from anti-DC receptor targeting were significantly improved in animals previously primed with Gag nanoparticles and PR8 H1N1 infection. High recall HI serum responses were observed with anti-DC-HA

Conclusion: This study provides the context for development of vaccines based on HIV Env fused to DC-targeting antibodies.

P14.12 LB
Safety and Immunogenicity of DNA and MVA HIV-1 Subtype C Vaccine Prime-Boost Regimens: A Phase I Trial in India


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Background: With over 2.5 million HIV-infected people and evidence of ongoing transmission, the search for a preventive HIV vaccine for Indian people gains importance. We have earlier reported moderate immunogenicity of MVA vaccine. Evidence elsewhere indicates superior immunogenicity of heterologous prime-boost strategy. We assessed the safety and immunogenicity of HIV-1 subtype C based DNA and MVA prime-boost regimens in Indian adults.

Methods: Sixteen healthy HIV-uninfected volunteers (12 vaccine, 4 placebo) were randomly assigned to vaccine or placebo in group A (DNA at 0, 1 and MVA at 3, 6 months) or group B (MVA at 0, 1, 4 placebo) were randomly assigned to vaccine or placebo in group A (DNA at 0, 1 and MVA at 3, 6 months) or group B (MVA at 0, 1, 6 months). All vaccines were administered intramuscularly in the deltoid muscle. Reactogenicity was assessed at 3, 7 and 14 days post-vaccination; adverse events up to 9 months and serious adverse events through out the trial. T-cell and antibody responses were assessed pre and 1 and 2 weeks post each vaccination, then at 9, 12 and 18 months.

Results: Local and systemic reactogenicity profiles were comparable between groups, and were mostly mild and transient. No serious adverse events were reported. In group A vaccine recipients IFN-gamma ELISPOT response was detected in 0%, 25%, 100% and 100% participants post 1st, 2nd, 3rd and 4th vaccinations and in 60%, 63.6% and 91.7% group B participants post 1st, 2nd and 3rd vaccinations, respectively. Responses were directed to multiple HIV proteins (magnitudes of 108 – 250 SFU per 10^6 PBMC) in most volunteers. HIV-specific antibodies were detected in 10/12 and 11/12 vaccine recipients in groups A and B, respectively, and neutralizing antibodies to tier-1
viruses were detected against HIV SF162 and MW-965 in most individuals.

Conclusion: Both vaccination regimens were found to be safe and well-tolerated. Heterologous DNA-MVA prime boost strategy elicited comparable T-cell immune responses to the homologous MVA strategy.

**P14.13 LB**

**Evaluation of Peripheral Blood and Mucosal HIV-1 Viral Load in Volunteers Who Became HIV-1 Infected During Participation in the Thai Phase III Trial**


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Background: The Thai Phase III trial of ALVAC-HIV® and AIDSVAX B/E® was the first study to show efficacy for prevention of HIV infection in a community-based cohort. During the trial, 132 participants became HIV-infected and 120 of these volunteers agreed to participate in RV152 to evaluate the effect of this prime-boost regimen on clinical disease progression, immunologic and virologic outcomes.

This study compared HIV-1 viral load in peripheral blood and mucosal compartments between vaccine and placebo recipients.

Methods: HIV-1 viral load was measured on entry into RV152 to evaluate the effect of this prime-boost regimen on clinical disease progression, immunologic and virologic outcomes.

This study compared HIV-1 viral load in peripheral blood and mucosal compartments between vaccine and placebo recipients.

Methods: HIV-1 viral load was measured on entry into RV152 from 42 vaccine and 58 placebo recipients’ paired peripheral blood (N = 100) and mucosal samples [seminal plasma (SP); N = 64 or cervicovaginal lavage (CVL); N = 36] using the Amplicor 1.5 HIV viral load RNA assay (range: 1.70–5.88 log_{10} copies/ml).

Results: Viral load was detected in blood (96%), SP (59%) and CVL (36%) of infected subjects. There was no difference in blood viral load between the vaccine (mean: 4.05 log_{10} copies/ml) and placebo (mean: 4.19 log_{10} copies/ml) groups (p = 0.87). However, within the mucosal compartments, the rate of undetectable viral load was higher in the vaccine group compared to the placebo group (SP: 57% versus 28%, OR = 3.5, p = 0.02; CVL: 71% versus 59%, OR = 1.7, p = 0.45; Overall: 62% versus 40%, OR = 2.5, p = 0.03). The mucosal viral load was lower in the vaccine group compared to the placebo group for SP (mean 2.22 log_{10} versus 2.71 log_{10}, p = 0.05) but not CVL (p = 0.99) nor overall (p = 0.25). Mucosal viral load was consistently lower than that of blood in both SP (mean difference = 1.9 log_{10}, p < 0.0001) and CVL (mean difference = 2.43 log_{10}, p < 0.0001).

Conclusion: The RV144 vaccine regimen lowered the detectable rate and magnitude of viral load in SP but not blood. Ongoing studies are evaluating the robustness of this effect and potential explanatory factors such as mediating immune responses.
Background: CSIC is an NNRTI with potent antiviral activity in cell culture against cell-free and cell associated HIV-1, and demonstrated a potent memory or protective effect against HIV infection. In this study CSIC has been evaluated for its cytotoxicity and antiviral activity in a cervical tissue derived organ culture. CSIC formulated as a vaginal ring was assessed for in vivo toxicity in rhesus macaques (RhM) as well as antiviral activity in an ex vivo challenge experiment against RT-SHIV.

Methods: CSIC was evaluated in organ culture for toxicity and its ability to block HIV-1 transmission across cervical mucosa. Silicone rings containing CSIC were applied intravaginally in 4 RhM for 14 days. Every three days, blood and cervicovaginal lavage (CVL) samples were analyzed for serum CSIC levels and for CVL antiviral activity. Vaginal and cervical biopsies taken prior to and fourteen days post-application of the ring were subjected to challenge with RT-SHIV in an ex vivo organ culture model.

Results: Unformulated CSIC blocked transmission of both R5 and X4 HIV-1 across cervical mucosa in our ex vivo organ culture model. Furthermore, CSIC did not induce proinflammatory cytokine response in tissues up to 72 hr after exposure. All CVL samples collected from the animals during CSIC ring exposure contained substantial amounts of CSIC and were found to have high antiviral activity. There was no toxicity observed from CSIC formulated rings during vaginal exposure. Importantly, cervical and vaginal biopsies from CSIC-instilled animals prevented RT-SHIV transmission in ex vivo organ culture compared to pre-exposure base line samples.

Conclusion: CSIC showed potent antiviral activity with no detectable cytotoxicity in vitro and ex vivo organ culture. The presence of antiviral CSIC in CVL and cervical/vaginal tissues with no toxicity throughout a fourteen day exposure period in vivo suggests that ring-formulated CSIC warrants further development as a topical microbicid.

P15.13 LB
Knowledge of HIV/AIDS Transmission, Prevention, Treatment and Sexual Behaviour among PLWHA in Eastern Uganda

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Background: Individual sexual behavior and knowledge about HIV are major determinants of HIV prevalence in a population. HIV among adults is mainly transmitted through heterosexual contacts. Most prevention programs in Uganda therefore focus on strategies to reduce sexual transmission through the ABC strategy. This study presents findings from data collected from 4 districts of Eastern Uganda in 2009 to explore knowledge of HIV/AIDS transmission, prevention, treatment and sexual behaviour among PLHIV.

Methods: An LQAS baseline survey was conducted during November and December 2009 in 4 districts namely; Busia, Butaleja, Pallisa and Sironko. Nineteen PLHIV were drawn from PHA Organization’s registers from each of the 4 districts giving a total sample of 76. The LQAS baseline survey was conducted for selected HIV/AIDS, TB and Malaria indicators.

Results: Seventy Six percent of the PLHIV were women, 28% were male. Most of PLHIV interviewed were aged 35–44 (48%) and a majority were Widowed (43%). About 41% had not completed primary level education. On levels of knowledge of prevention, abstinence was mentioned by at least 90% of PLHIV, condom use was reported by 95%. While 71% rejected that HIV can be transmitted through a mosquito bite, 90% rejected that the virus can be transmitted through sharing utensils. However although 67% rejected Witchcraft, 28% accepted it as a transmission mode. At least 90% mentioned MTCT as another mode while 92% knew where to access information.

Conclusion: The survey findings show that although knowledge of HIV/AIDS transmission, prevention and treatment among PLHIV is very high, PLHIV still engage in risky sexual behavior. This is likely to fuel drug resistance to the first line treatment currently being used in the country, thereby defeating the current secondary prevention programs. There is need to initiate programs that will help to transform high levels of knowledge into behavior change among PLHIV.

P17.28 LB
Occult Replication of a Conditionally-Live Attenuated SIV Profoundly Upregulates T Effector Memory Cell Frequency

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Background: The most potent protection against infection with virulent SIV, including protection against mucosal challenge, is conferred by “vaccination” with live attenuated virus. Although this approach is precluded for HIV because of safety concerns, understanding the mechanisms of superinfection resistance may inform rational vaccine design.

Methods: In order to uncouple antigen exposure from active viral replication we compared peripheral and intestinal T cell phenotype and SIV peptide-specific responses following infection of macaques with wild type SIVmac239, attenuated SIVmac239 nef or with a doxycycline (dox)-conditional replication variant of SIVmac239 nef designated SIVrtTA. Global (antigen-non-specific) T cell phenotype was assessed for central memory (Tcm) (CD28+ CD95b7 Tem) and effector memory (Tem) (CD28– CD95b7 Tem) and pre-exposure base line samples.

Results: In animals in which on-going virus replication was permitted (SIVrtTA + dox & SIVmac239 Nef), the proportion of CD4 and CD8 Tem were reduced in the majority of animals while Tem proportions increased. In animals infected with SIVrtTA in which dox had been withdrawn for 8 weeks prior to analysis, these changes were not seen. Moreover animals infected with wild type virus had elevated CD4 and CD8 Tem. Analysis of gut mucosal homing (α4b7 and b7) T cells showed similar polarised changes.

Conclusion: Overall, we found that active replication of SIVrtTA and SIVmac239 Nef had a profound impact on global T cell phenotype and antigen-specific polyfunctionality in the periphery and the gut. The use of these SIV mutants will contribute to the understanding of the mechanisms of superinfection resistance.
P17.29 LB

In Vivo Electroporation Induces Broad HIV-1 Envelope Epitope Responses to ADVAX HIV-1 DNA Vaccine in Humans

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Background: DNA-based vaccines have been found safe, stable, and are unencumbered by pre-existing vector-specific immune responses, although previous clinical studies suggested they were poorly immunogenic. Administration of the HIV-1 clade B/C ADVAX DNA vaccine (Gag-Pol, Nef-Tat and Env) by electroporation has been shown to be safe and to result in increased and more durable cellular immunogenicity over intramuscular administration. The majority of responses induced were of a poly-fenomenal phenotype in CD4+ T-cell populations. Here, we investigate the breadth of T-cell responses against the ADVAX envelope (gp160) protein along with the identities of regions recognized through epitope mapping.

Methods: PBMCs isolated from 16 recipients of ADVAX EP were selected based on ELISpot responses >100 SFU/10^6 PBMC and subjected to peptide mapping using a matrix containing sequences corresponding to the ADVAX gp160 insert. Potential positive peptides were deconvoluted and, whenever possible, responses confirmed using short-term T-cell lines generated following 9-day culture with the whole gp160 pool with IL-2.

Results: Following deconvolution in 11/16 individuals, the breadth of gp160-specific responses was comparable to the epitope breadth induced by MVA and Ad5 vectors in other trials (mean breadth = 2.8 epitopes/volunteer). The majority of responses focused on V3/C4 and V2 regions. 6/11 volunteers tested recognized the a4b7 integrin-binding domain of gp120. A trend towards an association with increased vaccine dose and breadth was seen.

Conclusion: Together these data indicate that electroporation of the ADVAX DNA vaccine induces T-cell responses towards the epitope breadth induced by MVA and Ad5 vectors in other trials. The majority of responses focused on V3/C4 and V2 regions. The trend towards an association with increased vaccine dose and breadth was seen.

P17.30 LB

Senegal Multi-Site Evaluation of the Point-Of-Care PIMA Instrument for CD4+ T-Cell Enumeration Using Venous and Capillary Blood

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Background: CD4+ T-cell enumeration is used as a criterion to initiate antiretroviral therapy (ART) in HIV patients and to monitor treatment efficacy. However, simple, affordable and reliable point-of-care instruments adapted to resource-limited settings are still lacking. PIMA is a new point-of-care CD4 instrument using disposable cartridges and battery-powered analyzer.

Methods: Whole blood samples were taken by venipuncture or by fingerprick, from 300 subjects, including HIV-infected patients and HIV(-) controls. CD4+ T-cell counts were measured on PIMA (using venous or capillary blood) and on FACSCount (using venous blood, considered as the reference). External quality controls were from AFREQUAS.

Results: CD4+ T-cell counts were similar on PIMA and FACSCount using either venous blood or fingerprick samples (concordance correlation coefficient 0.93 and 0.88 respectively), with better accuracy for low CD4+ T-cell counts. Considering clinical decision to start ART at 200 CD4+ T-cells/µl, kappa coefficient for agreement was 87% for venous and 85% for capillary blood, and 78 and 71% respectively at a 350 CD4+ T-cells/µl cut-off. Sensitivity was 90%/91% and specificity 98%/96% at 200 cells/µl for venous/capillary blood respectively, and 98%/91% and 79%/ 80% at 350cells/µl. Repeatability (precision) on venous blood resulted in a coefficient of variation (CV) of 4%. Using fingerprick blood, the error frequency (aborted analysis) was 14%.

Conclusion: PIMA device is of good value to follow-up adult HIV-infected patients in resource-limited settings. Besides affordability, easy and safe practice, we demonstrated that performance was similar to FACSCount for relevant clinical CD4+ T-cell counts. However, technical error prevention requires good training for fingerprick samples.

P17.31 LB

Efficacious SIV-Specific CD8+ T Cell Responses in Heterozygous Animals are Weighted Toward Peptides Presented by a Single MHC Haplotype


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Background: Certain MHC class I alleles are correlated with remarkable control of HIV and SIV, indicating that specific CD8+ T cell responses can effectively control viral replication; however, we do not understand why some individuals with these MHC class I alleles do not control their viral loads.

Methods: We used animals with defined MHC haplotypes (A and B) and viral inhibition assays to directly assess CD8+ T cell efficacy ex vivo. We isolated CD8+ T cells and CD8-depleted target cells from the blood of six homozygous AA, six homozygous BB and six heterozygous AB animals. We added CD8+ T cells to infected target cells in every possible combination. We then measured the ability for CD8+ T cells to reduce viral replication through intracellular p27 staining.

Results: While CD8+ T cells from homozygous AA, BB, and heterozygous AB animals were equally capable of suppressing viral replication on autologous and AB target cells, we found that AB heterozygotes suppress viral replication most effectively on AA target cells. Surprisingly, the same AB effector cells did not effectively inhibit viral replication on BB target cells, revealing a striking absence of effective B restricted CD8 + T cell responses.

Conclusion: These results indicate that the greater potential breadth of CD8+ T cell responses present in heterozygous animals does not...
necessarily lead to greater antiviral efficacy and suggest that CD8+ T cell responses in heterozygous animals have a skewed focus toward epitopes restricted by a single haplotype. Such ‘MHC haplodonorance’ may help explain why only some individuals with ‘protective’ MHC class I alleles durably control HIV replication.

P17.32 LB
Primary Immune Responses to Vaccinia Virus Vaccination: The Role of Cytotoxic Effector CD4+ T Cells in the Generation of Human T Cell Memory

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Background: To understand the developmental stages of CD4+ T cell responses to viral infection and their differentiation into long-term memory cells, we studied individuals receiving primary vaccinia virus (VV) vaccination. This provides an attractive model for the study of human antiviral T cell responses as vaccination results in an acute infection that is cleared and leads to long-term protective immunity.

Methods: At the peak of the primary effector cell response to VV, day 13 post-vaccination, we purified activated effector (CD45RO+CD38+++) and naive (CD45RO-CD38dim) CD4+ T cells from 2 subjects, extracted mRNA and conducted microarray analysis using the Affymetrix, HU133 Plus 2.0 microarray.

Results: In the activated effector CD4+ compared to naive CD4+ T cells many of the genes up-regulated were associated with cell division and activation, such as Ki-67, CD38 and CCR5, as expected, hence validating the arrays. Surprisingly, there was a strong up-regulation of cytokine-lymphocyte (CTL) associated genes in the activated effector CD4+ T cells. Of the top 40 differentially expressed genes granzyme K ranked 1st with fold change (fc) of 40 compared to naive CD4+ T cells. Killer cell lectin-like receptor subfamily B member 1 (KLKB1/CD161) fc:26.3, Rab27a:19th fc:8.2, granzyme A:26th fc:13.9 and granulysin:36th (fc:7.9). These fold changes are being validated by qPCR. Preliminary analysis at 13 days post vaccination with VV revealed >10% of activated VV-specific CD4+ T cells and >50% of activated CD8+ T cells expressed granzyme K.

Conclusion: Generation of anti-viral human CD4+ T cell memory during primary immune responses is poorly understood. The role of CTL associated genes in this process has not been explored. Understanding their role in the generation of an effective memory may lead us closer to the development of a more effective HIV vaccine, such as enhancing the modestly efficacious HIV-1 vaccine used in the RV144 study.

P17.33 LB
Ubiquitination of the HIV Gag Protein Results in an Increased Expression of Inhibitory Markers on Stimulated T Cells

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Background: The development of an HIV vaccine that generates a large pool of CD8+ T cells recognizing multiple cytotoxic lymphocyte (CTL) epitopes is a primary strategy in HIV vaccination protocols. Broadening of the CTL repertoire ensures responses to HIV proteins that are able to rapidly mutate as a result of selective pressures imposed by the immune system. Consequently, our strategy employed ubiquitination of the gag protein, with the aim of targeting the protein to the proteasome, thus enhancing MHC class I antigen processing and thereby broadening the CD8 T cell repertoire.

Methods: T cells were primed and boosted weekly with monocyte derived dendritic cells (mDCs) transfected with either rAd5 CN54 HIV ubiquitinated gag or rAd5 CN54 HIV non-ubiquitinated gag for a total of 4 weeks, and then assayed for antigen-specific responses by restimulation with peptides. The production of IFN gamma was determined by ELISPOT and intracellular staining and differences in activation status were determined by the expression of surface markers by flow.

Results: Although ubiquitination enhanced proteasomal targeting, unexpectedly, IFN gamma production was diminished in T cells primed and boosted against ubiquitinated gag proteins. To understand the absence of an enhanced T cell response, we investigated the expression of several markers, including CD38, TIM3, and CTLA-4 by flow cytometry. Preliminary results indicated that at 4 weeks post in vitro culture, T cells primed against ubiquitinated gag proteins and restimulated with peptides, expressed an increased frequency of some inhibitory markers as compared to T cells primed against non-ubiquitinated gag proteins. Interestingly, expression of inhibitory markers did not influence the proliferative capacity of lymphocytes as cell numbers increased progressively.

Conclusion: These results suggest that ubiquitination of the HIV gag protein may lead to chronic stimulation of T cells in vitro, which may lead to their exhaustion and consequently their reduced responsive capacity.

P18.21 LB
A Phase IIIB, Randomized, Double-Blind, Multicenter, Immunogenicity Study of Vacc-4x Versus Placebo in HIV-1-infected Patients


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Background: Vacc-4x is a peptide-based HIV therapeutic vaccine to conserved domains on p24Gag. Recently conserved ‘sectors’ on HIV p24, critical for virus viability and thereby immunologically vulnerable have been identified. Elite controllers target immune responses to such regions. The Vacc-4x peptides lie within a number of these conserved sectors of HIV p24. The co-primary endpoints of this study were to compare changes in CD4 counts and return to ART between treatment and placebo groups during a 24 week treatment interruption.
Secondary endpoints included safety, viral load and immunogenicity.

Methods: This prospective, randomized, double blind phase IIB clinical study (NCT00659789) was carried out in 13 European and 5 US centers recruiting 135 patients on ART. After 6 immunizations on ART over 28 weeks, treatment was interrupted for up to 24 weeks (to week 52) (Vacc-4x n = 88; placebo n = 38). Immunological analyses (ELISPOT, proliferation, intracellular cytokine staining) were carried out at central laboratories.

Results: There were no Vacc-4x-related serious adverse events. Of the 135 patients recruited (male n = 92; female n = 43), 126 patients completed the study. Median prestudy CD4 count was 712 (Vacc-4x) and 619 cells/mm³ (placebo), and median CD4 nadir 300 (Vacc-4x) and 285 cells/mm³ (placebo). There was no statistically significant difference between the two groups regarding change in CD4 counts (p = 0.12) or ART resumption (p = 0.89) during treatment interruption. A statistically significant treatment difference between Vacc-4x and placebo groups for viral load (VL) was found for patients who achieved a 6 month ART-free period (p = 0.0022). There was a positive correlation between ELISPOT responses and lower viral load in the Vacc-4x group compared to placebo (p = 0.02). Long-term follow-up of patients up to week 104 was completed in June 2011.

Conclusion: Vacc-4x was found to be safe and well tolerated. The Vacc-4x group experienced a significant reduction in viral load compared to placebo.

P18.22 LB
The Improvement of Five HIV-1 B’/C Subtype Genes Modification on the Express Level In Vitro in HIV Vaccine

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Background: Since the first reported AIDS case in 1981, AIDS has been spreading at an alarming rate in the world, seriously threatened human health. HIV vaccine has not been developed yet in the past decades, because of the specific biological characteristics and the lack of awareness of the mechanism on HIV infection and immune protection. The neutralizing antibody induction is important for the development of traditional vaccine, but for the HIV vaccine, besides the induction of cross-protective neutralizing antibody, wide spectrum cellular response is also important for the high efficiency protection. It’s getting an important strategy for HIV cellular immune vaccine to modify cellular immune antigen, use multi-antigens and combine immunization of “prime-boost”with different vaccines.

Methods: we selected five major cellular immune antigens of HIV-1 B’ / C subtype as target genes, which are gag, pol, rev, tat and nef, to modify and optimize their gene sequences, codon bias and express structure, and then constructed DNA vaccines or recombinant vaccinia virus(rVV) to assess the express level of the genes in vitro, respectively.

Results: Results shows that all optimized and non-optimized genes can express in DNA and rVV, and genes express better in DNA than rVV. Gag and pol had higher express level after gene optimization. The significant promote of express level is observed in pol gene, single pol gene had higher express level than gagpol fusing gene, but similar result was not observed in gag. As for rev, tat and nef, optimized single gene express slight higher level than optimized hRTN(fusing gene by rev,tat and nef), and both optimized single rev, tat, nef gene and fusing hRTN had better express level than non-optimized RTN.

Conclusion: These data suggest that all the optimized HIV genes may be good vaccine candidate antigens for use as a protective HIV vaccine based on the cellular mechanism.

P18.23 LB
Fragmentation of SIV Gag induces Broader T cell Responses In Vitro

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Background: Emergence of cytotoxic T lymphocyte (CTL) escape mutants during the natural course of SIV and HIV-1 infection suggests that CTL-exerted pressure plays an important role in the control of viral replication. Thus, broadening of the CTL response in a prophylactic setting is an attractive goal of T cell based vaccination against HIV-1. Antigenic competition is known to narrow the breadth of T cell responses. Therefore, we assessed whether gene fragmentation broadens the T cell repertoire.

Methods: Ad5 vectors expressing SIVmac251 gag genes were used in this study. The vectors either expressed full length SIV-gag or one of 7 SIV-gag segments. These mini-genes were equal in size and neighbouring fragments overlapped by 10aa. Human dendritic cells (DC) were generated from monocytes and transduced with the different vectors separately. They were then co-cultured with autologous naïve T cells that were isolated by negative selection. T cells were expanded over 3–4 weeks by boosting with autologous Ad5-transduced DC on a weekly basis. CD8 T cell responses against SIV overlapping peptides were then monitored by IFN-γ ELISPOTS. Their phenotype and ability to produce IFN-γ, TNF-x, and IL-2 was also measured by flow cytometry.

Results: No significant differences were observed between full length and fragmented SIV-gag constructs in terms of their ability to induce CD4 and CD8 T cell expansion. The generated cells exhibited an effector phenotype and had similar cytokine profiles in terms of IFN-γ, IL-2, and TNF-x. However, full length SIV-gag constructs generated CTL responses that recognised between 3 and 7 epitopes whereas fragmented SIV-gag vectors induced broader responses against new epitopes averaging 16 peptides. The magnitude of the response against most of the recognised epitopes was found to be higher when Ad5-vectors expressing fragmented genes were used.

Conclusion: Antigen fragmentation augments the magnitude and breadth of CTL responses possibly through elimination of antigenic competition.

P18.24 LB
Comprehensive Cross-Clade Elimination of Cells Infected with Globally Diverse HIV-1, HIV-2 and SIV Isolates by HERV-K-Specific CD8+ T-cells


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LATE BREAKER POSTER ABSTRACTS

P18.25 LB
Robust Immunogenicity after HIV DNA Vaccination with IL-12 Plasmid Cytokine Adjuvant Delivered via Electroporation in HIV Uninfected Adults


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Background: The genetic diversity of HIV antigens represents a paramount challenge in the development of vaccines. Here, we establish rationale for circumventing this obstacle by eliciting cellular immune responses against stable human endogenous retrovirus K (HERV-K) antigens. Protein-level expression of HERV-K antigens has not been observed in healthy tissues, but does occur in various malignancies. We hypothesized that HIV would disrupt control of HERV-K expression within infected cells, targeting them for elimination by HERV-K-specific CD8+ T-cells.

Methods: HERV-K expression was assessed by qPCR, western blot, immunohistochemistry and mass-spectrometry. HERV-K-specific T-cell responses were identified by ELISPOt and a HERV-K-Env-specific CD8+ T-cell was cloned by IFN-γ capture. Additional HERV-K-specific T-cell lines and cloned were obtained by a dendritic-cell based expansion method. Recognition and elimination of HIV-infected cells were assessed by flow cytometry.

Results: HIV-infection resulted in HERV-K Gag and Env protein expression in primary CD4+ T-cells. HERV-K Gag and Env specific CD8+ T-cells specifically responded to, and eliminated, HIV-infected cells in an MHC-I-restricted manner. This recognition was dependent upon Vif, however expression of Vif alone was insufficient to recapitulate a response. A HERV-K-Env-specific CD8+ T-cell clone displayed comprehensive elimination of cells infected with a panel of 23 diverse HIV-1 and HIV-2 primary isolates as well as with SIVmac251.

Conclusion: The de-repression of HERV-K expression in HIV-infected cells, for which Vif is necessary but insufficient, constitutes a marker of infection that can be targeted by HERV-K-specific CD8+ T-cells. The unprecedented breadth of reactivity of HERV-K-specific T-cell responses against diverse lentiviruses, both implied by the proposed mode of action and observed in the current study, comprises an enticing advantage over HIV-1-specific T-cell responses which could be exploited in the development of HERV-K-targeted vaccines to treat or prevent HIV infection. These data also provides rationale for considering a role for HERV-K-specific T-cell responses in natural control of HIV.

Background: DNA vaccination in humans has demonstrated relatively poor levels of cellular immune responses. HVTN 080 tested the ability of HIV DNA combined with IL-12 plasmid, and delivered via electroporation (EP) to induce cellular immune responses in HIV-uninfected adults.

Methods: HVTN 080 was a prospective, randomized, double-blind, placebo-controlled trial of 48 healthy adults. Subjects received 3 vaccinations of PennVax™B (PV), which consisted of 1 mg each of HIV DNA Consensus B Gag, Pol and Env either alone (10 subjects) or in combination with 1 mg of GENEVAX™ IL-12 plasmid (30 subjects) at 0, 1, and 3 months via EP. Eight subjects received placebo. Pain was assessed via a 10-point visual analog scale immediately and at 5 and 25 minutes post-EP. Immune responses were measured by intracellular cytokine staining (ICS) assay. HVTN 070 evaluated 4 vaccinations (0, 1, 3, and 6 months) with 6 mg of PV and 1.5 mg of IL-12 plasmid in 30 subjects without EP.

Results: The median pain score was 5.4 immediately, which decreased to 0.8 at 5 and 25 minutes. There were no significant vaccine-related adverse events. Two weeks after the 3rd vaccination 81% of PV + IL-12 subjects had a CD4+ T cell response, and 52% had a CD8+ T cell response. In contrast, response rates were significantly lower (CD4 responses 41%; p = .005 and CD8 T cell responses 4%; p = .002) when the same vaccine was given as 4 vaccinations at double the dose without EP (HVTN 070). Conclusion: HIV DNA vaccine + IL-12 plasmid delivered via EP led to frequencies of cellular immune responses equal or greater to those reported from current vector-based HIV vaccines such as adenovirus or traditional DNA vaccination without EP, either alone or followed by vector-based 'boost'. Further trials delivering HIV DNA with or without IL-12 plasmid using EP as a vaccine strategy are merited.

P18.26 LB
Immunological Correlates of Protection Against Acquisition of HIV-1 Infection in the iPrEx Trial


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Background: Pre-exposure prophylaxis for HIV-1 prevention in men who have sex with men (MSM) has been shown to reduce overall HIV-1 incidence by 44%. We hypothesized that exposure to HIV-1 before enrollment could have stimulated cell mediated immunity, which contributed to the observed protection.

Methods: We studied immune responses in highly exposed MSM enrolled in the iPrEx study who either seroconverted (SC; N = 93) or remained seronegative throughout follow up for up to 132 weeks (ESN; N = 485). Pre-infection time-points from SC participants were matched with time-points from ESN controls and analyzed for HIV-1 specific T-cell responses to p24, Nef, Vif, integrase (Int), reverse transcriptase (RT), and protease (Protease) using a standard IFN-γ ELISpot assay. Non-parametric tests were used to assess statistical significance of the comparisons between responses in SC and ESN participants.

Results: ESN participants had a 3-fold greater mean aggregate HIV-1 specific T-cell response compared to SC participants, 297
P18.27 LB
HIV T-Cell Vaccines Induce Epitope Hotspots That Differ From Those Observed In Natural Infection and Target Variable Regions of the HIV Genome

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Background: Currently available T-cell based HIV vaccines induce detectable responses to a median of 1-2 epitopes. To analyze these vaccine effects, we generated and compared population-based epitope maps from three T-cell HIV vaccine trials to those of natural infection.

Methods: The vaccines used were Merck-Ad5 Gag/Pol/Nef (HVTN502/Step and Merck016) and VRC-Ad5 Gag/Pol/Env (HVTN054). We applied a group testing approach of IFN-γ ELIspot using overlapping peptides spanning the vaccine inserts and analyzed epitope mapping data from 71 HVTN502/Step, 72 Merck016 and 39 HVTN054 participants and 372 HIV-infected individuals. Epitope hotspots were defined as sites targeted more frequently than predicted by vaccine sequence alone. Statistical tests were developed both for hotspot existence and for hotspot comparison between vaccines.

Results: We found strong evidence of multiple hotspots in all Step vaccine inserts: Gag, 4; Pol, 1; and Nef, 4 (p-values < 0.0001-0.03). All vaccine inserts had at least one epitope hotspot. Several vaccine-induced hotspots were not present in natural infection or were specific to single vaccine trials. A statistical test measuring the significance of targeting frequency disparity between Merck and VRC vaccine-induced hotspots revealed differences for Gag and Pol (p = 0.0035 and p = 0.0004, respectively). Analysis of hotspot conservation patterns identified a bias toward conserved peptide properties at 9-mer beginning with A-132 LATE BREAKER POSTER ABSTRACTS

P18.28 LB
HVTN503/Phambili Vaccine Trial: The effect of the HIV-1 Clade B-Based Vaccine on Breakthrough Founder Viruses from a Clade C Infected Population


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Background: The HVTN 503/Phambili trial (MRKAd5 gag/pol/nef HIV-1 subtype B vaccine) did not prevent infection nor reduce viral load set-point. While 80% of the vaccinees had insert-matched Gag-specific responses, only 50% recognized Gag subtype C PTE peptides. We characterized HIV-1 breakthrough infections from South Africans participants to determine if vaccination exerted a selective pressure on the infecting virus.

Methods: 262 HIV-1 genome sequences were obtained from plasma at HIV-1 diagnosis from 20 placebo and 23 vaccine recipients. A pre-specified analysis plan was implemented to investigate possible vaccine sieve effects.

Results: Of the 43 HIV-1 infections, 40 were subtype C and 3 were non-C: 24% vaccine and 26% placebo recipients were infected with heterogeneous founder populations. An average of 35% of 9mers in Nef founder sequences (12% in Gag) differed from the corresponding insert 9mer by >2 amino acids. Unlike for the Step trial, for the analysis based on predicted epitopes, we found no significant difference in epitopic distances to the insert between vaccine and placebo group sequences. Analysis of site-specific sieve effects revealed evidence for 3 signature sites: Gag 54, Gag 472 and Nef 23. A separate Bayesian analysis supported the findings at these sites (posterior probability >0.999) and at several additional sites. Additionally, we found significantly different physio-chemical peptide properties at 9-mer beginning with Gag 471 (q-value = 0.14).

Conclusion: Evidence for MRKAd5 sieve effects were previously demonstrated in the Step trial. Our results demonstrate much weaker MRKAd5 sieve effects in the Phambili trial, which may be explained by 1) few infection endpoints and thus limited statistical power; 2) incomplete vaccination courses; and 3) use of a subtype B immunogen in a predominantly subtype C infection. These findings underscore the importance of matching vaccine inserts to the predominant HIV-1 subtype of the region.

P19.30 LB
Preexisting Immunity to Multiple Adenovirus Serotypes is Not Associated with Increased HIV-1 Acquisition in Three HIV-1 Vaccine Efficacy Trials

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Background: The Step study of the MRKAd5 HIV-1 gag/pol/nef vaccine raised the possibility of enhanced HIV-1 acquisition risk in vaccinees who were both Ad5 seropositive and uncircumcised. Here we examined whether preexisting immunity to multiple adenovirus serotypes was intrinsically associated with
enhanced HIV-1 acquisition in the VAX003, VAX004, and Step studies.

**Methods:** We performed case-controlled studies to assess the association between baseline neutralizing antibodies (NAbs) to Ad serotypes 1, 2, 5, 6, 26, 35, and 48 and subsequent HIV-1 infection among 1,570 adults enrolled in the VAX003 and VAX004 trials of the HIV-1 rgpl20 vaccine and the Step study of the MRKAd5 vaccine. Cases represent all subjects who became HIV-1-infected. Controls were matched for demographic variables, and, for the Step study, also Ad5 serostatus and circumcision status.

**Results:** NAb titers to Ad serotypes 1, 2, 5, 6, 26, 35, and 48 were determined for 669 HIV-1-infected cases and 901 matched HIV-1-uninfected controls. Ad seroprevalence ranged from rare (Ad2, 87%) to common (Ad2, 87%) and proved comparable between cases and controls (< 5% difference) for all serotypes. There was no association between baseline NAbs and HIV-1 acquisition for any of the seven serotypes tested (P > 0.05 for all comparisons). Odds ratios ranged from 0.8 to 1.2 and 95% CI crossed 1.0 for all serotypes. Sub-analyses by trial, vaccine/placebo, and circumcision status also found no association (P > 0.07 for all comparators, adjusted for multiplicity.)

**Conclusion:** Ad5 seropositivity was not associated with increased risk of HIV-1 acquisition in the VAX003 and VAX004 studies. Moreover, seropositivity to six other adenovirus serotypes was not associated with increased HIV-1 acquisition in the VAX003, VAX004, and Step studies. These data demonstrate that Ad seropositivity is not intrinsically a marker for increased risk of HIV-1 acquisition, including following vaccination with rgpl20 or an Ad vector from a different serotype.

**P19.32 LB**

**HIV-1 JR-FL Env Trimers Expressed by DNA Electroporation Alone or Boosted With Soluble Trimers Elicit Neutralizing Antibodies**

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**Background:** All known HIV-1 gp120-directed broadly neutralizing antibodies efficiently recognize fully cleaved JR-FL spikes, however, the non-neutralizing gp120-directed antibodies cannot. Therefore, as an immunogen, cleaved, functional spikes may selectively present neutralizing epitopes to B cells, perhaps more efficiently eliciting neutralizing antibodies. However, attempts to make soluble versions of Env that fully mimic the viral spike are yet unsuccessful.

**Methods:** To present functional, cleaved spikes to the immune system, we inoculated both non-human primates (NHPs) and rabbits with JR-FL Env-expressing plasmid DNA by electroporation. In vitro, this plasmid encodes for fully cleaved, cell surface, trimeric JR-FL Env, but only when expressed from a mini-LTR in a non-codon optimized state. DNA priming was followed by boosts with soluble JR-FL gp140 trimers in adjuvant. In vivo, the LTR-driven, non-codon-optimized Env plasmid DNA, likely requires tat co-transfection in trans, and rev expression in cis, for efficient Env expression. A control codon-optimized and CMV-driven Env plasmid was used; however, in vitro this plasmid expresses inefficiently cleaved JR-FL cell-surface trimers.

**Results:** We report that the DNA priming elicited reasonable ELISA binding titers in both NHPs and, somewhat surprisingly, also in rabbits. The elicitation of neutralizing antibodies in the NHPs was exceedingly high against Tier 1 isolates following protein boosting. In rabbits, neutralization from the non-codon-optimized DNA was dependent upon co-transfection with the tat expressor plasmid, but not so for the codon-optimized construct. Reasonable Tier 1 neutralizing antibodies were elicited in rabbits after 3 DNAs, with modest binding titers, and weak, sporadic neutralization of Tier 2 isolates. The neutralizing activity generally increased following protein boosting.
Conclusion: We conclude that DNA priming by electroporation is an interesting means to present functional, cleaved HIV-1 Env spikes to the B cell immune system to prime or initiate trimer-elicited neutralizing antibodies in both rabbits and, importantly, primates.

P19.33 LB
Evaluations of Subtype C HIV-1 Envelope Glycoproteins as Immunogens for Vaccine Development

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Background: A successful vaccine against HIV-1 will likely require both a cellular and a neutralizing antibody (nAb) component. Despite substantial efforts to produce a potent vaccine immunogen capable of inducing broadly nAbs, little success has been achieved. The inability to generate an appropriate antigen has been hindered by enormous antigenic variability and immune-evasion mechanisms exhibited by the viral envelope glycoprotein (Env). The screening of new immunogens and their through in vitro and in vivo evaluations provides rationale for immunogen selection for future pre-clinical and clinical studies.

Methods: Besides rational antigen design, our approach has been to screen multiple recombinant EnvS from acute subtype C HIV-1 isolates for identifying superior antigen that generates better Env specific neutralizing response in small animals. In conjunction, we have also evaluated multivalent and sequential Env vaccinations with novel formulation strategies to elicit improved antibody response.

Results: We observe heterogenous expression of gp120s from acute subtype C HIV-1 isolates, predominantly as mixtures of monomer and dimers. The dimers, which are aberrantly disulfide-linked proteins, constitute 15–70% of stable cell-line expressed gp120s. Although in vitro, both gp120 forms differ in ligand-binding abilities, in vivo they are similarly immunogenic. Considering >70% monomer and >5mcg/ml expression and other criteria, two gp120 proteins were selected for future production.

Conclusion: The process of screening, in vitro characterization, generation of stable cell lines and immunogenic evaluations of the proteins in various small animals have led to the selection of top two HIV-1 subtype C gp120s for production to support Pox prime-Protein boost Phase 2b clinical trial in Southern Africa.

P20.07 LB
Significant Contribution of Subtype G to HIV-1 Genetic Complexity in Nigeria

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Background: Global vaccine concepts either include the subtypes A, B and CRF01_AE for insert design or mosaic inserts derived from published databases with low West African subtype representation. The conventional view that West African HIV variants are largely CRF02_AG would justify the inclusion of subtype A sequences in a global design. We challenge this justification by presenting the significant contribution of subtype G in the HIV-1 epidemic in four Nigerian cities using a multiregion hybridization assay (MHA) that can identify and differentiate between subtype G and CRF02_AG.

Methods: Viral RNA was extracted from plasma of 71 volunteers for a cohort for prevalence, risk factor, and subtype study conducted in Nigeria. Twelve were from Kaduna, 18 from Abuja, 30 from Makurdi, and 11 from Enugu and all were subjected to the G/CRF02_AG MHA, a high throughput assay. This MHA was designed to contain 7 regions: 3 (pol [RT], pol [INT], tat) identify V3-V5 region of viral envelope from HIV-1 infected Indian children.

P20.06 LB
Genetic Analysis of the Viral Envelope (V3-V5 region) in HIV-1 Infected Indian Children

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Background: HIV-1 infection in India is predominantly due to clade C viruses. Our objective was to assess the variability in the
both subtype G and CRF02_AG, and 4 (gag, pol [RT2], vpr, env [gp120]) differentiate between the two subtypes. For differentiating regions, probes are designed to be subtype specific.

**Results:** Overall, subtype G represents 23% while CRF02_AG contributes 41% and their recombinants represent 23%. 13% of the samples are neither subtype G nor CRF02_AG. Notably, 13 out of 15 recombinants contain subtype G gp120 sequences. Our data agree with previous reports showing a higher percentage of subtype G in southern Nigeria (Enugu) as compared to the north (Kaduna).

**Conclusion:** Subtype G and subtype G-positive gp120 recombinants represent a significant portion of HIV-1 infections in Nigeria, suggesting that the total number of subtype G infections in Western Africa may be more of a burden than previously thought and should inform global vaccine designs.

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**P20.08 LB**

**Generating Viruses Matching RV144 Vaccine Components to Explore Efficacy**

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**Background:** The results from the RV144 Thai vaccine trial have shown a modest effect in preventing HIV infection. However, the underlying mechanisms of protection remain to be identified. Developing vaccine homologous reagents for in vitro immunogenicity assays is a priority for the field. Here we present the development and characterization of vectors expressing functional CM244 envelopes (component of AIDSVAX B/E).

**Methods:** CM244 env was isolated from different sources: infected A3R5 cells (AD), PBMC virus passaged once from 1989 (OR), later passaged PBMC (ec1, NW) and a synthetic gene (v.i.). Sequenced plasmids encoding these envs were tested for infectivity and site-directed mutagenesis was used to correct non-functional and poorly infectious envelopes and/or to change amino acids to match the immunogen sequence. Neutralization assays were performed in TZMbl cells using pseudoviruses (PV) and in PBMC using infectious molecular clones (IMC).

**Results:** Among CM244 clones, OR was the best PV env candidate (highly infectious and nearly homologous to the vaccine insert) and was inserted into clade CRF01_AE and clade C Renilla Luciferase backbones to generate chimeric IMCs. The synthetic env matching the vaccine insert (v.i.) did not mediate infection and showed specific mutations, including some affecting the CD4 binding site. Sequence comparisons revealed that clones with different cellular origin had unique mutations; in particular, a three amino acid variation occurred in the V1/V2 region, affecting the recently identified “peptide 49” that may be associated with cellular immune responses. Preliminary neutralization results showed no major differences between PVs despite these mutations. However, when PV were compared with IMCs, striking differences were observed, including two-log increases in sensitivity of IMC to HIV + serum and monoclonal antibody b12.

**Conclusion:** These data highlight the importance of development of IMC for use in primary cell assays, as evidenced by differences observed in neutralization when the same envelope is tested in different platforms.

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**P20.09 LB**

**The Prevalence of Low and High Viral Load in a Cohort of African Volunteers During the First Year of Infection with HIV-1 Subtype A1, C and D**


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**Background:** In trials evaluating HIV vaccine efficacy, the potential influence of HIV-1 subtype on viral load (VL) during early HIV breakthrough infection may be important in vaccine evaluation.

**Methods:** HIV-infected volunteers in Kenya, Rwanda, South Africa, Uganda, and Zambia were enrolled into a seroconverter cohort within one year of estimated date of infection (EDI). Plasma VL post-EDI was measured monthly for 3 months, quarterly until 24 months, and 6-monthly thereafter. Acute and early HIV infection was defined as ≤90 days and 91–365 days post-EDI, respectively. VL data were analyzed as log10-transformed data. For each period, high and low VL was defined as a mean VL ≥5.0 × 10⁶ and VL ≤3.3 × 10⁶ copies/mL, respectively. Only visits before ART initiation were included.

**Results:** Of 463 volunteers, 177(38.2%) were infected with HIV-1 subtype A1, 216(46.6%) with subtype C, and 70(15.1%) with subtype D. 407 volunteers had acute infection (AI); 158(38.9%) had subtype A1, 183(44.9%) had C and 66(16.2%) had D; 157(38.6%) had high VL and 42(10.3%) had low VL; neither varied by subtype (p = 0.6 and 0.8, respectively). Median VL during AI(5.1x10⁶ copies/mL) was subtype-independent (p = 0.3). During early infection (EI), 115/463(24.8%) volunteers had high VL, which did not vary by subtype (p = 0.2). Low VL during EI was significantly associated with subtype A1(26/177, 14.7%) vs. subtype C(13/216, 6.0%, p = 0.01) but not associated with subtype D (p = 0.1). Controlling for sex and infecting subtype, women were significantly more likely (OR 2.9, p = 0.001) to have low VL during EI, while individuals with subtype C infection (OR 0.31, p = 0.001) were significantly less likely. Median VL during EI, 4.5x10⁶ copies/mL, did not vary by subtype (p = 0.07).

**Conclusion:** During early HIV infection in this African cohort, we observed a significant association between subtype A1 and low VL. The potential confounding effect of infecting HIV-1 subtype should be considered when evaluating HIV vaccine efficacy using viral load as surrogate endpoint in breakthrough infections.
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