Session 1: Vaccine Concepts and Design Part 1

0A01-01

Mapping the specificity of neutralizing HIV immune plasma

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Objective: We devised a set of assays and reagents to better define neutralization by HIV+ plasma and vaccine sera. The fine definition of neutralizing specificities will provide key information for rational vaccine design.

Methods: We devised 3 general methods: 1) modified neutralization assays to define neutralization mechanism (standard, post-CD4 and post-CD4CCR5 binding formats) 2) virus capture competition to determine the ability of serum to compete for the binding to virus by well defined monoclonal antibodies 3) trimer binding assays using blue native PAGE. We have developed a panel of trimer mutants to knock out particular epitopes of known neutralizing antibodies like b12, 2G12 as well as CD4.

Results: We find that the majority of HIV+ plasma neutralization is directed to gp120 and is active in standard neutralization formats, rather than post-receptor binding formats. Trimer binding is revealing evidence for b12-like antibodies in HIV+ plasma (sensitive to mutation at residue 368 of gp120). However, b12 and HIV+ plasma binding to an expanded panel of mutant trimers indicate some important differences in their peripheral points of contact.

Conclusion: HIV+ plasma neutralization appears to be mostly directed to the CD4 binding site, similar to b12. However, the precise epitopes are a little different, consistent with differing cross-neutralizing breadth. These new mapping methods, in particular the trimer binding assay, are beginning to reveal the nature of neutralization against complex nonlinear epitopes that can not be easily approximated by peptides or other traditional mapping approaches. We are also developing panels of trimers of Envs from different clades to expand the global utility of this method for evaluating the humoral response.

0A01-02

Glycopeptide analysis of individual glycosylation sites of HIV envelope oligomers that induce breadth in neutralizing antibody responses

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Objective: To correlate differences in immunogenicity of gp160-based oligomers using glycopeptide profiles.

Background: Recently, CON-S gp140CFI was designed based on a soluble form of the HIV-1 gp160 group M consensus; it elicited cross-subtype neutralizing antibodies of significantly greater breadth and titer than wild-type clade B envelope protein, JRFL.[1] However, the structural features responsible for the difference in recombinant Env immunogenicity are not known. Since these proteins are ~50% carbohydrate, glycosylation analysis of specific glycosylation sites are of interest to correlate with Env immunogenicity, and as an indicator of correct protein folding.

Methods: Purified CON-S gp140CFI and JRFL gp140CF were produced as recombinant proteins. They were denatured, reduced, and alkylated prior to digestion with trypsin. The glycopeptides for each protein were fractionated by HPLC and analyzed using MALDI and ESI mass spectrometry. The spectral data was interpreted using GlycoPep DB.

Results: For the more effective immunogen, eight of thirty potential glycosylation sites in CON-S were unoccupied. One important open site is adjacent to the V3 loop, and V3 of CON-S is known to be responsible for eliciting a major species of neutralizing antibodies in guinea pigs. By contrast, this site was occupied in the poor immunogen, JRFL Env. There is an established correlation between glycan type and the glycan's three dimensional environment: High mannose glycans are generally present at occluded glycosylation sites, while complex glycans predominate at exposed glycosylation sites. For CON-S, the glycosylation pattern closely matched these expectations, indicating that CON-S has a well-conserved three-dimensional structure. JRFL Env, by contrast, has very heterogeneous glycosylation, indicating heterogeneous structures with non-native folding.

Conclusion: The open glycosylation site at the V3 loop in CON-S may explain why it is more effective than JRFL at eliciting antibodies from V3. In contrast to CON-S Env, the glycosylation of JRFL Env was heterogeneous at almost every glycosylation site. These data indicate a high degree of structural heterogeneity for JRFL. Poor conservation of structural epitopes likely resulted in poor neutralizing antibody immunogenicity of JRFL Env.

[1]J. Virol. 2006, 353, 262.

Funded by NIH grants R01GM077226, PO1AI61734, and a Collaboration for AIDS Vaccine Development Grant from the Bill and Melinda Gates Foundation.

0A01-03

A new concept for HIV vaccine: the critical role of an NKp44 ligand in the CD4 t depletion by NK cells in SHIV162P3 infected macaques

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Objective: HIV infection leads to a state of chronic immune activation and progressive deterioration in immune function, manifested most recognizably by the progressive depletion of CD4 T cells. Recently, we have shown that a cellular ligand for the NCR NKp44 (NKp44L) is expressed during HIV-1 infection and is correlated with both the progression of CD4 T cell depletion and the increase of viral load. The ligand's expression is strongly induced by a highly conserved motif of the HIV-1 envelope gp41 protein, called 3S. In addition, the production of anti-3S antibodies was detected in around 30% of HIV-infected patients and was inversely correlated with both the CD4 cells count and NKp44L expression on CD4 T cells.
Methods: Ten Macaques were either immunized with 3S-KLH or KLH alone and then infected with SHIV162P3. Blood samples were collected each week up to day 42. Cell surface expression of NKp44L was analyzed using flow cytometric analysis, the engagement of NKp44L during the NK lysis process was evaluated using a standard 51Cr release assay, and the level of anti-3S antibodies production was detected by ELISA in the serum samples.
Results: All animals immunized with 3S-KLH developed a strong immune response against 3S, whereas the animals immunized with KLH alone did not. After SHIV infection, CD4 T cells depletion increased with time in control animals. By contrast, anti-3S immunization inhibits CD4 T cells death, and the subsequent CD4 cells decline, without affecting viral replication. In support of our hypothesis, anti-3S immunization suppresses NKp44L on CD4 T cells, and then prevented the NK lysis activities.
Conclusion: These data which indicate that NK cells may have a deleterious role on CD4 T cells, and explain, at least partially, their depletion, raise new questions about the pathogenesis of HIV and present new concept of HIV vaccine strategy, based on 3S immunization, non against the pathogen, but against the pathogenesis.

0A01-04

Development of IgG1 b12 scaffolds and HIV-1 env-based outer domain immunogens capable of eliciting and detecting IgG1 b12-like antibodies

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Background: HIV-1 Env-based vaccine candidates generally elicit strain-specific antibodies against the variable regions. Nevertheless, neutralizing antibodies with high potency and breadth have been identified in some HIV-1+ individuals. Among them, IgG1 b12 recognizes a subdomain of the CD4 binding site and has been shown to neutralize a range of HIV-1 primary isolates. Recently, a crystal structure of IgG1 b12 in complex with a cavity-filled, disulfide-bond-stabilized HIV Env core was elucidated. Information obtained from the structure has defined the molecular nature of the interaction between this antibody and HIV envelope and now guides the design of immunogens that might elicit antibodies with similar properties.
Methods: Based on structural information, the feasibility of developing immunogens capable of inducing neutralizing antibodies similar to IgG1 b12 was explored. Two approaches have been taken, including the design of scaffolds containing the CD4/ IgG1 b12 binding loop and the development of immunogens based on the outer domain (OD) of the HIV Env core. A panel of b12 scaffolds and OD constructs were first evaluated by immunoprecipitation and Biacore analysis for binding to IgG1 b12, followed by immunogenicity studies in guinea pigs.
Results: Among five b12 scaffolds developed, two bound to IgG1 b12 with low affinity. One b12 scaffold, termed Sca 2, was used to detect IgG1 b12 like antibodies in vaccine-generated immune serum. Antibodies capable of binding the Sca2 in those immune serum were found with reasonable frequency. Different versions of OD constructs were also generated. Momomeric ODs had lower affinity to IgG1 b12 than oligomeric form of ODs. Immune serum from guinea pigs immunized with different ODs were analyzed for presence of antibodies similar to IgG1 b12. Interestingly, some monomeric ODs were able to induce production of antibodies capable of recognizing b12 Sca2 with higher efficiency than oligomeric form of ODs. The neutralizing activity of those immune serum is under evaluation.
Conclusion: Structural analogs of the target region recognized by the b12 antibody have been developed. Such scaffolds may be useful both in eliciting antibodies with specificity similar to IgG1 b12 and in detecting similar antibodies in serum. OD based immunogens can elicit antibodies recognizing the CD4 binding loop but whether those antibodies will be able access the same region on functional viral spike remains unknown at present.

OA01-05

Characterizing broadly reactive neutralizing antibody responses during HIV and SHIV infections

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Background: Most HIV-infected patients develop autologous neutralizing antibodies (NAbs) within a year following infection. In a few cases, patients also develop cross-reactive NAbs. The virologic and immunologic factors conducive to the development of such cross-reactive responses are currently undefined.
Objective: To identify factors conducive to the development of cross-reactive NAbs and to define their epitope specificities.
Methods: We analyzed the serum neutralizing activities of patients chronically infected with clade B HIV-1, with diverse viral loads and peripheral CD4+ T cell numbers. Some HIV-infected patients were categorized as 'Long Term Non-Progressors' since they remained clinically stable for at least a decade by maintenance of normal CD4+ T-cell counts (>500 cells/ul) and control of viremia without antiretroviral therapy. A TZM-bl based neutralization assay was used to identify patients with broad neutralizing responses. Broad neutralizing activity was determined by the ability of patient sera to neutralize multiple viral variants over time. Epitope mapping studies were used to further characterize the antibodies elicited by these patients.
Results: 9 of 31 patients studied to date neutralized 70-100% of the clade B viruses tested thus far. Some of these patients also displayed neutralizing activities against clade A isolates. In parallel, we identified rhesus macaques that developed cross-reactive NAbs within two years of infection with the CCR5-tropic SHIVSF162P4 virus. The breadth of these responses was similar to those observed in some HIV-1 chronically infected patients. Extensive mutations in the gp120 were observed over time, including changes in glycosylation pattern, which resulted in the emergence of viruses resistant to neutralization by several known broadly reactive NAbs. Epitope mapping studies indicate that these animals developed 2G12-like antibodies as well as high titers of CD4-induced antibodies.
Conclusion: Although we have not yet mapped the epitope specificities of NAbs elicited in HIV-infected patients with cross-reactive neutralizing responses, overall, our results suggest that continuous viral replication, viral Env evolution and escape from broadly reactive NAbs all contribute to the development of broadly reactive NAbs during HIV infection. They also indicate that the delayed development (i.e., during chronic infection) of such broadly reactive NAbs does not affect viral replication.

OA01-06

Targeting high mannose glycan epitopes on gp120 using a yeast glycosylation mutant as a novel antigen

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Background: The broadly neutralizing monoclonal antibody, 2G12, was found to bind to a cluster of at least three high mannose glycans exposed on the envelope glycoprotein gp120. Numerous glycan constructs have been designed to mimic the 2G12 epitope, but these have yet to produce antibodies that cross-react to gp120 or neutralize the virus.
Objective: To elicit antibodies to high mannose glycan epitopes presented on gp120.
Methods: A mutant strain of Saccharomyces cerevisiae with three deletions in the glycosylation pathway (delta-mnn1mnn4och1) was created and the N-linked glycans were analyzed by MALDI-TOF profiling. The yeast mutant was tested for 2G12 binding in whole cell ELISA, immunofluorescence, and Western blotting. Rabbits were immunized with whole, heat-killed cells of the glycosylation mutant or wild type (WT) yeast. The sera were tested for binding to gp120 by ELISA and pseudovirus neutralization in the NIAID/Duke (D. Montefiori) screen.
Results: Carbohydrate profiling of delta-mnn1mnn4och1 yeast shows the strain exclusively produces high mannose type N-linked glycans, with 95% represented by Man8GlcNAc2. Western blotting shows that 2G12 binds to several proteins present in the lysate of the engineered yeast, but not lysate from WT yeast or other glycosylation mutants, with the glycans necessary for this binding. Whole-yeast ELISA and immunofluorescence show that these proteins are localized to the cell wall with 2G12 binding still occurring under native conditions. Rabbits immunized with the glycosylation mutant showed the production of antisera (week 5) that binds to JR-FL gp120 expressed in 293-T cells ($P<0.001$), while sera from WT immunized rabbits showed no binding ($P<0.001$). Further, this sera cross-reacted with other mammalian cell-expressed Env glycoproteins from Clades B and C of HIV-1, and SIV. Neutralization showed that sera from mutant immunized rabbits blocked infection of both Clades tested (B and C) at a modest titer, with one rabbit showing broader neutralization (2/4 from Clade B and 3/3 from Clade C).
Conclusion: A yeast glycosylation mutant was created that can mimic the 2G12 epitope and elicit antibodies that cross-react to mammalian cell-expressed Env proteins from HIV-1 and SIV. This data represents the first instance in which neutralizing antibodies that target the glycan shield of gp120 were raised in animals, thus underlining the potential of the yeast mutant to produce a safe and cost-effective HIV vaccine.

0A01-07

Focusing the immune response on the V3 loop of the HIV-1 gp120 envelope induces cross-clade neutralizing antibodies

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Objective: Experiments were performed to determine if a DNA prime and protein boost immunization regimen, designed to focus the humoral immune response on a single neutralizing epitope of HIV-1 gp120, would result in cross-clade neutralizing antibodies.

Methods: Rabbits were immunized with three priming doses of gp120 DNA plasmids derived from HIV env genes from a virus carrying a clade A Env bearing the GPGR motif at the tip of the V3 loop and/or with env from a clade C virus bearing the V3 GPGQ motif. The rabbits subsequently received two booster immunizations with one or more recombinant fusion proteins consisting of a truncated form of the MuLV gp70 Env protein and V3 sequences from viruses of either HIV clades A, B or C (V3-FPs).

Results: Immune sera from animals receiving various prime/boost regimens neutralized several primary isolates, including strains heterologous to those from which the immunogens were constructed. Neutralizing activity was primarily due to V3-specific antibodies as shown by peptide blocking experiments. Immune sera from rabbits receiving several of the prime/boost regimens also displayed neutralizing activity against pseudoviruses carrying the SF162 env whose V3 was replaced with the consensus V3 regions from several clades. The broadest and most potent neutralizing responses were generally elicited using the gp120 DNA from clade C and a combination of V3-FPs carrying V3 sequences from clades A, B and C. Potent cross-clade neutralizing activity was demonstrated in the sera of these rabbits against V3 chimeric pseudoviruses carrying the consensus V3 sequences from clades A1, AG, B, AE, and F; the geometric mean titers to achieve 90% neutralization ranged from 1:1232 (clade B) to 1:156 (clade A1).

Conclusion: The results demonstrate that cross-clade HIV neutralizing antibodies can be elicited by focusing the humoral immune response on a neutralizing epitope such as V3.

0A01-08

Crystal structures of HIV-1 neutralizing anti-V3 monoclonal antibody 2557 in complex with V3 peptides

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Human anti-V3 monoclonal antibody (mAb) 2557 was derived from a clade AG HIV-1 infected patient from Cameroon, and it reacts strongly with subtype A and subtype B V3 loops expressed as fusion proteins with a truncated form of MuLV gp70; this mAb neutralizes several viruses from clades A, B, and C (Gorny et al., J. Virol. 2006). We have crystallized the Fab fragment of mAb 2557 in complex with V3 peptides derived from clades B and C and determined the structures to better than 2Å resolution. Structural analyses of these crystal structures revealed that the backbones of the peptides maintain a conserved structure similar to that observed in the Fab 2219/V3 structures (Stanfield et al, J. Virol., 2006). The contacts of the V3 peptide with the mAb can be divided into 3 successive regions: (i) the very tip of the crown, GPGQ/GPGQ, is placed against a universal Tyr residue; (ii) the tip is followed by the beta sheet with a hydrophobic face and a hydrophilic face that interacts with the antibody by van de Waals and specific interactions; (iii) the next region has charged residues that interact with the mAb by salt bridges. This is the first crystal structure of a neutralizing mAb from a patient infected with a non-B clade virus, and the atomic details of the V3 peptides complexed with the mAb may help facilitate structure-based HIV vaccine designs.

Session 2: Clinical Trials Part 1

0A02-01

Cellular and humoral immunogenicity of ADMVA, a clade C/B MVA-based HIV-1 candidate vaccine, in healthy volunteers

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Background: We are completing an 18 month Phase-I trial of ADMVA, a Clade-C/B-based HIV-1 candidate vaccine expressing Env, Gag, Pol, Nef, and Tat in a modified vaccinia Ankara vector. ADMVA was constructed based on CHN019, a circulating recombinant form from Yunnan, an area of high HIV-1 incidence in China.

Objective: To evaluate the safety and immunogenicity of ADMVA in healthy adult volunteers not infected with HIV.

Methods: ADMVA or placebo was administered intramuscularly to 48 healthy volunteers at low risk for HIV-1 at months 0, 1 and 6. In each dose group - $1x10^7$ (low), $5x10^7$ (mid), or $2.5x10^8$ pfu (high) - twelve volunteers received ADMVA and four received placebo in a double-blinded design. Subjects were followed for 18 months for local and systemic reactogenicity, adverse events, and clinical laboratory parameters. Humoral immunogenicity was evaluated by anti-gp120 binding ELISA and IFA as well as by an HIV-1 neutralization assay (Monogram). Cellular immunogenicity was assessed by an IFNγ ELISpot assay. Anti-vector immunity was measured by an anti-vaccinia binding ELISA assay.

Results: Overall, the vaccine was safe and well tolerated, with no vaccine-related serious adverse events or cardiac adverse events. Local or systemic reactions were reported by 77% and 78% of volunteers respectively, the majority of which were mild and transient. The IFNγ ELISpot response rate to any HIV antigen was 3/12 (25%) in the low-dose group, 4/12 (33%) in the mid-dose group, and 8/13 (62%) in the high-dose group. Responses were often multigenic and occasionally persistent. Analyses on the late samples for the high-dose group are still ongoing. There were no IFNγ ELISpot responses in placebo recipients. Binding antibodies to gp120 were detected in 7/12 (58%), 7/12 (58%) and 11/13 (85%) in the low-, mid-, and high-dose groups, respectively. Binding antibodies persisted up to 12 months after vaccination. All placebo recipients tested antibody negative. However, despite having anti-gp120 antibodies, only two volunteers had detectable neutralizing antibodies for clade-matched viruses.

Conclusion: ADMVA is safe, well tolerated, and elicits humoral and cell-mediated immune responses persisting up to one year after vaccination. Prime-boost regimens are envisaged.

0A02-02

Safety and immunogenicity of rMVA-HIV and rFowlpox (FPV)-HIV vaccines, alone or in combination, in healthy vaccinia-naïve HIV-1 negative participants

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Background: Several second generation pox vectors have been developed as HIV vaccines. This study was designed to evaluate the safety and immunogenicity of two separate replication defective pox vectors: one a Modified vaccinia Ankara (MVA), the other a Fowlpox virus (FPV). Each has matching HIV inserts from a perinatally-transmitted subtype B isolate (C58A1).

Methods: The 4 study vaccines were: 1) MVA-env/gag, 2) MVA-tat/rev/nef-RT, 3) FPV-env/gag, 4) FPV tat/rev/nef-RT; the controls were MVA and FPV without HIV inserts. Healthy vaccinia-naïve study participants (ppts) were enrolled into this 2-part study and received vaccination vs control IM (env/gag into left deltoid; tat/rev/nef-RT into right deltoid) at 5 timepoints (month 0, 1, 3, 5 and 7). Part A (5 groups: 10 active, 2 control/group) examined 5 doses of FPV-HIV (10^9 pfu/ml), 2 doses of MVA-HIV (10^7, 10^8 or 10^9 pfu/ml) boosted by 3 doses of FPV-HIV (10^9 pfu/ml), and 5 doses of MVA-HIV (10^9 pfu/ml). Part B (3 groups: 25/5 active vs control, all doses 10^9 pfu/ml) examined FPV-HIV x 5, MVA-HIV x 5, and MVA-HIV x 2 boosted with FPV-HIV x 3. Serial safety assessments included close monitoring for myopericarditis. Sera and PBMCs were collected at day 0 and 2 weeks after the 3rd and 5th immunizations for humoral and cellular (IFN-γ ELISpot and ICS using consensus B peptide pools) assays.

Results: Among the 150 ppts enrolled, 138 received at least 3 vaccinations. Safety data are blinded to receipt of vaccine/control. Study products were well-tolerated, including MVA-HIV at the 10^9 pfu/ml dose. No ppt had any indication of myopericarditis. Severe local/systemic reactions occurred in only 6/150 (4%); moderate local/systemic reactions occurred in 48% and 37%, respectively. Unblinded IFN-γ ELISpot data are available post-3rd vaccination. Assays were positive in 5/9 (56%) and 12/22 (55%) ppts in the MVA-HIV 10^8 and 10^9 pfu/ml prime/FPV-HIV boost groups, respectively. Among ppts who received MVA-HIV and FPV-HIV alone, assays were positive in only 5/22 (23%) and 2/22 (9%), respectively. The false positive rate was low (2/109) at study day 0.

Conclusion: MVA-HIV and FPV-HIV vaccines were well-tolerated in vaccinia-naïve healthy subjects. IFN-γ ELISpot responses were more frequent in ppts primed with 10^8 or 10^9 pfu/ml of MVA-HIV followed by a single dose of FPV-HIV than in those who received the same vector x 3. Results of ICS assays will help define the precise character of these responses.

0A02-03

Improved modes of delivering a safe and highly immunogenic multigene multiclade HIV-1 DNA plasmid vaccine boosted with HIV-1 MVA

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Background: DNA priming with MVA boosting has been reported to be poorly immunogenic in human HIV-1 vaccine trials, despite good results in non-human primates. The current study aims to optimize these modes of immunization. Objectives To investigate four different modes of delivering plasmid DNA prior to a MVA boost, both expressing HIV genes.

Methods: 40 healthy HIV negative volunteers, 7 women and 33 men, were randomised to 4 groups injected id or im with Biojector. They were primed with 3 injections with 7 DNA plasmids produced by KI/SMI and Vecura; plasmids containing gp160 of HIV-1 subtypes A, B, C and rev B were given in the left arm (with or without adjuvant rGM-CSF, sargramostim) and plasmids containing p17/p24 gag A, B and Rtmut B in the right arm at months 0, 1 and 3. The same volunteers were re-randomized to a single boost with MVA with HIV-1 genes env, gag, pol of CRF01A_E produced by NIAID and WRAIR at month 9, either with 10^7 pfu id or with 10^8 pfu im. IFN- γ and IL-2 ELISPOT responses were measured using fresh cells and peptide pools representing HIV-1 p17B, p24A, p55A, gp120A/B, gp120 B, gp41B and 2 RTB pools. Criteria for positive ELISPOT were >55 spots/ 10^6 PBMCs and 4 times the background.

Results: The vaccines were safe, but GMCSF was associated with some adverse events. Thirty-four of 37 evaluable (92%) had a positive IFN- γ ELISPOT response after the boost, 32 to Gag and 24 to Env, while 11/38 were reactive after HIV-DNA alone. One mg HIV-DNA id was as effective as 4mg im as a prime to the HIV-MVA boost. Addition of GMCSF was of no benefit. Prior remote smallpox vaccinations did not affect the ability of HIV-MVA to boost. 10^8 pfu HIV-MVA im gave a superior boost to 10^7 pfu id. Age above 40 was associated with markedly lower ELISPOT responses. Gender did not seem important. These results were supported by IL-2 ELISPOT (68% responders) and HIV-1 specific lymphoproliferation assays (92% responders). Only one volunteer remained non-reactive to HIV antigens. In diagnostic HIV serology 6 of 37 had a Gag response and one vaccinee reacted with gp120 3 months after the boost.

Conclusion: This HIV-1 DNA prime MVA boost approach was highly immunogenic. The highest vaccine induced immune responses were seen in vaccinees under 40 years of age given HIV-DNA either id (low dose) or im (high dose) and the HIV-MVA boost im.

0A02-04

HIV-1 DNA prime followed by recombinant MVA boost is well tolerated and immunogenic when administered to healthy HIV-1 seronegative adults in HVTN 065

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Background: We examined the safety and immunogenicity of an HIV-1 DNA/recombinant MVA prime-boost vaccine (GeoVax, Inc.) in healthy adult participants. A SHIV prototype had earlier induced robust immune responses in rhesus macaques that protected against the development of AIDS.

Methods: Vaccinia-naïve participants were vaccinated at months 0 and 2 with HIV-1 DNA and at months 4 and 6 with HIV-1 recombinant MVA. Ten of the participants received 0.3mg DNA followed by 10^7TCID_{50} MVA while 30 received 3mg DNA followed by 10^8TCID_{50} MVA. An additional 2 placebo recipients were included in the low and 6 in the high dose groups. While only the DNA preparation contained the tat, rev, and vpu genes, both the DNA and MVA constructs contained the same gag, protease, reverse transcriptase, and env genes. Participants were evaluated for safety throughout the study via clinical follow up and routine laboratory testing. Cardiac troponin levels and ECGs were performed at baseline and following MVA vaccinations. Participant samples were evaluated for vaccine-induced T cell responses by gamma interferon (IFN-γ) ELISpots and intracellular cytokine staining (ICCS) 2 weeks after the first and second MVA boosts.

Results: All analyses were performed and are presented in a blinded fashion. Both the low and high dose DNA and MVA preparations were well tolerated. Injection site pain and/or tenderness was either not present (39.5%) or rated as mild (52%) or moderate (8.5%). No systemic symptoms were noted in 50% of participants and the remainder had either mild (33.3%) or moderate (16.7%) symptoms. Side effects and tolerability were not significantly different between the low and high dose groups. There were no cases of myocarditis. Baseline IFN-γ ELISpot responses were uniformly negative. Following DNA prime and MVA boost, 5 of 9 (55.6%) of the participants in the low dose group had measurable responses by IFN-γ ELISpot (median response of 285 spot forming cells/106 PBMCs). Three of the responses were directed to Gag while another two were Env-specific. Immune testing of the high dose group as well as ICCS analysis is ongoing.

Conclusion: This HIV-1 DNA/MVA vaccine strategy is well tolerated at both the low and high dose formulations. Analyses from the low dose group demonstrated T cell responses in the majority of participants. These data support further development of this product as a candidate for a preventative AIDS vaccine.

OA02-05

Increase in cell-associated infectivity following therapeutic immunization in HIV-1-infected young adults on suppressive HAART

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Background: Persistence of replication-competent HIV-1 in CD4+ T cells and low-level viremia (LLV) preclude virus eradication and discontinuation of treatment. Therapeutic HIV-1 vaccines could alter the frequency and magnitude of CD4+ T cell-associated infectivity in patients suppressed on HAART by activating CD4+ T cells or enhancing HIV-1 specific immune responses.
Methods: We assessed changes in LLV and the frequency of replication-competent HIV-1 in CD4+ T cells (IUPM) in a longitudinal study of 20 infected young adults (18-24 years of age) enrolled in a Phase I trial of recombinant MVA vaccines (entry and 1 mo) and FP vaccines (2 and 6 mos) expressing HIV-1 proteins. Blood samples were assayed twice before and 4 times after vaccination through 40 weeks of study. An ultra-sensitive viral load assay with a detection limit of ≥6.5 copies/ml and a limiting dilution culture assay were used to measure LLV and IUPM, respectively. The prevalence of detectable IUPM (>0.1) and LLV (>6.5) before and after vaccination were analyzed using repeated measures (GEE) logistic regression models
Results: 143 culture assays and 115 ultra-sensitive plasma viral loads were assessed. At baseline, the mean IUPM was 0.48 (range ≤ 0.1 to 1.6) and 50% (9/18) of the study participants had levels of cell-associated infectivity ≤0.1 IUPM. Relative to baseline, levels of cell-associated infectivity was statistically different at wks 6, 24 and 40 (P=0.0009).in subjects with ≤0.1 IUPM levels pre-vaccine.. All study subjects had clinically undetectable viral loads to <50 copies/ml on HAART at screening and entry. The mean prevalence of LLV (detectable viremia between ≥ 6.5 and <50 copies/ml) before vaccine was 23%. At weeks 2, 4, 6 and 24-post vaccination, the prevalence of detectable viremia between 6.5 and <50 copies/ml was 17%, 16%, 25% and 24% respectively (P>0.4 relative to baseline for all time points). Only one of the study participants had a "blip" above 50 copies/ml (436 copies/ml) during study treatment.
Conclusion: "Blips" in cell-associated infectivity was observed amongst therapeutic HIV-1 vaccine study participants; this was particularly evident in those with undetectable (≤0.1) IUPM levels. Importantly, "blips" in cell-associated infectivity were not associated with breakthrough viremia or the development of drug resistance.

OA02-06

Therapeutic vaccination with MVA-nef: a randomized, controlled phase II study in 77 HIV-1 infected patients followed by structured HAART interruption

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Background: Cell mediated immunity (CMI) plays an important role in the effective control of HIV-1 replication during acute and chronic infections. MVA BN®, a viral vector which is safe and fails to replicate in human cells, has shown potent ability to induce cell mediated immune response in previous phase I/II studies. This phase II trial was initiated to assess the immunogenicity and safety of the MVA vector expressing HIV-1-LAI nef in HIV-1 infected patients.
Methods: In this single blind, controlled, randomized, multicenter phase II trial 77 HIV-1 infected patients received three s.c. vaccinations of either 1E8 MVA BN®, 1E8 MVA-nef or 5E8 MVA-nef (n=26, 25 and 26, respectively) at week 0, 8 and 16. Unsolicited and solicited AEs were captured. Viral load (VL) and CD4/CD8 counts were measured continuously during the study. Antibody response against MVA was measured by ELISA. Patients that had received all vaccinations and maintained HIV-1 RNA levels <50 copies/ml were offered a Structured Treatment Interruption (STI) at week 20 and were followed up until week 52 with close monitoring of VL as well as CD4/CD8 counts.
Results: MVA-nef proved to be safe and well tolerated. All patients reported transient injection site reactions, the majority mild to moderate; only few patients experienced systemic reactions. 60/77 subjects were pre-immune to vaccinia (childhood smallpox vaccination). All 77 subjects completed the immunisation schedule consisting of three vaccinations. 37 subjects interrupted HAART 4 weeks following the third vaccination (9:13:15 for 1E8 MVA BN®, 1E8 MVA-nef, 5E8 MVA-nef, respectively). During the 32 week post-STI period, 12 of 37 subjects restarted HAART and subsequently returned to VL below detection limit. Data collected from the 25 patients continuously off HAART until week 52 indicate significantly better control of the VL in the group vaccinated with 5E8 MVA-nef compared to the MVA BN® vaccinated control group. All patients developed a strong MVA-specific humoral immune response. Data on Nef-specific cellular immune responses will be available soon.
Conclusion: The results of this study confirm data of a previous Phase I trial indicating a trend in controlling VL during STI after therapeutic immunisation with MVA-nef. Results of the ongoing immune analysis may allow correlating viral control to Nef-specific cellular immune responses and to assess the influence of anti-vector immunity on the Nef-specific immune response.

0A02-07

Immunogenicity and clinical effects of GTU-MultiHIV B clade DNA vaccine in treatment-naïve HIV infected subjects

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Background: The development of an effective, safe and affordable HIV vaccine remains the best hope of controlling HIV, which globally continues to cause significant mortality and morbidity.

Objective: To evaluate the clinical effects, and immunogenicity of a DNA vaccine expressing complete sequences of Rev, Nef, Tat, p17/p24 proteins, and an epitope stretch of previously identified CTL epitope-rich regions encoded by pol and env of a subtype B-HIV-1 isolate Han-2 in subtype C HIV infected, treatment naive individuals.

Methods: 60 subtype C infected men (16%) and women (82%) with CD4 counts greater or equal 0.350 x 10^9/L and viral loads less or equal 50 copies/ml at the screening visit were randomly allocated to receive investigational vaccine or placebo either intradermally (1.5mg) or intramuscularly (3mg) using the Biojector device. Immunizations were administered at 0, 1 and 3 months. Safety and tolerability of the investigational vaccine were evaluated by clinical and safety blood monitoring and local and systemic reactogenicity and collection of other adverse events. Efficacy endpoints were evaluated by viral load, CD4+, CD3+ and CD8+ cell counts. Immunogenicity was evaluated by measuring cytotoxic T-lymphocyte activity assessed by IFN-γ ELISPOT assay and by measuring the antibodies against HIV-1 specific proteins (Nef and Gag). Preliminary safety and efficacy data are reported.

Results: There were no vaccine related serious adverse events. Half of the subjects reported reactogenicity events immediately after any of the injections which were more frequent in the ID group compared to the IM group. However, there were no differences in the number of reactogenicity events between active vaccine and placebo in either ID or IM group.

In subjects that receiveing vaccine there was a 0.4 log decrease in viral load compared to placebo (p less 0.01; repeated measures ANCOVA over all post-baseline visits).

Conclusion: GTU-MultiHIV B clade is safe and well tolerated. This therapeutic vaccine offers the potential to control HIV viral load at relatively low levels preventing transmission of infection to partners or from mother to child. The vaccine appears to maintain CD4 counts suggesting that this vaccine may be used either alone or in combination to supplement the therapy of HIV infected individuals. This is important in developing countries where access to antiretroviral therapy is limited.

Session 3: T Cell and Mucosal Immunity

0A03-01

Induction of long-lived polyfunctional SIV-specific CD8+ T cells in the genital mucosa by live-attenuated lentivirus immunization

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Background: Although robust CD8+ T cell responses are induced in cervicovaginal tissues by SIV infection, they are delayed relative to virus replication. Thus, a vaccine-induced T cell response in the genital tract could control viral replication before infection is established. Infection with attenuated SHIV89.6 protects monkeys from uncontrolled viral replication after vaginal challenge with SIVmac239.
Objective: To define the timing and quality of the SIV-specific CD8+ T cell response in the cervicovaginal tissues of SHIV-vaccinated rhesus macaques after intravaginal SIVmac239 challenge.
Methods: Vaccinated and non-vaccinated animals where necropsied at 0, 7 and 14 days after intravaginal SIVmac239 challenge, and the lymphocytes from the cervicovaginal tissues where analyzed by polychromatic flow cytometry after Gag specific stimulation.
Results: Polyfunctional Gag-specific CD8+ T cell responses were present before challenge (day 0, n=6), at day 7 (n=6) and 14 (n=4) post-challenge (PC) of all SHIV-immunized animals examined. Although some vaccine naive animals had responses at 7 and 14 days PC, the responses were less frequent and more monofunctional compared to vaccinated monkeys. IL-2 secretion was consistently more common in Gag-specific CD8+ T cells of vaccinated monkeys. In addition, at 14 days PC the frequency of SIV-specific Bcl-2+ CD8+ T cells was lower in vaccine naive animals compared to vaccinated animals. There was also an increase of caspase-3 expression and effector phenotypes in unstimulated CD8+ T cells from unvaccinated monkeys, but not vaccinated monkeys, at 14 days PC.
Conclusion: These findings suggest that expansion of CD8+ T cell memory and effector pools at the local sites immediately after challenge help contain virus replication in vaccinated monkeys.

0A03-02

Intramuscular administration of adenovirus vector-based HIV vaccine candidates induces potent and persistent mucosal CD8+ T-cell responses

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Background: Mucosal surfaces and associated lymphoid tissue represent primary sites of HIV transmission, early replication, and massive and irreversible destruction of memory CD4+ T-cells. Induction of effective mucosal immune responses is therefore a priority for an HIV vaccine, but most vaccine candidates are administered intramuscularly rather than being routed directly to mucosal surfaces. The magnitude, durability, and phenotype of mucosal CD8+ T-cell responses elicited by intramuscular administration of recombinant adenovirus (rAd) vectors have not been fully characterized.
Objective: To evaluate the magnitude and phenotype of mucosal cellular immune responses elicited by intramuscular administration of various rAd vector-based HIV vaccine candidates.
Methods: C57BL/6 mice and rhesus macaques were immunized intramuscularly with various rAd vectors expressing SIV Gag. Lymphocytes were isolated from peripheral blood, lymph nodes and a variety of mucosal tissues. Gag-specific CD8+ T-cell responses were evaluated by tetramer staining and multiparametric flow cytometry.
Results: Intramuscular administration of replication-incompetent, recombinant adenovirus serotype 5 (rAd5) vectors expressing SIV Gag in C57BL/6 mice induced potent CD8+ T-cell responses in multiple mucosal compartments, including the small and large intestine and the vaginal tract. The magnitudes of these responses were generally comparable with those in peripheral blood and spleen as determined by tetramer binding assays. Rare serotype rAd26 and rAd35 vectors as well as hexon-chimeric rAd5HVR48 vectors also induced high frequency mucosal CD8+ T-cell responses. Gag-specific mucosal immune responses persisted for at least 24 weeks after a single injection and were characterized by the progressive accumulation of effector memory T-cells at mucosal surfaces and central memory T-cells in lymph nodes and Peyer's patches. Pilot studies in rhesus macaques similarly showed that intramuscular administration of rAd5HVR48-Gag elicited potent CD8+ T-cell responses in duodenal mucosa that were approximately half the magnitude of responses observed in peripheral blood.
Conclusion: Intramuscular immunization with rAd5 and novel rAd vector-based vaccines induces potent, persistent, and dynamic mucosal cellular immune responses in both mice and rhesus macaques.

0A03-03

Systemic delivery of plasmid-encoded CCL27 and CCL28 adjuvant mucosal immune responses to DNA vaccines

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Background: The induction of a mucosal immune response is a crucial goal for HIV vaccines, as the mucosa is the primary site of HIV transmission and initial viral replication. There is a great need for a safe, effective, and systemically administered vaccine to induce HIV-1 specific immunity at mucosal sites. Mucosal immune cell homing is controlled by a unique subset of chemokines including CCL27 and CCL28 and the shared receptor CCR10. Gut lymphocytes expressing CCR10 include IgA secreting cells, trafficking them to the mucosal tissues where the chemokines are expressed.
Objective: To systemically co-immunize with CCL27 or CCL28 in combination with our HIV-1 Gag DNA vaccine and elicit mucosal immunity.
Methods: Wild type BALB/c or CCR10-/- mice (BALB/c background) were immunized with intramuscularly three times with our HIV-1 Gag in combination with CCL27 or CCL28. Immunizations were two weeks apart and both cellular and humoral immune analysis was carried out one week following the final boost. Assays include IFN-γ ELISPOT, ICS, Proliferation (CFSE), B-cell ELISPOT, and antibody ELISA.
Results: In BALB/c mice, co-immunizations with mucosal-derived chemokines resulted in redirection of CCR10+ and α4β7+ immune cells to the injection site. Co-immunization with CCL27 and CCL28 increased cellular immune responses including enhanced cytokine expression by antigen-specific T cells from the spleen, lung, and lamina propria. In addition, enhanced humoral immune responses were also observed including elevated antigen-specific IgG and secretory IgA responses in secondary lymphoid organs, B cells from the gut, peripheral blood, and fecal extracts. Interestingly, as CCR10 is the receptor for both CCL27 and CCL28, enhanced CTL and antibody responses elicited are abrogated in CCR10-/- mice.
Conclusion: We demonstrate that a novel systemic administered DNA vaccination strategy utilizing co-delivery of mucosal-derived chemokine adjuvants, produces mucosal immune responses. The observed responses are due to the specific effects of CCL27 and CCL28 on immune cells expressing, CCR10, as responses are abrogated in knockout mice. Ongoing experiments will further characterize antigen specific responses in the mucosa using the LCMV transgenic mouse model. These studies are important for understanding lymphocyte homing and commitment as well as generating mucosal responses from a systemically delivered vaccine. Support by N01-A1-15429, NIAID-HVDDT to DBW

0A03-04

Increased frequencies of regulatory T cells and limited function of CD4+ effector T cells in infant macaque blood: implications for SIV pathogenesis

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Background: HIV-1-infected infants often have limited control of virus replication and faster disease progression. The mechanisms for these age-related differences in pathogenesis have not been clearly defined. One possibility is that effector T cell responses are limited by the immaturity of the infant immune system. In addition, however, our group recently reported that fetal human tissues contain a large number of regulatory T cells. These cells may also serve to limit vital effector responses or might limit detrimental T cell activation among "bystander" T cells.
Objective: To assess the influence of a large regulatory T cell population and altered cytokine production on SIV disease progression, T cell responses, and T cell activation.
Methods: Effector T cell phenotypes and function in uninfected infant macaques were followed for one year. Regulatory T cells were identified and their number and function compared to those of adults. Finally, adult and infant macaques bearing different numbers of regulatory cells were infected with SIV. Effector responses were assessed in vitro in the presence or absence of T-regs, in order to determine whether a large population of regulatory cells in infants affects immune responses to SIV. Expression of T cell activation markers was also followed.
Results: The frequency of regulatory T cells and their suppressive activity is significantly higher in infant than adult macaques. Furthermore, infant T cells have a reduced capacity to produce certain cytokines, in particular IFN-γ. SIV-infected infants had a more rapid clinical course. T cell responses to SIV were largely absent after the first month of infection; however, anti-SIV responses among CD4+ cells could be restored by depletion of T-regs. Ongoing active suppression of CD4+ T cell responses was associated with failure of CD8+ responses, which were not restored by T-reg depletion. Widespread T cell activation was not reduced by the presence of more T-regs.
Conclusion: T cell effector functions in SIV-infected infant macaques are limited both by intrinsic factors and by abundant, highly-functional regulatory T cells. In infected infants, T-regs directly suppress CD4+ T cell responses to SIV. CD8+ T cell responses are initially detected but fail within the first month of infection. These responses are not directly suppressed by T-regs and likely fail instead due to lack of support from suppressed CD4+ cells or to functional immaturity of CD8+ cells.

OA03-05

T-cell recognition of nef epitope variants in early HIV-1 infection

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Objective: To understand how broad recognition of circulating HIV-1 variants may be achieved with T-cell based vaccines, we examined the ability of T-cells from early-infected persons to recognize a broad spectrum of potential T-cell epitopes (PTE) within the context of the infecting autologous virus population.
Methods: Nef-specific T-cells were identified by their ability to produce IFN-γ or granzyme B, a key mediator of cytotoxic cell death, when stimulated with a novel peptide panel providing 70% coverage of PTE among subtype B isolates. T-cell recognition of a total of 150 nef variants encompassing 35 epitopes was assessed in 17 subjects with demonstrated Nef responses. For each of the 35 domains the optimal epitope was either confirmed experimentally or inferred based on the subject's HLA type and published MHC class I restricted epitopes. Autologous sequences were amplified by standard nested PCR in eight subjects. Amino acid substitutions were scored for similarity using the BLOSUM matrix. Scores of +2 and +3 were imputed conservative, 0 and +1 semi-conservative and -1 and below non-conservative.
Results: With the referent being either the autologous (when available) or the subtype B consensus, 73 of the 115 variants examined contained one substitution, 35 contained two, and seven contained three. T-cells recognized 52.1% of the variants containing one substitution with a < 0.5 log difference in response magnitude in contrast to 25.7% (P=0.0098) that contained two and none (P=0.0125) that contained three. Variants containing conservative substitutions were recognized at a significantly higher frequency [62.2% (23 of 37)] vs. those containing semi-conservative [37.9% (11 of 39), P=0.0029] and non-conservative substitutions [7.5% (3 of 40), P<0.0001]. Even when variants were recognized, there were significant differences in magnitude and/or avidity of both IFN-γ and granzyme B responses. Effects on both the binding of the peptide variants with the HLA molecules and that of the peptide-MHC complexes with the T-cell receptors contributed to the difference in responses to the variant epitopes.
Conclusion: While Nef-specific T-cells capable of recognizing multiple variants are commonly induced during early infection, semi- and non-conservative substitutions and those affecting more than one residue are infrequently tolerated. Our findings suggest that induction of broad immunity will require vaccines that encompass multiple sequence variants.

OA03-06

CTL epitope analysis in MRKAd5 HIV-1 gag/pol/nef vaccine recipients

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Objective: To assess the breadth and specificity of the T cell response in recipients of Merck Adenovirus type 5 (MRKAd5) vaccine vectors expressing HIV-1 clade B gag, nef and pol.
Methods: Peripheral blood mononuclear cells (PBMC) were collected from HIV-negative phase I study volunteers who received MRKAd5-gag/nef/pol vaccine at doses of $3x10^6$ – $1x10^{11}$ adenovirus genomes (vg). Samples from a cohort of 70 vaccinees were selected for CTL epitope analysis based on positive responses to HIV gag, nef and/or pol 15mer peptide pools in a validated interferon-gamma (IFN-γ) ELISPOT assay. The assay was modified and validated for gag-nef-pol epitope mapping using 194 pools of eight 9mer peptides overlapping by 8 amino acids. The number and location of CD8+ T cell epitopes were evaluated in the context of high resolution HLA typing for each donor.
Results: Epitope analysis demonstrated that CD8+ T cell epitopes were recognized across the entire length of all 3 proteins, with a median of 3 epitopes per subject (1 each in gag, nef and pol) and a range of 0-30. Specific epitopes associated with acute infection were detected at a higher frequency than those associated with chronic infection. For example, the A*0301-restricted gag epitopes within amino acids (aa) 18-29 were detected in 10/14 subjects presenting this allele, but 0/35 A*0201 subjects responded to the gag SL9 epitope associated with chronic infection. In nef mapping, 6/11 subjects with A*1101 responded to the 84-92 epitope observed frequently in acute infection but none of these A*1101 subjects responded to the nef epitope at aa 73-82. Responses to novel epitopes were also observed in this vaccinee cohort, particularly within the nef sequence.
Conclusion: The MRK Ad5 trivalent vaccine induces a broad T cell response in terms of epitope frequency and recognition. The predominant epitopes appear to be those associated with acute infection as well as novel epitopes. When current Phase II proof-of-concept studies are analyzed for efficacy, measures of breadth and specificity may provide information about potential correlates of vaccine efficacy.

0A03-07

Primary CD8+ T cell responses to immune escape variants of HIV-1

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Objective: Host control of HIV-1 infection requires a robust and broad spectrum, anti-HIV-1 CD8+ T cell response. Loss of this T cell reactivity to constantly evolving variants of HIV-1 by undefined mechanisms leads to progression of HIV-1 infection (termed immune escape). We have examined whether immune escape of HIV-1 is related to the inability of HIV-1 variants to prime naive CD8+ T cells to become memory T cells capable of responding to these variants.

Methods: CD8+ T cells were obtained from several HLA A*0201 B*0702 HIV-1 seronegative subjects and from an HIV-1 progressor >1 yr before seroconversion (HIV-1 plasma RNA and antibody negative) in the MACS. The T cells were primed with autologous, CD40L-IFN gamma matured dendritic cells (DC) loaded with HIV-1 clade B gag, env and nef consensus overlapping peptides or HIV-1 gag, env and nef peptides derived from sequences of the HIV-1 infected MACS subject ("autologous HIV-1"). Primary T cell responses (IFN gamma ELISPOT and tetramer staining) before seroconversion in the MACS subject were compared to memory T cell responses detected during his 15 years of infection, before and after HAART.

Results: Robust, broad spectrum, primary CD8+ T cell responses were induced by peptide-loaded DC, which required help from autologous CD4+ T cells. Multi-epitope, primary responses to gag, env and nef were induced by the clade B peptides and the autologous HIV-1 peptides in CD8+ T cells from the seronegative donors and the HIV-1 subject obtained >1 year before seroconversion. This included primary T cell reactivity to autologous HIV-1 variants that exhibited immune escape, i.e., failed to activate memory T cell responses several years after seroconversion. Memory CD8+ T cell reactivity to some but not all of the autologous HIV-1 variants was restored in this MACS subject several years after he received virus-suppressive HAART.

Conclusion: These results show for the first time that a robust, multi-epitope, primary CD8+ T cell response can be induced to consensus and autologous HIV-1 strains, including immune escape variants. This study indicates that the human host has the necessary repertoire of naive CD8+ T cells to respond to HIV-1 variants, even though memory T cell reactivity is lost over time to these HIV-1 epitope sequences during progressive infection. This supports use of HIV-1 prophylactic and immunotherapeutic vaccines that target DC to prime broad T cell reactivity.

0A03-08

Correlation between intracellular epitope stability and HIV epitope hierarchy

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Background: Inducing protective HIV-specific CD8 T cell responses will likely be a critical arm of HIV vaccine design. The success of this vaccine relies not only on the identification of the proper epitopes but also on the capacity of vaccinated tissues to produce and present these epitopes to the immune system. Defining predictable events leading to efficient epitope presentation will be critical to selection of vaccine sequences. The degradation of proteins into epitopes through the MHC-I antigen processing pathway precedes epitope presentation to CTL. We previously showed that antigen processing contributes to HIV-1 immunodominance by modulating the production of epitopes. Whether intracellular stability of optimal epitopes may contribute to immunodominance by favoring the accumulation or destruction of epitopes has never been addressed.

Objective: To compare intracellular epitope stability with epitope hierarchy and to identify factors involved in epitope stability.

Methods: We combine novel assays of degradation of HIV peptides in PBMC extracts and computational analyses of HIV-1 sequences. Optimal epitopes were incubated with PBMC extracts for up to 60 minutes. The amount of epitopes remaining over time was assessed by HPLC profile analysis, where the surface of the peak corresponding to one peptide is proportional to the amount of epitope. In order to identify residues involved in epitope stability, we mutated two unstable epitopes and measured the stability of the mutants. Finally, we performed a computational analysis of stable and unstable epitopes to identify motifs involved in peptide stability.

Results: We analyzed 100 optimal HIV-1 epitopes and showed that the stability of epitopes is highly variable with 0 to 90% remaining after a 10-minute incubation with PBMC extracts. The stability of epitopes largely correlates with the dominance hierarchy for many HLA. Point mutations in unstable epitopes yielded an 8-fold increase of intracellular stability, thus identifying residues protecting epitopes from degradation. Computational analyses of stable and unstable epitopes lead to the identification of motifs involved in epitope stability.

Conclusion: These data are the first identification of a new factor contributing to HIV epitope hierarchy, namely the critical role of epitope intracellular stability, and suggest that defined changes to epitope sequences at non-essential positions may be used to optimize epitope production from vaccine vectors.

Session 4: Vaccine Concepts and Design Part 2

0A04-01

Correlates of protection after yellow fever vaccination : a benchmark for HIV vaccines

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Objective: The yellow fever vaccine strain 17D (YFV17D) is one of the most efficacious attenuated viral vaccines and thus could be an excellent model for the study of immune responses associated with an effective protection. Here, we used several techniques to study the development of T cell immune memory following vaccination with YFV17D, in a cohort of human volunteers.
Methods: We immunized 20 healthy volunteers with YFV17D, and blood was drawn from them at the time of vaccination and at 3, 7, 10, 14, 28, 60, 90, 180 and 365 days post-vaccination. Some of the peripheral blood mononuclear cells (PBMC) contained in the samples were isolated and their RNA was analyzed by cDNA microarray. The remaining cells were cultured with pools of overlapping peptides derived from YFV17D, stained and analyzed by multiparametric fluorocytometry. The kinetics of cytokine expression by those cells was studied by cytometric bead assay.
Results: RNA expression analyses revealed the identity of specific genes, mostly encoding anti-apoptotic proteins, that are up-regulated early in response to the vaccine (days 3 to 7). Similarly, we found that another set of genes coding for pro-apoptotic proteins were down-regulated during the same period. Incubation of the PBMC with YFV17D-derived peptide pools stimulated T cell proliferation as early as day 10 post-vaccination, demonstrating the development of an immune response specific to YFV17D epitopes. Staining of unstimulated cells for Ki67 revealed the expansion of large numbers of central and effector memory T cells as early as days 7 and 10 in the CD4 and CD8 compartments, respectively. Some of the Ki67+ cells were shown to be antigen-specific, as evidenced by their swift expression of the activation marker CD154 and the cytokines IL-2 and IFN-γ when stimulated with peptide pools. While the cytokine profile in the supernatant of peptide-stimulated PBMC varied depending on the volunteer and the peptide pool, both TH1 and TH2 responses were observed with a clear tendency towards an anti-inflammatory response.
Conclusion: Expression of YFV-signature genes occurred as early as day 3 and the induction of memory T cells occurs as early as day 10 following vaccination with YFV17D, and this protective immune response correlates with a Th1/Th2 cytokine profile. This work was supported by grants to RPS from Génome Québec /Génome Canada and CANVAC, the network for Vaccines and Immunotherapeutics.

0A04-02

Next-generation adenovirus-based HIV-1 vaccine candidates

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Background: We have been developing an HIV-1 vaccine based on a replication-incompetent human adenovirus type 5 expressing clade B gag, pol, and nef sequences. Two Phase II proof-of-concept studies to test the efficacy of this vaccine in predominantly clade B and clade C regions began in Dec 2004 and Feb 2007, respectively. A potential limitation of this vaccine involves the non-optimal immunological coverage elicited by the clade B vaccine against non-clade B HIV-1 infections.
Objective: The aim is to identify and experimentally test novel sequences which will enhance the cross-reactivities of the current clade B sequences both within a given HIV-1 clade and across clades.
Methods: An algorithm was developed to design novel sequences which, when added sequentially to the current clade B gag and nef vaccine sequences (the more variable of the 3 antigens in the current vaccine), improve the epitope (CD4+ and CD8+) coverage against a global HIV-1 database. The algorithm threads 16-amino acid (aa) fragments derived from a set of HIV-1 viral sequences collected from Merck studies and from the Los Alamos National Laboratory database. In the analysis, sequences were weighted such that each patient contributed equally (to correct for multiple sequences belonging to a single patient) and according to the global distribution of the different clades/subtypes.
Results: The resulting novel sequences (two per antigen) do not represent clade-specific consensus sequences. Instead, they maintain continuity with HIV-1 viral isolates across a 16-aa window; no artificial junctions are created, and T cell epitopes found only in clinical isolates are preserved. Addition of the two novel gag sequences to the CAM-1 (Clade B) gag in the current vaccine candidate improves the coverage from 71% to 87% of all potential 9-aa epitopes in the global database. When the novel gag sequences were incorporated into the replication-defective human adenovirus type 6 vectors and administered into rhesus macaques, the immunological reactivities of the PBMCs with peptide pools of representative clade A and clade C sequences improved significantly (by 3-4 fold) when compared to those from monkeys immunized with clade B vaccine alone. The approach is being extended to nef and the resultant multi-component vaccine product will be limited only by the size and number of transgenes which can be accommodated into the vector(s).

0A04-03

Induction of persistent multifunctional T cell responses with a multi-antigen DNA vaccine injected intradermally with electroporation in macaques

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Objective: To improve the immunogenicity of DNA vaccine, by using the binding capacity of the bovine papilloma virus E2 protein, and the electroporation (EP) to increase the DNA uptake after intradermal (i.d.) injection.

Methods: We have designed a MultiHIV antigen which includes complete sequences of rev, nef, tat, p17 and p24 proteins from HIV. This MultiHIV sequence was introduced in a vector containing the nuclear factor E2 from bovine papilloma virus, which interacts with its specific binding site in the vector and mitotic chromosomes. These interactions provide nuclear segregation of the vector in dividing cells. The vector was administered via i.d. injections into 8 cynomolgus macaques. EP of the skin at the injection site was performed for 4/8 vaccinated animals. 4 additional macaques were vaccinated with an empty vector under the same conditions. The follow up study of immune responses was analyzed by using ELISA to detect antigen specific antibodies (Ab) and interferon (IFN)-γ Elispot method to detect peptide specific T cells. Intracellular cytokine staining (ICS) was performed to identify T cell subpopulations secreting IL-2 or IFN-γ.

Results: After the 2nd i.d. injections and EP, all 4 animals showed Nef and Gag specific Ab responses. These responses were boosted after the third injection but then decreased. After i.d. injections without EP, the responses were weaker. IFN-γ T cell responses specific for all the plasmid-encoded proteins were detected by Elispot in all the vaccinated animals. EP induced stronger T cell responses, reaching 3932±780 SFC/million PBMC at the peak (mean±SD), that were still high 18 months after the third injection (1337±461 SFC/million PBMC at day 546 post vaccination). We have focused on Rev and Gag specific strong IFN-γ T cell responses. We have shown that they were mediated by CD8 T cells that recognized epitopes included in epitope enriched regions for human. ICS analysis showed that the vaccine-induced T cells were mainly CD8+ and 25-30% of these responding cells produced both IL-2 and IFN-γ.

Conclusion: Vaccination of macaques with a single plasmid encoding several HIV proteins induced Ab and long lasting T cell responses, including multifunctional CD8 T cell responses. The immunogenicity of the plasmid did not need boost with other powerful vector to be revealed. The mapping of recognized epitopes shows that the vaccine-induced T cells were relevant with the human anti-HIV CD8 T cell responses.

0A04-04

Enhanced immune responses to co-vaccination of HIV and IL-12 plasmids using constant current electroporation in non-human primates

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Responses induced by DNA immunization in larger animal models have been inexplicably weaker than expected based on the large amount of data collected in mouse models. We performed a study in non-human primates in which we examined the immunogenicity of HIV gag and env DNA cassettes using constant-current electroporation (CCE) compared to animals receiving the identical vaccines and vaccine dose via IM injection alone. Animals administered HIV vaccines using CCE had significantly higher antibody titers ($p<0.05$) as compared to IM injection alone. Significantly higher positive HIV gag and env ELISpots were also demonstrated after only a single immunization in the group immunized with CCE, and remained significantly higher than IM injection ($p<0.05$) following three immunizations. These results indicated that plasmid delivery with CCE greatly enhances the immune potency of DNA vaccines in non-human primates. In a subsequent study, we co-vaccinated using HIV vaccines and a IL-12-expressing plasmid as an adjuvant. After a single immunization using the HIV plasmids in conjunction with the IL-12 plasmid and CCE, ELISpot counts were several-fold higher than the previous study. Following a second immunization, the antibody levels were fully double the amount from the previous study at the same time point. Animals administered the HIV plasmid vaccines with IL-12 had significantly higher $CD8^+$ proliferation ($p<0.05$) and an increased number of $CD8^+$ T cells that were positive for IFNγ, IL-2 and TNFα by intracellular cytokine staining, indicating a robust, polyfunctional T-cell response. Finally, these ELISpot results were comparable to the reported values for vaccination using viral vectors. By using the CCE technology and the cytokine plasmid, this study provides the first exciting evidence for a functional therapeutic vaccine for HIV without using viral vectors.

0A04-05

Modulation of DNA vaccine-elicited CD8+ T lymphocyte epitope immunodominance hierarchies

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Background: Generating broad cellular immune responses against a diversity of viral epitopes is a major goal of current HIV-1 vaccine strategies. Virus-specific CD8+ T lymphocyte responses, however, are often highly focused on a very limited number of epitopes as a result of immunodominance constraints. It has proven difficult to develop strategies that augment the breadth of cellular immune responses elicited by candidate HIV-1 vaccines.
Objective: To investigate epitope modification strategies as a novel approach to modulate CD8+ T lymphocyte epitope immunodominance hierarchies elicited by DNA vaccines in mice.
Methods: C57BL/6 mice were immunized with DNA vaccines encoding either wildtype SIVmac239 Gag (Gag WT) or a modified SIV Gag (Gag dAL11) specifically lacking the dominant Db-restricted CD8+ T lymphocyte epitope AL11 (312-322; AAVKNWMTQTL). Responses to the dominant AL11 epitope and the subdominant Db-restricted CD8+ T lymphocyte epitope KV9 (76-84; KSLYNTVCV) were assessed by intracellular cytokine staining and ELISPOT assays. Mice were boosted with rAd5 vectors expressing Gag WT and challenged with $5x10^6$ pfu recombinant vaccinia-Gag.
Results: Mice immunized with a DNA vaccine expressing Gag WT developed dominant AL11-specific responses that were over 4-fold higher than subdominant KV9-specific responses as expected. In contrast, mice immunized with a DNA vaccine expressing Gag dAL11 generated a marked and durable augmentation of KV9-specific responses. In fact, mean KV9-specific responses elicited by the DNA-Gag dAL11 vaccine (0.73% of CD3+CD8+ lymphocytes) were comparable in magnitude to mean AL11-specific responses elicited by the DNA-Gag WT vaccine (0.69% of CD3+CD8+ lymphocytes). Interestingly, priming with the DNA-Gag dAL11 vaccine and boosting with the rAd5-Gag WT vector resulted in balanced, codominant responses against both epitopes. This regimen proved significantly more effective than the unmodified DNA-Gag WT prime, rAd5-Gag WT boost regimen in controlling a recombinant vaccinia-Gag challenge ($p<0.05$, ANOVA).
Conclusion: These data demonstrate that dominant epitopes can dramatically suppress the immunogenicity of subdominant epitopes in the context of DNA vaccines in mice. Epitope modification strategies can be utilized to enhance responses to subdominant epitopes and improve protective efficacy against a recombinant vaccinia challenge.

0A04-06

MVA versus NYVAC vectors expressing SHIV antigens induce preferential CD8+ versus CD4+ T-cell responses but give similar efficacy against SHIV89.6p

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Pox virus vectors have been proven to be very successful in DNA prime-pox virus boost settings. However, differences exist between various pox-based vaccine vectors that may have an impact on their immunobiology. Here we compared immunogenicity and efficacy of two lead HIV-1 pox vector T-cell based vaccine appraoches NYVAC and MVA, currently in phase I human trials, in the SHIV macaque model. A DNA-prime (0, 4 weeks), pox-vector boost (20, 24 weeks) immunisation schedule identical to Eurovacc clinical trials was used to compare MVA and NYVAC recombinants expressing HIV-1 Env and SIVmac239 Gag/Pol/Nef antigens. The study consisted of 3 groups of 7 animals each (group 1: MVA boost, group 2: NYVAC boost, group 3: controls). Cellular immune responses revealed marked boosting of IFN-Gamma as well as IL-2 and IL-4 vaccine-specific responses with similar kinetics induced by either poxvirus vector. However, a preferential induction of peptide specific CD8+ T-cell responses was observed in MVA boosted animals, while CD4+ T-cell responses tended to predominate in NYVAC boosted animals. HIV-1 neutralizing antibody titers were low and similar in both groups, but insufficient for protection from the HIV-1 env bearing SHIV89.6p challenge. Control of virus load and extended survival was achieved in both groups as compared to controls. Although in this study similar efficacy was achieved in both groups, the type of immune responses induced may have consequences on long term survival.

0A04-07

A heterologous rMuVgag/rVSVgag prime-boost vaccination regimen elicits a very robust gag-specific cellular immune response in rhesus macaques

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Previous work has shown that rVSV vectors expressing HIV-1 Gag can elicit 500-1000 gag-specific IFN-γ ELISPOTs/10^6 PBMCs following an IM prime-boost inoculation regimen in rhesus macaques. One strategy to enhance gag-specific immune responses is the administration of heterologous vectors in prime-boost vaccination regimens. Here, we describe the generation of a novel recombinant mumps virus vector expressing HIV-1 gag (rMuVgag), derived from the Jeryl Lynn vaccine strain of MuV, and the gag-specific immune responses elicited following vaccination of rhesus macaques with rMuVgag and rVSVgag in heterologous prime-boost regimens. Gag-specific humoral immune responses were modest following prime-boost vaccinations. However, in animals primed either once or twice with rMuVgag and boosted with rVSVgag, average peak cellular responses of 3000 and 3500 gag-specific IFN-γ ELISPOTs/10^6 PBMCs were detected post boost, respectively; declining to an average of ~700 IFN-γ ELISPOTs for each group at 4 weeks post boost. T cell depletion studies showed a bias towards a CD8+ response following rVSVgag boosting. These data further support the notion of using heterologous HIV-1 vaccine vectors in prime-boost vaccination regimens and indicate a possible role for rMuV as an HIV-1 vaccine vector.

0A04-08

Comparative efficacy of gag/pol/env vaccines derived from temporal isolates of SIVmne against cognate virus challenge

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Early and late isolates of HIV often differ in coreceptor usage, replicative capacity and neutralization sensitivity. While it seems reasonable that vaccines should prevent or limit infection by transmitted viruses, little is known about the relative immunogenicity and protective efficacy of vaccines derived from temporal isolates of lentivirus infection. We aim to examine this issue using the SIVmne model.

SIVmne170 is a molecular clone isolated from a pig-tailed macaque 170 wks after inoculation with SIVMneCL8. CL8 and 170 viruses exhibit phenotypes typical of early and late isolates, respectively. We examined the protective efficacy of vaccines derived from CL8 or 170 against CL8, 170, or chimeras 8/170 and 170/8, containing the 5' or 3' half of the respective parental genomes. Pig-tailed macaques (N=16/arm) were primed with recombinant vaccinia viruses expressing env and gag-pol genes of CL8 or 170, and boosted ~1 yr later with the cognate Env and Gag-Pol proteins. A month later, 4 animals in each arm were challenged by an IV inoculation of CL8, 170, 8/170 or 170/8.

Both SIV-specific antibody and cell-mediated responses were generated. Levels of response were similar in CL8- or 170- immunized animals. As reported, CL8 infection resulted in low (10^4-10^6/ml) peak viral loads (VL) with few persistent viremia, whereas 170 infection led to high and persistent VL (10^6-10^8/ml). Infection with the chimeric viruses resulted in persistent viremia, with mean VL ~10-fold lower than that in 170-infected animals. CD4+ T cell decline was observed within 9 months of infection in the majority of animals infected with 170 and the chimeric viruses, but not in CL8-infected ones. As expected, CL8 vaccines protected animals against the CL8 virus, but not 170. Surprisingly, 170 vaccines not only failed to protect against the 170 virus, but also the less pathogenic CL8. There was only a modest (1 log10) reduction in mean VL in immunized animals challenged with the chimeric virus 8/170. On the other hand, both CL8- and 170-immunized animals showed substantial (>3 and 2 log10, respectively) reduction of VL after challenge with the 170/8 chimera, consistent with the notion that the envelope antigen in the early virus represents an important target for protective immunity.

Together, these results underscore the potential importance of targeting transmitted viruses through judicious choice of immunogens from early isolates for vaccine development.

Session 5: B Cell Immunity

0A05-01

Phospholipid binding and neutralization by anti-gp41HIV-1 envelope antibodies

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Background: Anti-HIV-1 gp41 envelope (env) antibodies (Abs), which bind to linear and/or conformational epitopes, are induced in HIV-infected individuals. A subset of anti-HIV-1 gp41 Abs with binding specificities within gp41 cluster II that react with the membrane proximal external region (MPER) (aa644-667) have been described. Anti-gp41 Abs are largely non-neutralizing and only rarely are broadly neutralizing (2F5, 4E10, Z13). 2F5 and 4E10 Abs also show reactivity towards phospholipids and other autoantigens. Thus, we have probed the relationship between phospholipid reactivity and neutralization and compared peptide epitope, phospholipid and peptide-lipid conjugate binding of 2F5 to gp41 cluster II Abs.

Methods: Anti-gp41cluster II Abs (98-6, 167-D, 126-6) were derived from HIV-infected individuals and murine anti-MPER Abs (5A9, 13H11) were made following immunization with group M consensus Env ConS gp140 oligomer. 2F5 peptide-lipid conjugates were prepared by incorporating the 2F5 nominal epitope peptide into synthetic liposomes via a lipophilic linker. Binding kinetics and antibody cross-blocking were measured using a surface plasmon resonance binding assay. Neutralization assays were performed with pseudoviruses in TZM/bl cells or mitogen-activated peripheral blood mononuclear cells (PBMCs).

Results: In comparison to 2F5 Ab, both human cluster II and murine MPER Abs bound to the 2F5 nominal epitope peptides with faster dissociation kinetics. In crossblocking experiments, binding of 2F5 was blocked partially by cluster II Abs, while 2F5 completely blocked the binding of cluster II Abs. 98-6 and 13H11 did not reciprocally inhibit each other's binding to gp41 epitopes. However, 98-6 and 13H11 acted synergistically to completely block 2F5 binding. 98-6 showed strong reactivity with cardiolipin and bound to 2F5 peptide-lipid conjugates. In contrast, 5A9 and 13H11 did not bind to phospholipids nor to 2F5 peptide-lipid conjugates. None of the human cluster II Abs or murine MPER Abs had neutralizing activity against the HIV-1 primary isolate B.6535 pseudovirus in the TZM/bl assay. However, in the PBMC assay, human Ab 98.6 neutralized the primary isolate B.6535 (ID50= 3.5µg/mL).

Conclusion: HIV-1 neutralizing activity is rare among cluster II anti-gp41 mAbs. Only anti-gp41 Abs (2F5, 98-6) that bound with high avidity to both MPER epitopes and phospholipids neutralized HIV-1.

Supported by CAVD grants from the Bill & Melinda Gates Foundation.

0A05-02

Difficulties in eliciting broadly neutralizing anti-HIV antibodies are not explained by cardiolipin autoreactivity

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Objective: In a recent study Haynes et al. (Science 2005; 308:1906-1908), difficulties in eliciting broadly neutralizing antibodies to HIV were linked to the binding of prototypic broadly neutralizing monoclonal antibodies to autoantigens, and in particular, to the binding of two anti-gp41 antibodies, 2F5 and 4E10, to the autoantigen cardiolipin. We employed a number of assays to understand whether the lipid reactivity observed for 2F5 and 4E10 is indicative of autoreactivity, polyreactivity, or perhaps a generalized lipid affinity that may assist their recognition of membrane proximal epitopes.

Methods: 2F5 and 4E10 were evaluated for autoreactivity using assays developed for the diagnosis of antiphospholipid syndrome (APS). As an indication of general lipid affinity, we measured the binding of 2F5 and 4E10 to liposomal bilayers of differing composition by surface plasmon resonance (SPR) spectroscopy. 2F5, 4E10 and other broadly neutralizing anti-HIV antibodies were analyzed for polyreactivity against a microarray panel of 400 recombinantly expressed E.coli proteins using biochip technology.

Results: 2F5 showed completely negative results in the APS and SPR studies, indicating that it is neither autoreactive nor absolutely requires phospholipid binding for epitope recognition. In contrast, 4E10 showed weak activity in the APS studies and bound to more than one lipid in the SPR studies. Moreover, the activity displayed by 4E10 in the APS studies more closely resembles that of anti-phospholipid antibodies elicited during many infections, than that of autoimmune APS antibodies - at variance with the notion that difficulties in eliciting 4E10-like antibodies can be attributed to tolerance mechanisms. The microarray studies indicated that in general, broadly neutralizing anti-HIV mAbs are not exceptionally polyreactive.

Conclusion: These results suggest that autoantigen mimicry cannot be reliably invoked as a general mechanism for HIV immune evasion.

0A05-03

Role of NK cells in HIV inhibition by antibodies

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Objective: NK cells are described to be able to directly lyse infected cells and to induce antibody dependant cytotoxicity (ADCC) after binding of antibodies to epitopes present at the surface of infected cells. In this study we analyse the role of NK cells in neutralizing experiments performed on PHA-stimulated PBMC.

Methods: PBMC were depleted or not from NK cells before PHA stimulation. In some experiments, purified NK cells were cultured in IL-2 and added back to depleted PBMC at various concentrations. HIV infected cells were detected by intracellular p24 staining after a single cycle of infection.

Results: We found that 1) in the absence of antibodies, the percentage of infected cells decreases when NK cells are present in the PHA-stimulated PBMC and 2) in the presence of HIV specific antibodies, ADCC could be detected. For non neutralizing antibodies, we found a 50 to 80% reduction of the percentage of infected cells in presence of NK cells. This inhibition is observed at high concentration of antibodies and only in PHA stimulated PBMC that contain high percentage of NK (after addition of purified NK cells or donors that have more than 20% NK cells within the PBMC population). However, for highly active neutralizing antibodies, the neutralizing titer is similar whether NK cells were present or not, certainly because ADCC is not detected at low antibody concentrations.

Conclusion: When high percentage of NK cells are present in PHA stimulated PBMC, ADCC due to NK cells could be responsible for the low inhibitory activity observed with some non-neutralizing antibodies at high concentration.

0A05-04

Mapping autologous neutralization targets in newly transmitted clade C primary HIV-1 isolates

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Objective: Progress towards a successful vaccine for HIV-1 has been stymied by the inability of current vaccines to induce a protective humoral response. Conserved neutralization targets identified to date are either masked on typical primary isolates, or are poorly immunogenic. However, even highly masked primary isolates have been found to possess sensitive neutralization targets that are recognized by autologous patient sera. The objectives of this study were to identify the location and nature of such targets in a panel of envs isolated from clade C-infected patients shortly after infection.

Methods: Initial studies were focused on two sets of envs and sera isolated from a cohort in Zambia (Li et al., J. Virology, 80:5211-18, 2006). Sera samples 53M and 133M both possessed high autologous neutralizing titers, but while 133M serum did not neutralize any of the heterologous envs, 53M serum also had a lower neutralizing activity for a number of additional primary envs. Using conserved or engineered restriction sites, exchanges were made between the two envs of the gp120 N-terminal fragment (N-term through C2, including V1/V2), the gp120 C-terminal region (C2 through C5, including V3, V3', V4 and V5) and gp41 region (C5 through C-term). The sensitivities of these env chimeras to the parental sera were then quantitated in a single cycle entry assay using pseudotyped virus.

Results: Although both sera possessed substantial cross-reactive anti-V3 antibody titers, the V3 domains of these envs were masked and did not mediate neutralization. Substitution of all three regions of 53M env resulted in reduced neutralization by the autologous serum, and insertion of all three 53M regions into 133M env resulted in gain of neutralizing activity. For 133M Env, only the gp120 C-terminal fragment exchange resulted in gain or loss of neutralization activity by the autologous serum.

Conclusion: These results suggested that the high autologous neutralizing activity of 53M serum was directed towards three distinct targets, located in the gp120 N-terminal, gp120 C-terminal and gp41 regions, while the autologous neutralizing activity of 133M serum was primarily directed towards the gp120 C-terminal region. The cross-neutralizing activity of 53M serum was presumably mediated by antibodies directed against one or more of the regions identified. Ongoing experiments aimed at defining the nature of the epitopes involved in this neutralization will be discussed.

OA05-05

Recombinant baculovirus derived HIV-1 virus-like particles elicit potent neutralizing antibody responses

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Objective: Virus-like particles (VLPs) represent an attractive HIV-1 vaccine platform, but have been limited by the amount of envelope (Env) glycoprotein that can be incorporated. Using chimeric env constructs in which transmembrane (TM) and cytoplasmic (CT) domains were replaced with those of other viral (influenza HA, mouse mammary tumor virus, baculovirus gp64) proteins, we recently increased the Env content of recombinant baculovirus (rBV) derived HIV-1 Gag/Env VLPs by more than 10-fold. Here, we investigated the potential of these second generation VLP preparations to elicit neutralizing antibody responses in guinea pigs.

Methods: Guinea pigs (GP) (n=32) were primed twice (wk 0 and 4) with ConSΔCFIgp145 DNA (400μg), divided into four groups, and then boosted twice (wk 8 and 12) with three different rBV derived VLP preparations containing chimeric ConSΔCFI Env (VLP-HA, VLP-SP64 and VLP-MMTV, respectively), as well as with oligomeric ConSΔCFI gp140 protein (ConS) for comparison. Immunogens were equilibrated for their Env content and given at 100 μg per dose. ConS gp140 protein (but not the VLP preparations) was adjuvanted with CpG. Sera were collected at weeks 6, 10 and 14 from each animal and assayed for binding and neutralizing antibodies.

Results: VLP-HA, VLP-SP64, VLP-MMTV as well as the ConS gp140 protein elicited high titer gp120 binding antibodies, with geometric mean titers (GMT) reaching of 0.8, 1.2, 2.4 and 4.9 x 10^6, respectively. VLP-HA, VLP-SP64, VLP-MMTV, and ConS gp140 also elicited neutralizing antibodies to SF162, which after the first boost reached IC50 values (GMT) of 426, 1470, 3667 and 3764, respectively. Further studies of sera from VLP-MMTV immunized GP (post boost 2) revealed neutralizing antibodies to additional tier 1 viruses, including Bal, SS1196, TV1 and 92BR025 (IC50 values of 64, 113, 217 and 61, respectively). A select number of these animals also exhibited low level neutralizing activity against 8 of 13 tier 2 viruses (subtype B and C). The breadth and magnitude of this response was comparable to that of animals immunized with the ConS gp140 protein.

Conclusion: When primed with DNA, non-adjuvanted Gag/Env VLPs are as potent as CpG adjuvanted gp140 oligomeric protein with respect to eliciting neutralizing antibody responses to both tier 1 and tier 2 viruses. Given the known additional advantages of particulate immunogens, rBV derived VLPs should be evaluated as components of future AIDS vaccines.

OA05-06

Evaluation of early subtype A, C, and D HIV-1 envelope variants to identify strategies to expose neutralization epitopes

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Objective: To isolate and evaluate the neutralization profiles of HIV-1 envelope variants from early in infection from individuals infected heterosexually with subtypes A, C, and D HIV-1 in Kenya. To use these variants to identify viral features that will allow better targeting of vaccines to transmitted strains.

Methods: Full-length HIV-1 envelope variants from early after infection were cloned by single copy nested PCR and used to generate pseudoviruses. Pseudoviruses were tested for their coreceptor specificity and their susceptibility to neutralization by heterologous plasma pools, and a subset was also tested for susceptibility to autologous plasmas and monoclonal antibodies. Site-directed mutagenesis was used to engineer mutations found in neutralization-sensitive variants to define residues important in determining neutralization susceptibility.

Results: Pseudoviruses with subtype A, C, and D HIV-1 envelopes from early in infection had IC50 values to a heterologous plasma pool ranging from <1:50 to 1:1600. Several candidate envelope variants from each subtype representing a range of neutralization phenotypes were identified. Some isolates were unusually sensitive to neutralization, but most were relatively neutralization resistant. One unusually neutralization-sensitive variant was a CCR5-tropic subtype A variant. Within the early subtype A variant, two amino acid mutations in gp41 were found to confer sensitivity to neutralization by antibodies that bind to both gp41 and to gp120. Transferring these mutations to diverse viral isolates from multiple subtypes resulted in the exposure of neutralization epitopes throughout the envelope protein, including epitopes recognized by heterologous plasma samples from unrelated HIV-infected individuals.

Conclusion: The envelope variants identified here will supplement subtype A, C, and D panels for screening vaccine candidates for their ability to elicit relevant neutralizing antibody responses. Characterization of these early envelope variants has led to the identification of structural features that enhance exposure of conserved neutralization epitopes on diverse HIV-1 envelope proteins. Incorporation of such features into novel immunogens may enhance the generation of broadly neutralizing antibodies to HIV.

0A05-07

Dynamic process of autologous and heterologous neutralizing antibody development in drug-naïve HIV controllers

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Background: Neutralizing antibodies (NAbs) with the capacity to neutralize divergent, or heterologous, isolates are an important target for an effective HIV vaccine. The development of NAbs is a process that spans months to years, and only a subset of patients develop NAbs capable of neutralizing heterologous isolates.

Objective: The goal of this study is to examine the kinetics of development of NAbs in a cohort of elite (VL <50 C/ml) and viremic (VL 50-2000 C/ml) controllers to determine how changes in the Envelope proteins of predominant members of the quasispecies affect escape from NAbs. We also compared autologous neutralization (ANAb) and heterologous neutralization (HNAb) profiles of antibodies in plasma from controllers that had occasional viral breakthrough with those with no breakthrough.

Methods: Patients (n=11) chronically infected with subtype B (15-22 years), are asymptomatic, drug naïve, and have CD4 counts >400/mm^3 and average viral loads <2,000 C/ml. HNAbs were measured in patient plasma against the clade B pseudovirus panel using the TZM-bl assay. Cloned full-length gp160 env genes (n=10 per sample) resulting in functional pseudoviruses were used to measure ANAbs from plasma of 4 patients at 2-3 late time points 4-18 months apart.

Results: Plasma from all 11 patients neutralized at least 3 and a median of 6 clade B panel viruses, with titers in the hundreds, similar to the positive control plasma pool. Viremic controllers with viral blips virus (>10^4) had high ANAbs, while those with controlled replication had undetectable or low titers. Plasma-derived env gp160 clones showed continuous viral evolution, coinciding with viral breakthrough. Maturation of ANAb responses over time was seen against a subset of Env variants at one or more time points, with increasing titers at later time points, in some cases extending to neutralization of heterologous viruses. Some sensitive variants persisted in these patients, while containment of earlier variants and evidence for emergence of resistant variants was seen in viremic controllers with no breakthrough virus. Detailed analyses of specific mutations responsible for escape and containment are in progress.

Conclusion: The role of NAbs in viral containment in HIV controllers remains a key question. These data support a model where continuous low-level viral replication and selection results increasing ANAbs that can broaden to recognize a majority of heterologous viruses.

0A05-08

HIV neutralization is profoundly affected by the cell model system used in vitro when a multiclade virus panel and polyclonal antibodies are employed

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Objective: A HeLa-derived (TZM-bl) luciferase reporter cell line-based assay employing pseudoviruses generated in 293T cells has been advocated as a standardized method for assessing antibodies elicited by HIV vaccine candidates. This assay has many advantages over the traditional peripheral blood mononuclear cell (PBMC)-based assays, to include reproducibility and ease of transfer to new labs. Balanced against this, it has long been recognized that the methods utilized to assess HIV-1 neutralization are directly affected by the variables inherently surrounding functional antibody assays, to include incubation time, cell density, and endpoint, as well as the viruses and the target cell used. Our objective was to compare neutralization data obtained using polyclonal plasma and viruses of multiple clades in the TZM-bl pseudovirus assay and a PBMC assay.

Methods: Neutralization was measured using 6 plasma pools from different geographic regions and a panel of 54 primary isolates from 6 subtypes, or pseudoviruses prepared from a single env clone from each of the 54 isolates. Pools showing >50% neutralization at a 1:40 dilution against a given primary isolate or pseudovirus were titered to obtain IC_{50}s and IC_{80}s, and these values were compared by linear regression analysis.

Results: The titers obtained in the two assays showed no correlation (R^2=0.0018 for IC_{50}s, R^2=0.0035 for IC80s). Using IC_{50}s, 40% of the pairs were positive in one assay, and negative in the other. The subtype B isolates were the most sensitive in the pseudovirus system and were rarely found to be positive only in the PBMC assay. One of the geographic antibody pools appeared to be highly potent in the TZM-bl assay and was responsible for 49% of the values that were positive in both assays. Comparative neutralization experiments using PBMC derived virus on TZM-bl cells and pseudovirus on PBMC are underway to verify that this differential effect is cell line dependent.

Conclusion: These data suggest that caution should be used when choosing a neutralization assay platform. Parallel assessment of vaccine-induced neutralizing antibodies in both primary cell assays, as well as reporter cell line-based pseudovirus assays, may be prudent until a correlate of protection can be identified.

Session 6: Animal Models

0A06-01

Immununogenicity of HIV virus like particles bearing various forms of env proteins in guinea pigs and macaques

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Objective: To compare the ability of various virus like particle immunogens to elicit neutralizing antibodies in guinea pigs and macaques.

Methods: We immunized 20 guinea pigs 3 monthly doses of various types of VLPs in Ribi or CpG adjuvants. Particles included "WT-VLPs" (bearing unmodified trimers), "SOS-VLPs" (bearing disulfide-shackled functional trimers), "UNC-VLPs" (bearing uncleaved Env), and control "naked VLPs" (bearing no Env). Particles were normalized to 5µg equivalents of Env per dose. Soluble gp120 (100µg per dose) was used as a control immunogen. We are also immunizing 4 macaques with WT-VLPs and SOS-VLPs (2 animals for each type), using 20µg Env equivalents per dose and Ribi as an adjuvant.

Results: Env-VLPs rapidly elicited high titer anti-Env antibodies in guinea pigs. UNC- and SOS-VLP sera reacted strongly with the V3 loop and to some extent, the gp120 coreceptor binding site. However, reactivity to the gp41 immunodominant domain was absent in SOS-VLP sera. Gp120 and WT-VLP guinea pig sera were less focused on the V3 loop, but had modest reactivity with epitopes that overlap CD4 binding site. Some Env-VLP sera neutralized sensitive primary isolates, including 1196 with IC_{50} titers of ~1:100. For comparison, we are also testing the immunogenicity of WT-VLPs and SOS-VLPs in macaques. So far, after 2 VLP immunizations, all 4 macaques have generated high anti-Env titers. Mapping the specificity of these sera has revealed a striking differences from what we observed in guinea pigs with the same immunogens. Rather than predominantly V3 loop-specific antibodies, all 4 monkey sera contained predominantly CD4 binding site-overlapping antibodies. Furthermore, in preliminary neutralization studies, strong neutralization (IC_{50} ~1:1,000) was observed in one of the WT-VLP immune sera against primary isolate 92US715. Two of the other 3 sera also neutralized this virus, albeit more weakly. There was, however, so far no activity against the matched JR-FL isolate. We are presently trying to map the specificity of the 92US715 neutralizing activity.

Conclusion: Our results indicate that different animal species can respond to the same antigen with markedly different antibody specificity profiles. This suggests small animal vaccine evaluation studies may not accurately predict the nature of the antibody responses to a given immunogen in primates.

0A06-02

Characteristics of SIV-specific cellular immune response induced by different regimens of recombinant DNA and herpes simplex virus (HSV)d106 vectors

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Background: We have previously reported on the immunogenicity of replication-defective HSVd106 vector-based SIV vaccines. In order to determine the optimal protective regimen, different prime-boost immunization regimens are being tested in rhesus macaques.

Methods: Twenty herpes B virus negative rhesus macaques were enrolled into four vaccination groups and one control group. Each group contained two Mamu-A*01-positive and two Mamu-A*01-negative rhesus macaques. The vaccines consisted of six plasmid DNA constructs expressing SIV Gag, Env and a Rev-Tat-Nef fusion protein, three HSVd106 vectors expressing the same SIV proteins, and a control HSVd106 empty vector, all administered via the intramuscular route. All macaques received two priming doses at 0 and 4 weeks and two booster inoculations at 12 and 24 weeks, and are due to be challenged intrarectally with SIVmac239 at week 36. The vaccination groups consisted of a DNA prime-DNA boost, DNA prime-HSV boost, HSV prime-DNA boost, and a HSV prime-HSV boost immunization regimen. Immune responses to SIV were monitored during the vaccination phase.

Results: All four vaccination regimens induced SIV-specific IFN-γ, IL-2, and perforin ELISPOT responses in the peripheral blood. With the exception of one DNA vaccinated animal, lymph node ELISPOT responses were only observed after vaccination with HSVd106-SIV vectors. The highest magnitude of Gag- and Env-specific IFN-γ ELISPOT responses were elicited in the DNA prime-HSV boost regimen one week after the first booster inoculation and peaked at 5,813 and 1,550 spot forming cells per 10^6 PBMC respectively. While IFN-γ ELISPOT responses were dominant in the DNA-primed vaccinated macaques, a more 'balanced' IFN-γ, IL-2 and perforin ELISPOT response was observed in the HSV-primed macaques. Tetramer-positive CD8+ T lymphocytes were detected in the rectal mucosa of 7/8 Mamu-A*01-positive macaques, but did not appear to be enriched with the exception of one macaque in the HSV prime-HSV boost group. The DNA prime-HSV boost immunization regimen induced both transitional effector memory (CD95+CD28+CCR7–) and central memory (CD95+CD28+CCR7+) CD8+ T cells.

Conclusion: These data confirm our previous findings on the strong immunogenicity of DNA and HSVd106-SIV vectors. Induction of central memory T lymphocytes and immune responses at lymphoid and mucosal sites appear to be additional advantages of using HSVd106 vectors as a vaccine for AIDS.

0A06-03

Envelope is a primary determinant of lentiviral vaccine efficacy

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Lentiviral envelope antigenic variation and the resulting immune system evasion are believed to present a major obstacle to the development of an effective vaccine. While this speculation is widely assumed by the scientific community, there is to date no rigorous experimental data assessing the effect of increasing levels of lentiviral envelope variation on vaccine efficacy. It is our working hypothesis that envelope is the primary determinant of vaccine efficacy. We previously reported that a particularly successful experimental live-attenuated equine infectious anemia virus (EIAV) proviral vaccine, derived by mutation of the viral S2 accessory gene (EIAVD9), provided 100% protection from disease after virulent virus (EIAVPV) challenge. In the current study we sought to comprehensively test our hypothesis by challenging vaccinated animals with proviral strains of defined, increasing envelope variance. We cloned envelope SU genes (6% and 13% divergent from EIAVD9/EIAVPV SU), which had arisen naturally during experimental infection of ponies with EIAVPV into the backbone of our standard molecular clone (SU homologous to EIAVD9). The new virus stocks generated from these molecular clones (EV0, EV6, EV13) were characterized both in vitro and in vivo for viral dynamics and for immunogenicity. Standard viral replication kinetics as well as immunogenicity and the ability to cause typical acute and chronic disease was observed for all three strains. Hence we vaccinated 24 outbred ponies with EIAVD9 and subsequently challenged three different groups of eight animals each after seven months with either viral strain EV0, EV6, or EV13. Analysis of day of challenge viral (vaccine) loads and immune parameters did not distinguish one challenge group from the other; however, the groups displayed markedly different levels of disease upon challenge. One EV0, three EV6, and five EV13 animals displayed clinical signs of EIA upon challenge. Further analysis of these samples demonstrated a direct, significant linear correlation of env divergence from vaccine strain and protection from disease (r^2= 0.998, P = 0.02). The results of this study indicate and validate for the first time that envelope antigenic variation poses a major obstacle for lentiviral vaccine development, even utilizing a live-attenuated vaccine which to date has demonstrated the highest levels of vaccine immunogenicity.

0A06-04

Immunization of macaques with single-cycle SIV by two different regimens reduces viral loads after an intravenous challenge with SIVmac239

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Separate groups of rhesus macaques were immunized by repeated versus prime and boost regimens with strains of SIV that are limited to a single cycle of infection to compare the effects of frequency of inoculation versus infectious dose of the inoculum on the ability to elicit protective immunity. Six animals (Group A) were inoculated intravenously at 8-week intervals with 6 identical doses containing a mixture of three different envelope variants of single-cycle SIV (scSIV); scSIVmac239, scSIVmac316 and scSIVmac155T3. Six additional animals (Group B) were primed intravenously with a mixture of the same three envelope variants that included a truncation of the gp41 cytoplasmic tail to increase envelope incorporation into virions. These animals were then boosted on weeks 12 and 24 with scSIV trans-complemented with the vesicular stomatitis virus glycoprotein (VSV G scSIV). To prevent neutralization of the inoculum by VSV G-specific antibodies, different serotypes of VSV G were used for each boost. For Group A, virus-specific T cell responses were detectable in peripheral blood after the first and second doses by MHC class I tetramer staining and IFNγ ELISPOT assays. CD8+ T cell responses to the Mamu-A*01 Gag181-189 epitope peaked at 0.9% to 0.13% of the CD8+ T cell population three weeks after the first dose. Surprisingly, additional rounds of inoculation did not result in further recall responses. In contrast, virus-specific T cell responses in Group B were more than 10-fold higher after boosting with VSV G scSIV. Peak Mamu-A*01 Gag181-189-specific CD8+ T cell responses one week after each boost ranged from 2.0% to 13.1% of the CD8+ T cell population in blood. Additional T cell responses to each of the 8 viral proteins expressed by scSIV were also observed by whole-proteome IFNγ ELISPOT assays and CD4+ T cell responses to Gag were detectable by intracellular cytokine staining for TNFα. Twelve weeks after the last inoculation, both groups were challenged intravenously with SIVmac239. With the exception of a single animal in Group A, all of the animals became infected. However, Groups A and B exhibited 9.7- and 8.5-fold reductions in acute phase (week 2) and 22.4- and 42.5-fold reductions in chronic phase (week 8) viral loads respectively relative to unvaccinated control animals. Thus, despite differences in the magnitude of virus-specific T cell responses, both immunization regimens afforded partial control of SIVmac239 replication after challenge.

OA06-05

Acute intestinal CD4+ T-cell depletion is not predictive of SIV virulence

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Background: Acute HIV/SIV infection induces massive mucosal CD4+ T cell depletion, which is believed to determine infection outcome. Since this occurs within weeks of infection, it is not clear why progression to AIDS occurs after a prolonged incubation.
Objective: To investigate the predictive value of acute mucosal CD4+ T-cell depletion for the outcome of SIV infection.
Methods: Our study included: rhesus macaques (Rh) infected with pathogenic SIVsmm/SIVmac (n=8); Rh infected with 5 different primary SIVsmm isolates derived directly from sooty mangabeys (n=26); African green monkeys (AGMs, n=18), Rh (n=3) and pigtailed macaques (PTMs, n=6) infected with SIVagm. sab (which infects AGMs and is R5-X4 dual tropic); PTMs infected with SIVrcm (which infects red-capped mangabey and uses CCR2 co-receptor in vitro). Viral load (VL) dynamics, as well as lymphocyte and macrophage populations and subsets were quantified in blood, lymph nodes and intestine.
Results: High VL levels during acute infection resulted in similar acute intestinal CD4+ T-cell depletions of 60-95%, irrespective of NHP species, viral strains or clinical outcomes. While common patterns of acute SIV infection were seen, the outcomes of chronic infection varied widely. Rh infected with pathogenic SIVmac/smm progressed to AIDS in 8-12 months; Rh infected with less pathogenic SIVsmm strains progressed in 3-4 years. AGMs were persistently infected without disease progression for 5 years, Rh cleared SIVagm infection and SIVagm-infected PTMs progressed to AIDS or controlled the infection. PTMs infected with SIVrcm showed persistent infection. Interestingly, Rh infected with SIVsmm with low VL peaks maintained CD4+ T-cell pools during acute infection, but showed disease progression during the chronic infection. NHPs that showed better control of VLs during the chronic phase, partially restored intestinal CD4+ T-cells after 200 days p.i. Restoration was associated with normal/low levels of immune activation. In NHPs that progressed to AIDS, this restoration was transient.
Conclusion: We provide compelling evidence that acute mucosal CD4+ T cell depletion is a common feature of SIV infection in pathogenic, persistent non-pathogenic or controlled infection. Therefore acute mucosal CD4+ T cell depletion may be necessary but it is not sufficient to result in disease progression. The outcome of chronic SIV infection is dependent of viral replication, immune activation, and possibly other unknown factors.

OA06-06

Reduced immunogenicity but enhanced control of SIV challenge by a DNA/MVA vaccine in the presence of preexisting immunity to vaccinia

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Background: Here, we evaluate how preexisting immunity to vaccinia affects the immunogenicity and efficacy of a DNA/MVA SIV vaccine in macaques.
Methods: Three groups of macaques, eight per group, were studied. The non-Dryvax group received the DNA/MVA SIV vaccine (DNA at weeks 0 and 8, and rMVA at weeks 16 and 24). The Dryvax group received the Dryvax vaccine (current smallpox vaccine) one and one half years prior to the DNA/MVA vaccine. The control group did not receive any vaccine. All macaques were challenged intrarectally with SIV251.
Results: Dryvax vaccination elicited a robust long-lived vaccinia-specific CD8 and CD4 responses. In the non-Dryvax group, gag-specific CD8 and CD4 responses peaked following the second MVA boost with geometric mean frequencies of 0.14% and 0.47% of total CD8 and CD4 cells, respectively. These responses were 5-10 fold lower in the Dryvax group. Despite post vaccine cellular immunity being 5-10-times higher in the non-Dryvax animals, viral control was 100-fold better ($p<0.01$) at peak viremia and 20-fold better at set point in the Dryvax than the non-Dryvax group. Retrospective analysis of vaccine elicited SIV-specific responses revealed enhanced expression of the lymph node homing receptor CCR7 on gag-specific CD4 T cells in the Dryvax group compared to the non-Dryvax group ($p<0.01$). A significant inverse correlation was observed between the frequency of gag-specific CD4 T cells co-expressing CCR7 following vaccination and peak viremia following challenge ($p=0.02$). A significant inverse correlation was also observed between the frequency of gag-specific CD8 T cells co-expressing IFN-γ and IL-2 and peak viremia at 2 weeks following challenge ($p<0.01$). No correlation was observed between the frequency of IFN-γ positive cells and plasma viremia.
Conclusion: Our results demonstrate that preexisting immunity to vaccinia lowers the magnitude of vaccine-elicited T cells but enhances control of SIV challenge by a DNA/MVA vaccine. This enhanced control correlated with better lymph node homing potential of the elicited T cells. These results demonstrate that preexisting immunity to the vaccine vector modulates the quality of insert-specific response that may play a critical role in viral control.

0A06-07

High levels of SIV-specific CD8+ T cells in the mucosal tissues of rhesus macaques immunized with MVA-vectored candidate AIDS vaccines

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Background: Several recent studies have shown that pathogenic HIV/SIV infections are associated with a rapid, dramatic, virus-induced depletion of memory CD4+ T cells from mucosal associated lymphoid tissues (MALT). These studies led to a model in which the MALT CD4+ T cell depletion plays a key role in the pathogenesis of AIDS. As such, a desirable property of a candidate AIDS vaccine would be to generate high numbers of HIV-specific memory CD8+ T cells in the mucosa that may limit this early loss of CD4+ T cells.

Objective: The aim of this study was to assess the level of SIV-specific CD8+ T cells in mucosal tissues of rhesus macaques (RMs) immunized with two MVA-vectored candidate AIDS vaccines.

Methods: Two groups of five MaMu-A01 RMs were inoculated either i.m. or i.d. with $2x10^8$ PFU of "wild type" MVA (wt-MVA) or an MVA vector with the Uracyl DNA Glycosylase (UDG) gene deleted (MVAΔUDG). Both vectors expressed SIVmac239 gag and tat. All animals received three immunizations six weeks apart. Tissues examined included peripheral blood (PB), lymph nodes (LN), rectal mucosa (via rectal biopsies, RB) and lung (via broncho-alveolar lavage, BAL). Lymphocytes isolated from these tissues were examined by flow cytometry and gag- and tat-specific CD8+ T cells were assessed by tetramer staining.

Results: In all animals studied, both wt-MVA and MVAΔUDG induced detectable levels of gag-specific CD8+ T cells, with levels of 0.25-2.2% in PB; 0.03-0.55% in LN; 0.03-1.09% in BAL; and 0.33-16.2% in RB at day 7 after the third immunization. Tat-specific CD8+ T cells were also detectable in all tissues of all animals, albeit at lower levels. Interestingly, the highest levels of SIV-specific CD8+ T cells were found in the rectal mucosa, with the majority of these cells displaying a phenotype (CD28-CD95+) indicative of full differentiation to "effector" or "effector-memory" T cells.

Conclusion: Repeated immunization with MVA vectors that express SIV-gene products results in levels of SIV-specific "effector" CD8+ T cells in gut-associated lymphoid tissues that are higher than those observed in PB, LN, and BAL. Challenge studies are in progress to determine whether and to what extent these intestinal mucosal SIV-specific CD8+ T cells protect from the early depletion of MALT CD4+ T cells described in unvaccinated SIV-infected RMs.

0A06-08

Optimization of a prophylactic DNA vaccine induces high levels of cellular immune responses in an SIV macaque model

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A major challenge in the development of an effective vaccine for HIV is to stimulate potent cell-mediated immunity while maintaining a high level of safety. An earlier vaccine study completed in our laboratory included co-immunization of a DNA-based vaccine alone or in combination with an IL-12 (pmacIL-12) or IL-15 (pmacIL-15) expressing plasmid. Vaccines induced antigen specific IFN-gamma producing cells. Subsequently, using CFSE staining and flow cytometry we assessed lymphocytes proliferative capacity following in vitro SIVgag stimulation. Lymphocytes isolated from the groups co-injected with either pmacIL-12 or pmacIL-15 had higher cellular proliferative responses compared to the antigen vaccine alone. Importantly pmacIL-15 induced the highest CD8 antigen-specific proliferative response. And, these animals demonstrated the greatest control of viral replication. In the current study we set out to further improve our cellular immune responses. We utilized electroporation to improve DNA vaccine delivery of a poly-optimized DNA vaccine. The SIV-gag, SIV-env, and SIV-pol constructs were optimized by four optimization approaches. Our results demonstrate a 10 fold increase in the average number of IFN-gamma producing cells compared to the previous study. Indeed utilizing the ELISPOT assay we established the induction of approximately 7,500 vaccine specific cells per 1 million PBMCs. This was noted after just 2 immunizations. Other correlates of protection were also studied by means of CFSE proliferation assays and gene array multiplex analysis. We established high levels of antigen specific lymphocyte proliferation in vitro and antigen specific expression of a series of immunological molecules. The observed differences in the immune profiles are reflective of how the antigen specific lymphocytes control viral replication. In conclusion by using both optimization of the expression vectors concurrently with electroporation delivery we can likely decrease the number of immunizations and the dose required to successfully impact viral replication in an SIV Rhesus macaque model. This ultimately has important implications for HIV-1 vaccine development.

Session 7: Acute HIV/ HIV Transmission

0A07-01

Fluorescent detection of HIV particles in human tissue explant cultures and the rhesus macaque genital tract: model to understand sexual transmission

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Objective: Sexual transmission accounts for over 80% of all HIV infections. How HIV breaches the genital epithelium of men and women remains poorly defined. The objective of this study is to define the interaction of HIV with human and macaque genital tissue to gain insights into the mechanism(s) of sexual transmission of HIV.
Methods: Previous attempts to observe HIV interactions with tissue explants and primate models have been hindered by high background. To study single virion interactions with genital tissues, we developed a new technology utilizing photoactivatable GFP (PA-GFP) labeled HIV and fluorescent deconvolution microscopy. Photoactivation of PA-GFP is achieved by exposure to 400-430nm light. Using this system, tissue autofluorescence is defined, the sample is activated and newly detected signal is labeled HIV. To study initial HIV interactions with the genitalia, we incubated human foreskin and cervical explants for 4-24 hours with PA-GFP labeled HIV. These experiments were replicated in the rhesus macaque non-human primate model. Following inoculation, genital samples were frozen, sectioned and stained, enabling observation of tissue structure, HIV target cells, and PA-GFP labeled virions.
Results: Within 4 hours HIV penetrates the superficial squamous epithelia of the ectocervix, in some cases deeper than 30 microns. Penetrating virions are observed between differentiated squamous epithelial cells. We also observe HIV penetration of the columnar endocervical epithelium with virions observed in the sub-basal milieu. Penetrating virions regularly co-localize with both Langerhans cells and exposed CD4 T-cells. In cervical explants, mucus harbors numerous virions, and when present reduces the incidence of epithelial penetration. Complimentary results were obtained using the rhesus macaque model.
Conclusion: HIV can clearly penetrate the intact squamous and columnar epithelium of the genital tract in both human explants and living macaques gaining access to underlying target cells. The presence of mucus decreases access of virus to the columnar epithelium. Breaks in the epithelial barrier facilitate the association of virus with tissue resident T cells. Observing early HIV interactions with genital tissues has provided important insight into HIV transmission across epithelial barriers. A better understanding of the initial events of HIV sexual transmission is essential for the development of a successful vaccine.

0A07-02

Identification and characterization of transmitted/early virus envelopes in 96 subjects with acute HIV-1 infection

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Objective: Characterization of the virus responsible for HIV-1 transmission could be important in vaccine design. We sought to identify and characterize the transmitted/early virus in primary HIV-1 infection.
Methods: Single genome amplification (SGA) followed by direct DNA sequence analysis of complete env amplicons from plasma virion (vRNA) in 96 acutely infected HIV-1 clade B patients before or after seroconversion.
Results: 2,590 full-length env sequences were determined. Each sequence was shown to lack detectable “double-peaks” on sequence chromatograms and thus corresponded to single vRNA templates. The average number of env sequences analyzed per subject was 27 (range 10-62). Within individual subjects, env diversity ranged from 0.1% to 7.6% and was bimodally distributed (mean of 0.2% in 77 subjects and 2.8% in 19 subjects). In each of the 96 subjects, one or more distinct monophyletic env lineage could be identified, reflecting productive infection by either a single virus (77 subjects) or more than one virus (19 subjects). Viruses comprising each lineage exhibited surprisingly little env diversity (mean 0.2%; range 0–1.2%) and invariably included unique sets of identical or near identical sequences. In 13 of 19 multiply infected subjects, recombinant viruses were identified. In one subject, we determined 133 env sequences from three time points preceding antibody detection. The proportion of identical (consensus) sequences from all three timepoints was 65–70%, with overall diversity of 0.2%. Consensus env sequences from 17 subjects were cloned, expressed, and assayed in a single-infection cycle Env pseudotype assay. All consensus env genes were functional.
Conclusion: HIV-1 transmission most commonly resulted from productive infection by a single infectious unit. When infection resulted from more than one virus, the number of infectious units could be estimated by identifying distinct monophyletic viral lineages and ranged from 2 to 6. Surprisingly, in each of the 96 subjects, a “consensus” set of identical or near identical viral sequences (different in each subject) formed the core of individual viral lineages. Based on phylogenetic inference and analysis of rates of viral diversification, we conclude that these consensus sequences correspond to extremely early envs, and most likely, the actual transmitted envs. Further characterization of these env glycoproteins may represent a step forward in HIV-1 vaccine design.

0A07-03

Acute viremia is often polyclonal neutralization resistant virus populations with high env viability in the absence of neutralizing antibodies

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Objective: Early HIV-1 infection is characterized by spread from the initial site of infection to the GALT followed by peripheral blood viremia where there is a rapid and high titered growth in CD4+ T lymphocytes. Peak viremia drops as the CD4+ host cells become depleted. This study examines the viral envelopes and plasma neutralizing antibody from the earliest times of peripheral blood viremia.

Methods: Longitudinal samples were acquired from 12 plasmapheresis donors who became infected during the period of their regular donations. Plasmas were collected every 2-7 days during the pre-viremic period, through the acute viremia phase and, in some cases, for weeks after. The population of env sequences were amplified from viremic plasma and cloned into the PhenoSense env expression vector. Viral env was successfully amplified from 2-10 timepoints for each plasma donor. Virus env viability, tropism and sensitivity to neutralization with autologous and heterologous plasmas were assessed using the Trofile Tropism assay and PhenoSense Neutralization Assay. All gp160 env pools were sequenced. An in-house cell-to-cell fusion assay was used to assess the fusogenicity of the envs.

Results: All donors were infected by R5-tropic viruses except one which acquired a dual-tropic virus with weak X4-tropism. Autologous neutralizing antibody was absent in all individuals throughout the course of the study. Neutralization, using 5 heterologous broadly neutralizing plasmas, of the acute infection isolates along with a comparison panel of chronic infection isolates showed lower inhibition of the acute infection viruses when compared with chronic infection viruses (p=0.015). Consensus sequencing of the gp160 pools revealed mixed sequence in at least 4 cases. Clonal analysis of one of the mixed sequences revealed two major populations. Rapid selection against the more fusogenic env of the two resulted in almost complete displacement of that isolate over an 11 day period that preceded peak viremia.

Conclusion: Genetically mixed env populations were not uncommon in the earliest stages of viremia and at least one became more homogeneous during peak viremia. Sequence analysis suggests that there is minimal env evolution during this period but rapid expansion of the virus with the best replicative fitness in the peripheral blood. The viruses from earliest infection are overall less sensitive to neutralization with heterologous plasmas than viruses from chronic infection.

0A07-04

Molecular characteristics of the HIV-1 envelope glycoproteins of CRF01_AE variants transmitted from mother to child

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Background: Previous studies have shown that mother-to-child transmission of HIV-1 is often characterized by acquisition of a homogeneous viral quasipecies in the infant, suggestive of a genetic bottleneck.

Objective: To elucidate the molecular characteristics involved in HIV-1 mother-to-child transmission in a homogeneous population infected by CRF01_AE variants in Thailand.

Methods: 17 mother-child pairs were studied. They were participants of a clinical trial assessing various zidovudine durations for the prevention of mother-to-child transmission in Thailand (no breastfeeding). Envelope gene sequences covering the entire gp120 were amplified from both the proviral DNA of maternal PBMC collected before delivery and the plasma viral RNA of infants (first positive sample). The PCR products were cloned and sequenced. A total of 356 clones were available for analysis.

Results: Phylogenetic analysis indicated 2 patterns of transmission: 13 mothers transmitted a single variant and 4 mothers transmitted multiple variants to their infants. The mean genetic distances of viruses from infants with multiple variants were similar to that of the mothers (1.68 vs. 1.63; p=0.95), whereas viruses from infants infected by a single variant were highly homogeneous (mean genetic distance 0.70 in the infants vs. 3.49 in the corresponding mothers; p<0.001). The number of amino acids and potential N-linked glycosylation sites (PNGS) were similar in both the entire gp120 and individual regions of gp120 of all mother-child pairs. However, 4 PNGS, although irregularly present in the mothers, were conserved in all the clones from the babies when infected by a single variant. There were N241, N301, N354, and N384. This suggests that these PNGS would be associated to a selective advantage. In addition, one infant was infected by a single viral population issued from recombination between maternal variants.

Conclusion: Our results confirm that most of MTCT of HIV-1 results in acquisition of a single maternal variant. They suggest that 4 potential N-linked glycosylation sites were selected for transmitted viruses, supporting the hypothesis that the “glycan shield” on gp120 might confer a selective advantage.

OA07-05

Role of anti-V1V2 antibodies in autologous neutralization of acute HIV-1 subtype C viruses

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Background: The early autologous neutralizing antibody response in HIV-1 subtype C infections is often characterized by high titers, but the response is generally type-specific with little to no cross-neutralizing activity within the first year of infection. The specificity of these antibodies is largely unknown but they are likely to target the most variable regions of the envelope. We have previously observed a correlation between fewer N-linked glycosylation sites in V1V2 with higher autologous neutralizing titers, implicating V1V2 as a potential target of autologous antibodies.

Methods: Autologous peptides (15 mer overlapping by 5 amino acids) spanning the V1V2 region of 14 viruses from acutely infected participants were used in neutralization inhibition studies and ELISAs. Three pairs of V1V2 chimeric viruses were generated using overlapping PCR to swap the V1V2 region between viruses. Cloned envelopes were sequenced to confirm chimerism and pseudovirion stocks were generated by transfection in 293T cells. Autologous and heterologous neutralization assays were performed to compare chimeras with both parental viruses.

Results: In 14/14 participants, autologous V1V2 peptides failed to adsorb neutralizing antibodies, and binding ELISAs confirmed that in 13/14 participants, antibodies to linear V1V2 peptides were not detectable. In 4 of 5 participants a proportion (6-100%) of autologous neutralization appeared to be mediated by anti-V1V2 antibodies, as reflected by acquisition of partial or complete sensitivity in heterologous viruses in which the V1V2 was swapped in, and loss of some sensitivity in viruses where the autologous V1V2 had been swapped out. In one participant, there was no evidence that neutralization was mediated by anti-V1V2 antibodies. One set of chimeras acquired increased sensitivity to both parental sera possibly due to conformational changes exposing more conserved neutralization epitopes, although no substantial increased sensitivity was observed in heterologous neutralization experiments.

Conclusion: The region of envelope targeted by antibodies conferring autologous neutralization differs between individuals. In 4/5 individuals antibodies targeting the V1V2 region contributed to autologous neutralization; however these epitopes appear to be of a conformational nature. Future work will address the role of autologous neutralizing antibodies targeting the V3 and α2-V4 regions of the subtype C envelope.

OA07-06

Evidence of frequent HIV-1 superinfection in a cohort of Kenyan women

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Background: We previously showed that HIV-1 intersubtype superinfection occurred in 3 of 20 high-risk women examined; in each case the second virus of a different subtype was detected at about one year after initial HIV-1 infection. Little is known about the frequency of superinfection by more closely related viruses over a several years course of infection.

Objective: To determine how commonly superinfection, and particularly intrasubtype superinfection, occurs during the first 5 years after initial infection.

Methods: Retrospective analysis of 37 high-risk Kenyan women with extensive longitudinal follow-up was used to identify cases of HIV-1 superinfection. Partial gag and env proviral DNA sequences from blood samples collected soon after initial infection were compared to those present approximately five years later. Cases of superinfection were identified by analysis of viral sequences by phylogenetic methods and estimation of the sequence divergence. Viral sequences obtained from intermediate time points were analyzed to estimate the time of superinfection.

Results: Seven women were identified who showed evidence of superinfection with a second strain of HIV-1. Of those four were cases of HIV-1 intrasubtype superinfection. Phylogenetic analysis of early and late sequences showed separate clustering of either gag and/or env, with high sequence divergence. In all 4 intrasubtype superinfection cases, the time of superinfection was estimated to be 2.35, 2.41, 2.43 and 5.12 years since initial infection, and both the initial and superinfecting virus were present after superinfection. Phylogenetic analysis of viral sequences from 3 other subjects indicated probable cases of intersubtype superinfection. Interestingly in two cases, superinfection occurred during primary infection and the superinfecting virus recombined with the initial virus to generate an intersubtype recombinant, and both the initial and recombinant viruses were present up to 5 years post-infection. While in the third subject the superinfecting virus (at 5.2 yrs) replaced the initial virus.

Conclusion: Results from this study suggest that among high-risk HIV-1 infected women, superinfection is occurring frequently and even occurs with closely related viral strains of the same subtype. It appears that HIV-1 superinfection is not limited to a 'window of susceptibility' and occurs after the time is adequate for the development of an immune response to the initial virus.

0A07-07

Superinfection in cohabiting couples previously infected by genotypically different viruses

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Background: Both inter-subtype and intra-subtype superinfection have the capacity to facilitate more rapid diversification of HIV-1 through the generation of recombinant viruses, and this has the potential to make the development of effective vaccines against HIV-1 even more difficult. Through the study of superinfection it may be possible to understand the opposing roles of viral diversity and natural immunity in this process and from this knowledge inform vaccine development.
Objective: The goal of this study was to determine in cohabiting couples, in which the partners were infected with genotypically different HIV-1, (i) whether superinfection could be detected, (ii) its frequency and (iii) the virologic consequences of superinfection.
Methods: Approximately 15% of seroconverting individuals in the RZHRG Lusaka discordant couple cohort acquire their virus from someone other than their partner. In this preliminary study, we report on the analysis of 10 previously HIV-1 discordant couples where the seronegative partner was infected by a virus that was genetically distinct from that of their spouse. For each couple, samples were collected at 3 monthly intervals for at least 24 months from the newly infected partner. We used a novel gp41-based HMA assay to detect superinfection and the results were confirmed by phylogenetic analysis of the V1-V4 sequences from single genome amplified env genes and.
Results: In one case, superinfection of the chronically infected partner apparently occurred during the acute phase of his partner's epidemiologically-unlinked infection. In two newly infected partners (both female), we observed DNA heteroduplexes consistent with superinfection 3 and 6 months after the initial infection event. Sequencing confirmed superinfection, but in both cases the superinfecting virus was genetically distinct from that of the spouse. In all three cases, superinfection was accompanied by at least a 10-fold increase in viral load. Phylogenetic analyses are consistent with rapid recombination between the superinfecting strains in each individual.
Conclusion: In this retrospective study of a limited number of HIV-infected cohabiting couples, superinfection appears to be a frequent event. In one case there is evidence for superinfection of one spouse during acute infection of the other. The study of superinfection in this setting could provide insight into the correlates of immune-mediated protection against infection.

0A07-08

Detecting, confirming and characterizing extraordinarily low levels of HIV-1 infection by an ultrasensitive high throughput method

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Objective: Whether or not and how frequently extraordinarily low levels of HIV-1 infection (ELLHI) occur in the human population has been a critical controversy due to contamination and lacking of sensitive assays. We developed an ultrasensitive high throughput method for screening, confirming and characterizing ELLHI.
Methods: The newly developed method is the combination of our limiting-dilution nested PCRs for simultaneous detection of multiple HIV-1 genes with "clean" procedures, Real time PCR, and sequence confirmation systems. Special procedures were adapted into the method to prevent contamination. Subsequent sequencing and analysis of HIV-1 in PCR products were also included to verify the positive tests and to define the genetic characteristics of HIV-1 in ELLHI.
Results: We compared the new method with available assays including standard limiting-dilution nested PCR, which was considered the most sensitive assay available that can detect a single HIV molecule in patient's genomic DNA, and Real Time PCR. The new method detected ELLHI significantly better in patients' genomic DNA than the standard limiting-dilution nested PCR ($P < 0.001$) and real time PCR ($P < 0.0001$). The new method is optimized for high throughput, and is significantly faster than the standard limiting dilution nested PCR. We used this method to define ELLHI for up to 23 years in 10 patients whose viral load was undetected by conventional assays. We detected HIV-1 proviral DNA from patient PBMC (n=52) at sequential time points of these 10 ELLHI (0.7 – 64 copies/million cells). Sequencing of the positive PCR products indicated that HIV-1 gag p17 and env sequences from each patient formed a close cluster that are distinct from each other patient and control sequences in GeneBank and a local database, suggesting no detectable contamination. HIV-1 env and gag p17 sequence evolution rates (0.22 – 0.45%/site/year) were higher than zero ($P < 0.05$) in 6/10 ELLHI, which together with detectable residual plasma HIV RNA, suggest that HIV-1 replicated and evolved in at least 6 ELLHI at levels below detection of routine assays.
Conclusion: The new method is unique with the features needed for screening, confirming and characterizing ELLHI, which should prove useful in identifying ELLHI on a larger scale worldwide. These ELLHI who have remained disease-free for two or more decades offer a unique study model for the unusual control of HIV-1 infection.

Session 8: Clinical Trials Part 2

0A08-01

Adenoviral HIV-1 vaccine elicits durable CD8+ and CD4+ HIV specific responses in HIV-1 uninfected adults without pre-existing Ad5 antibodies, HVTN 054

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Objective: To evaluate the safety and immunogenicity of the NIAID Vaccine Research Center adenoviral vector HIV gag-polB/envA/envB/envC vaccine (rAd5-HIV) delivered IM at 2 doses in adults with undetectable levels of pre-existing adenovirus type 5 (Ad5) neutralizing antibodies (nAb).
Methods: The NIAID HIV Vaccine Trials Network enrolled 48 participants: 20 received 1 dose of 10^{10} PU rAd5-HIV (T1), 20 10^{11} PU rAd5-HIV (T2), and 8 placebo (C). Participants were monitored for reactogenicity and adverse events. PBMC were evaluated by IFN-γ ELISpot and CD4/CD8, IFN-γ/IL-2 intracellular cytokine staining (ICS) assays. These assays utilized potential T cell epitope peptide pools for Gag, Pol, and Env, representing peptides present in at least 15% of HIV-1 isolates in the Los Alamos Database. HIV antibody testing was performed using Abbot HIVAB HIV 1/2, BioRad Genetic Systems HIV 1/2 Plus O, and/or bioMerieux Vironostika HIV-1 EIAs.
Results: The vaccine appeared safe in participants without prior Ad5 nAb (presented at AIDS Vaccine 2006). IFN-γ ELISpot response rates to any of the 3 antigens at 4 weeks post injection (D28) were 0/7C, 14/16 T1 and 17/20 T2 with magnitudes up to 3200 SFC/10^6 PBMC to a peptide pool. 53% of vacinees recognized all 3 antigens, 22% recognized 2, 11% recognized 1, and 14% recognized 0. One year post vaccination the IFN-γ ELISpot response rates were 15/22 T1 and 13/20 T2. Response rates to any of the 3 antigens for CD4+ or CD8+ T cells by ICS for IFN-γ and/or IL-2 at D28 were 0/8 C, 17/19 T1, and 17/20 T2 with up to 4.2% of T cells responding to a peptide pool. 0/8 C, 12/19 T1, and 10/20 T2 had HIV-specific CD4+ T cell responses. 0/8 C, 15/18 T1, and 16/20 T2 had HIV-specific CD8+ T cell responses. One year post vaccination, 0/8 C, 18/19 T1, and 15/19 T2 tested positive for vaccine induced HIV antibodies by 1 or more EIA.
Conclusion: One dose of this rAd5-HIV vaccine elicited high frequency and magnitude of CD8+ T cell responses to HIV antigens in Ad5 nAb seronegative persons. These responses were discernible for up to one year, perhaps related to the associated CD4+ T cell responses. 75% of vaccinees recognized epitopes in 2 or more antigens. 87% of uninfected vaccinees developed antibodies detected by commercial HIV antibody tests. To date, these are the most potent vaccine induced HIV-1 responses reported in humans.

0A08-02

Safety and immunogenicity of the MRKAd5 gag HIV-1 vaccine in a worldwide phase I study of healthy adults (Merck V520-018/HVTN 050)

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Background: Phase I studies indicate the monovalent MRKAd5 HIV-1 gag vaccine is well tolerated and immunogenic in N. American populations. High prevalence of preexisting immunity to adenovirus type 5 (Ad5) may affect vaccine response rates. **Aim:** We analyzed the preliminary data through Week 30 of this international Phase I study testing the safety and immunogenicity of an Ad5 HIV vaccine candidate.
Methods: Healthy adults aged 18-50 at low risk for HIV infection were randomized 1:3:3 to receive placebo, 1x10^9 or 1x10^{10} viral particles (vp) of the MRKAd5 HIV-1 gag vaccine at Day 1, Week 4 and Week 26 in a dose-escalating staged study in 24 centers in Africa, Asia, Caribbean, N. and S. America. Enrollment was not stratified by baseline Ad5 titer. Adverse events (AE) and lab values were assessed after each dose. Immunogenicity was evaluated using an IFN-γ ELISPOT gag 15-mer assay. Positive ELISPOT responses were defined as >55 SFC/10^6 PBMC and ≥4-fold over mock control.
Results: 360 people (55% male, median age 30) were enrolled (87 each in Asia and N. and S.America; 75 in the Caribbean; 24 in Africa). The vaccine was generally well tolerated at both doses. The most common AEs were injection site reaction, headache, fever, and diarrhea. At Week 30, pooled ELISPOT responses were 57/133 (43%) in the 1x10^9 vp group and 108/139 (78%) in the 1x10^{10} vp group. Overall, responses to 1x10^{10} vp were 85% and 68% in subjects with low (≤200, n=75) and high (>200, n=62) baseline Ad5 titers, respectively. Response rates among subjects with high baseline Ad5 titers who received 1x10^{10} vp were: 23/26 (88%) in Asia, 2/7 (29%) in N. America, 10/13 (77%) in S. America, 7/12 (58%) in the Caribbean, and 0/4 in Africa.
Conclusion: The MRKAd5 HIV-1 gag vaccine was generally well tolerated and immunogenic in diverse world regions. The 1x10^{10} vp dose was generally more immunogenic than 1x10^9 vp. Although there may be a modest effect of high baseline Ad5 titers on ELISPOT responses, overall most subjects with high levels of preexisting Ad5 immunity had positive ELISPOT responses to 1x10^{10} vp. These data indicate that the MRKAd5 HIV-1 gag vaccine may be immunogenic in regions with high prevalence of Ad5 immunity. This international study of the MRKAd5 gag vaccine supports ongoing Phase II test-of-concept trials of a next generation MRKAd5 trivalent gag/pol/nef vaccine.

0A08-03

Safety and immunogenicity of VRC multiclade HIV-1 adenoviral vector vaccine alone or with VRC multiclade HIV-1 DNA plasmid vaccine in African adults

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Methods: Healthy adults aged 18-50 in Kigali, Rwanda and Nairobi, Kenya were randomized to either i) one intramuscular injection of $1x10^{10}$ or $1x10^{11}$ particle units of VRC multiclade HIV-1 recombinant adenoviral vector vaccine (rAd5, n=35) or placebo, or ii) 3 doses 4mg VRC multiclade HIV-1 DNA vaccine (DNA) followed by 1 dose rAd5 $1x10^{10}$ or $1x10^{11}$ as boost or placebo (n=79) (vaccine:placebo 3:1). Safety and tolerability were assessed clinically and by routine lab tests. Immunogenicity was evaluated by Ad5-specific neutralization (NT) assay and IFN-γ ELISpot on frozen PBMC with matched peptides reported as spot forming cells (SFC)/million PBMC.

Results: The study is ongoing; preliminary safety and group unblinded immunogenicity data are presented. Local reactions were experienced by approx. 75% of volunteers following any rAd5/placebo and almost all volunteers following any DNA/placebo, most events were mild. Systemic symptoms were reported by 67-72% following any rAd5/placebo and 89% of volunteers following any DNA/placebo, most events were mild. 342 adverse events, mostly mild, were reported within 28 days of any vaccination. There were no vaccine related serious adverse events.

Immune responses by ELISpot were detected in 6/13 (46%) and 7/13 (54%) recipients of rAd5 10^{10} and rAd5 10^{11} respectively, and in 0/9 placebo recipients. Median SFC were 85 and 77 per million PBMC respectively (range 39-297). Following 3 DNA/placebo there were 27/55 (49%) vaccinees and 1/20 placebo recipients with positive IFN-γ ELISpot responses. Median SFC were 92 (range 44-598). Following rAd5 10^{10} and rAd5 10^{11} boost, there were 18/25 (72%) and 18/26 (69%) responders respectively. Median SFC were 106 and 105 (range 41-1707).

At baseline, 74% of all volunteers had detectable Ad5 NT antibodies (Ab). In either DNA prime/rAd5 boost arms the impact of Ad5 NT Ab on immunogenicity amongst vaccine recipients was modest. In rAd5 alone arms numbers were too small to assess effect.

Conclusion: Vaccination with rAd5 either alone or as boost in combination with corresponding DNA vaccine appears safe and well tolerated. Data from African volunteers indicates good immunogenicity of DNA prime/rAd5 boost regimen, appears equivalent at the different rAd5 boost doses and only modestly affected by baseline Ad5 NT Ab. Future trials will focus on larger sample sizes and those at risk for HIV.

0A08-04

A phase IIA trial to evaluate a multiclade HIV-1 DNA vaccine followed by a multiclade rAd5 HIV-1 vaccine boost in HIV-1 uninfected adults (HVTN 204)

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Background: The NIAID HIV Vaccine Trials Network (HVTN) has undertaken a prospective, randomized, double-blind, placebo-controlled phase IIA clinical trial of the NIAID Vaccine Research Center's (VRC) 6-plasmid candidate DNA HIV-1 (envA, envB, envC, gagB, polB, nefB) (DNA-HIV) and rAd5 HIV-1 (envA, envB, envC, gagB, polB) vaccine (Ad5-HIV) in healthy uninfected people in diverse geographic locations.

Methods: A total of 480 participants (ppts) were enrolled irrespective of their baseline Ad5 titer; 240 in the Americas (US, Brazil, Jamaica & Haiti) and 240 in South Africa. Half in each region received DNA-HIV (4 mg IM by Biojector) at 0, 1 and 2 months, followed by Ad5-HIV (10^{10} PU IM by needle/syringe) at 6 months; the other half received placebo injections on the same schedule. Ppts were monitored for reactogenicity and adverse events throughout the 12-month trial; sera and PBMCs were evaluated by humoral (binding/neutralizing antibody) and cellular (bulk IFN-γ ELISpot and IFN-γ /IL-2 intracellular cytokine staining, using global Potential T cell Epitope [PTE] peptide pools) immune assays.

Results: At the US sites, the 180 allotted ppts completed enrollment on 7 Apr 06 while enrollment was completed in South Africa on 18 Dec 06 and in the Caribbean/South American region on 20 Mar 07. Preliminary blinded safety and immunogenicity results are available for the US cohort. Both vaccines were well-tolerated. DNA-HIV/placebo caused more local reactions; moderate pain in 10% of participants after 1st vaccination, decreasing with subsequent injections, while Ad5-HIV/placebo caused more systemic reactions; self-limited moderate malaise and/or fatigue in 9.3%. At days 0 and 210 (6 weeks after the rAd5-HIV boost), IFN-γ ELISpot assays were positive in 1.6% and 37.8% of study ppts (half of whom received placebo), respectively. Of those that were positive, responses to Env and Gag peptides were most frequent, seen in 73.2% and 80.4% of responders, respectively.

Conclusion: These preliminary interim data indicate acceptable safety and suggest that IFN-γ ELISpot responses using the PTE peptide pools could be positive in up to 75% of vaccine recipients. If further study data confirm these findings and results are favorable from other related protocols, evaluation of this regimen in a phase IIB efficacy trial will be proposed, and would be conducted by the Partnership for AIDS Vaccine Evaluation (PAVE) in distinct regions with diverse HIV-1 epidemics.

OA08-05

Safety and immunogenicity of an adenovirus type-6 (MRKAd6) HIV-1 gag/pol/nef trigene vaccine dose escalation study

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Background: Preexisting immunity to adenovirus type 5 (Ad5) may affect immune responses to Ad5-based HIV vaccines. Using alternate serotype vectors may facilitate vaccine development for populations with a high prevalence of Ad5 immunity.
Objective: We performed an analysis of the preliminary Week-30 data in the 1st human study to assess the safety & immunogenicity of the MRKAd6 trigene vaccine, an Ad6-vectored HIV vaccine containing 3 clade-B HIV-1 transgenes in a single construct: a nef expression cassette (human CMV promoter-HIV nef-BGH polyA) directly followed by a combined gagpol expression cassette (murine CMV promoter-HIV gagpol fusion-SV40 polyA).
Methods: HIV-seronegative healthy adults 18-50 years old at low risk of acquiring HIV infection with baseline anti-Ad6 titers ≤18 were randomized in a blinded, dose-escalating, placebo-controlled study to receive 3 injections of the MRKAd6 trigene vaccine (containing either 10^9, 10^{10}, or 10^{11} viral genomes [vg]) or placebo (P) in a 4:1 ratio at Weeks 0, 4, & 26. Safety was evaluated by assessment for clinical adverse events (AE) using a Vaccine Report Card and by safety laboratory evaluation after each dose. Immunogenicity was assessed by gag-, pol-, or nef-specific unfractionated IFN-γ ELISPOT assays at Week 30; a positive response was defined as ≥55 SFU/10^6 PBMC.
Results: Safety data through Week 30 were available for all 30 subjects (6 P and 24 vaccine recipients). No serious AE occurred. Fever ≥100°F was noted in 2 (8%) vaccine recipients. Injection-site reactions were the most commonly observed AE; their frequency increased with dose: 33% (P), 50% (10^9 vg), 88% (10^{10} vg) and 100% (10^{11} vg). No consistent laboratory abnormalities were observed. ELISPOT response rates to >1 vaccine antigen were lower at 10^9 vg (14%) than 10^{10} (63%) or 10^{11} vg (71%); vaccine recipients more often responded to nef (57%, 88%, 86%) than to gag (14%, 38%, 57%) or pol (14%, 50%, 43%).
Conclusion: The MRKAd6 trigene vaccine was generally well tolerated and immunogenic in subjects without preexisting Ad6 immunity. Injection site reactions were dose related. The 10^{10} and 10^{11} vg doses elicited comparable cell-mediated immune responses and appeared more immunogenic than 10^9 vg. ELISPOT response rates to nef were consistently higher than to gag or pol with the trigene construct. MRKAd6 is a promising vector for HIV-1 vaccines designed to induce cell-mediated immune responses.

OA08-06

Safety and immunogenicity of the MRKAd5 HIV-1 trigene and MRKAd6 HIV-1 trigene vaccines in combination (MRKAd5+6 trigene vaccine) in healthy adults

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Background: Preexisting adenovirus type 5 (Ad5) antibodies may affect responses to Ad5-vectored HIV-1 vaccines. A vaccine combining 2 Ad serotypes might overcome Ad type-specific immunity.
Objective: We performed a preliminary Week-30 analysis of the 1st human study of the MRKAd5+Ad6 trigene vaccine containing 3 clade-B HIV-1 transgenes as a single construct in each vector: a nef expression cassette (human CMV promoter-HIV nef-BGH polyA) directly followed by a gagpol expression cassette (murine CMV promoter-HIV gagpol fusion-SV40 polyA).
Methods: HIV-seronegative healthy adults 18-50 years old at low risk of HIV infection were randomized in a blinded, dose-escalating, placebo-controlled study stratified by neutralizing anti-Ad5 titers (≤200 or >200) & anti-Ad6 titers (≤18 or >18) to receive 3 IM injections of vaccine (containing 10^9 or 10^{10} total viral genomes [vg] as 1:1 mixture of Ad5 & Ad6 vectors) or placebo (P) at Weeks 0, 4, & 26. Clinical adverse events (AE) & safety laboratory tests were evaluated after each dose. Immunogenicity was assessed by gag-, pol-, or nef-specific unfractionated IFN-γ ELISPOT responses ≥55 SFU/10^6 PBMC.
Results: 97 subjects were randomized, yielding ~10 subjects in each of the 4 baseline Ad5/6 strata for each of the 2 vaccine dose groups & 16 subjects in the P group. 90 (93%) subjects completed 30 weeks of follow-up. No serious AEs were observed. Injection-site reactions were more frequent with 10^{10} vg (85%) than with 10^9 vg (65%) or P (44%). The most frequently reported systemic AEs were headache (38% P; 35% 10^9 vg; 27% 10^{10} vg) & fatigue (13% P; 15% 10^9 vg; 22% 10^{10} vg). ELISPOT response rates to nef at 10^{10} vg were 50% in low Ad5/low Ad6 stratum (n=8), 78% in lowAd5/high Ad6 stratum (n=9), 75% in the high Ad5/low Ad6 stratum (n=8), & 44% in the high Ad5/high Ad6 stratum (n=9). Gag response rates ranged from 11-38% at 10^9 vg to 22-44% at 10^{10} vg (n=8-10 per stratum).
Conclusion: The MRKAd5+6 trigene vaccine was generally well tolerated. Injection site reactions appeared to be dose related. ELISPOT response rates to nef were consistently higher than to gag or pol. At the 10^{10} vg dose, ELISPOT response rates in the high Ad5/low Ad6 stratum were similar to those in the low Ad5 strata. Although individual strata sample sizes were small, this exploratory study suggests that combining Ad5+6 vectors may be a useful vaccine strategy to address pre-existing Ad type-specific immunity.

0A08-07

A phase I clinical trial to evaluate the safety and immunogenicity of an HIV-1 DNA vaccine with a plasmid cytokine IL-2/Ig adjuvant (HVTN 044)

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Objective: IL-2 is an important immunostimulatory cytokine, but its half-life in vivo is short and the optimal timing of in vivo stimulation may be critical. To investigate the potential immunostimulatory effect of IL-2, we conducted a dose escalation study of a plasmid coding for a fusion protein of IL-2/Ig (a protein that has IL-2 functional activity and a longer half-life in vivo) administered simultaneously or 48 hours after a DNA HIV vaccine candidate to normal volunteers, and measured HIV-1 specific cellular and humoral immune responses.

Methods: The NIAID HVTN conducted a phase I clinical trial to evaluate the safety and immunogenicity of the HIV-1 DNA vaccine (Gag-Pol-Nef-multiclade Env; dose 4 mg) with the plasmid cytokine adjuvant (IL-2/Ig) given at 0, 1, 2, and 6 months in 70 HIV (-), healthy adults. Subjects received placebo (n=10, P); vaccine alone (n=10, D); dose escalation of adjuvant alone (n=10); adjuvant (at 0.1 mg [n=5], 0.5 mg [n=5], 1.5 mg [n=10], or 4.0 mg [n=10, T4]) concurrent with vaccine; or adjuvant (at 4.0 mg [n=10, T5]) given 2 days post vaccine.

Results: The participants were 67% (43) male, 51% (36) white, 31% (22) African-American, and had a median age of 26 years (range 18-40). No significant differences in local or systemic reactogenicity or other adverse events were observed between groups. 1 SAE/EAE (malaise T1) was considered probably related to vaccination. No anti-IL2 antibodies were detected. Many volunteers did not receive all vaccinations: 87% (61/70) received 3 and 70% (49/70) received 4. In the day 70 ITT analysis on fresh cells, cellular immune responses to the ENV-A antigen were detected by IL-2 Elispot in 0% (0/19) P, 40% (4/10) D, 40% (4/10) T4, and 80% (8/10) T5; and by IFN-γ Elispot in 0% (0/19) P, 80% (8/10) D, 50% (5/10) T4, and 100% (10/10) T5. The magnitude of median responses (SFCX10^6 PBMC) by IL-2 Elispot were 3 P, 32 D, 90 T4, 193 T5 (p=0.16 T5vsD); and by IFN-γ Elispot were 5 P, 127 D, 103 T4, and 380 T5 (p=0.14 T5vsD). In a pooled analysis DvsT5 for Elispot magnitudes looking at both IL-2 and IFN-γ responses p=0.04. Neutralizing antibodies were detected in 3 subjects, 1 each in groups D, T2, and T5, at day 182.

Conclusion: Both the adjuvant and vaccine were generally well tolerated. The plasmid IL-2/Ig adjuvant augmented cellular immune responses when administered 2 days after the DNA vaccine. Further characterization of the breadth and magnitude of these immune responses are underway.

Session 9: Immune Escape

0A09-01

Analysis of HLA class I associated mutations identifies CD8+ T cell selective pressures driving viral escape within highly conserved regions of HIV

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Objective: CD8+ T cell responses play a critical role in the containment of HIV. Recent data reveal that some CTL escape mutations in gag substantially impair viral replication which may represent a key component of this control. Here we attempt to identify additional CTL escape mutations within highly conserved regions of HIV likely to impair viral replication.

Methods: Full-length viral sequences and HLA types were derived from 99 chronic untreated HIV clade B infected subjects to identify HLA class I-associated mutations in HIV at the population level. Mutations associated with expressed HLA alleles were determined through Fisher's exact test and corrected for multiple comparisons, then mapped to previously described CD8+ T cell responses.

Results: We identified 430 HLA-associated sequence polymorphisms across HIV representing likely CD8 escape mutations or secondary compensatory mutations. Over 100 of these resided at amino acid residues >95% conserved. 18% of associations were located within previously described CD8 epitopes, and 4% flanked described CD8 epitopes within 3 amino acids. Overall, 16% of HIV residues contained an HLA association, with the majority of associations in gag, pol, and nef (88, 90, and 58 respectively), or restricted by HLA-B alleles (50%). Comparing the location of HLA associations with CD8 responses detected in 90 chronic clade B infected subjects identified a correlation between the average magnitude of the T cell response and the strength of the HLA-association (p=0.028). In addition, HLA associated mutations were found in 20 CD8 epitopes targeted during acute infection, including the single most immunodominant epitope in 9 HLA alleles. Among these were the B57-TW10 and B27-KK10 epitopes in gag for which we have previously demonstrated an impact of CTL escape mutations on viral replication. A trend was also observed in gag towards HLA-associated mutations restricted by 'protective' HLA alleles arising at more highly conserved residues.

Conclusion: These data identify a large data set of HLA-associated mutations across the HIV genome, and suggest that immunodominantly targeted eptiopes in acute HIV infection preferentially drive viral escape. The identification of HLA-associations at highly conserved residues will enable a broader assessment of the contribution that viral fitness constraints during CTL escape play in the control of HIV to guide current vaccine approaches aimed at limiting viral replication.

0A09-02

Longitudinal evaluation of HLA class I-mediated evolution in HIV-1 pol and nef in the first two years of infection: a multicenter study

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Objective: Population-based analyses of chronically-infected individuals reveal substantial levels of HLA class I-mediated imprinting on circulating HIV genomes. However, the time-course of selection of HLA-associated polymorphisms, and the relative contribution of HLA-restricted immune selection pressures to net HIV-1 evolution is unclear. We characterize HLA-mediated viral evolution in 75 recently-infected individuals and determine the proportion of evolution attributable to HLA-restricted immune pressure in the first 2 years of infection using phylogenetically-corrected methods.

Methods: HIV-1 RNA genotyping of protease (PR), RT and nef was performed on serial plasma samples collected from as early as 2 weeks after infection up to 2 years of followup (median 5 samples/subject). Baseline amino acid (aa) sequences were analyzed for HLA-associated escape mutations present at first sampling; subsequent aa substitutions were identified by comparison with baseline sequences. HLA class I alleles were determined by sequence-based typing. HLA-associated escape and reversion substitutions were strictly defined as those identified in a viral lineage-corrected, cross-sectional analysis of ~700 chronically-infected, treatment-naïve individuals.

Results: Baseline PR, RT and Nef aa sequences exhibited an average of 0.1, 0.4 and 1.4 HLA-associated escape mutations at the earliest timepoint. A total of 54, 127 and 270 substitutions were observed at 28, 80 and 110 unique codons in PR, RT and nef between baseline and final sequences, indicating modest levels of overall evolution in pol compared to nef. The proportion of substitutions attributable to HLA-restricted immune pressure was ~20% and 40% for pol and nef, respectively, estimates which are unlikely solely due to the high density of associations identified in nef in cross-sectional analyses ($p<0.05$). Escape and reversion events accounted for 35% and 65% of all HLA-associated aa changes during followup.

Conclusion: HLA class I-restricted immune selection accounts for a minimum of 20-40% of aa changes in pol and nef in the first two years of infection, indicating rapid and dynamic viral adaptation in the new host. Longitudinal analyses confirm the selection of specific HLA-associated substitutions identified in an independent cross-sectional study, indicating escape follows largely predictable patterns in context of specific HLA alleles. Common escape pathways should be considered in cell-mediated HIV vaccine design.

0A09-03

Debate on HIV envelope as a T cell immunogen has been gagged

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Background: HIV-specific T lymphocyte responses are important for the control of viremia, but the relative utility of responses to the various HIV proteins is controversial. An immune response that elicits a dire fitness cost to the mutating virus would be an ideal vaccine target. HIV envelope (env) is subject to both humoral and cellular immune responses, suggesting multiple rounds of mutation are needed to facilitate viral escape. The gag protein, however, has recently been shown to elicit a more effective immune response in humans, potentially because gag mutations carry greater fitness costs.
Objective: To evaluate the role of env-specific and gag-specific CD8 T cell responses in control of SHIV viremia and maintenance of CD4 T cell levels in prime/boost vaccinated macaques.
Methods: We studied 36 pigtail macaques for their T lymphocyte responses to HIV-1 env and SIV gag following prime/boost vaccination with DNA, recombinant fowlpoxvirus and vaccinia vaccines, followed by intrarectal challenge with $SHIV_{mn229}$. Vaccines expressed a heterologous env truncated in the middle third of the protein, precluding the stimulation of env-specific neutralising antibody responses.
Results: Eight CD8 and two CD4 Env-specific T cell epitopes were mapped. Three of the CD8 T cell epitopes, $RY8_{786-793}$, $SP9_{110-118}$ and $NL9_{669-677}$ were identified in multiple animals. Animals that responded to HIV-1 env, when compared to those animals without responses to Env, showed no significantly better control of viral load at week 15 post challenge (5.1-log_{10} vs 5.2-log_{10}) nor retention of CD4 T lymphocyte levels (4.8% vs 3.3%). These results are in contrast to gag-responding animals compared to gag non-responders. Mutations at the env-specific CD8 T cell epitopes was common (5 of 8 epitopes in 7 of 10 animals), but appeared to induce minimal fitness costs. This also contrasts to mutations in gag, which result in the rapid emergence of dominant escape motifs, suggesting significant selective pressure.
Discussion: Our results suggest that env-specific T cells have minimal impact on viremia, potentially because the responses are readily escaped with minimal fitness cost. Env may have limited utility as a T cell immunogen.

0A09-04

Does T cell function drive HIV-1 evolution?

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Background: The enormous mutability of HIV-1 presents one of the greatest challenges to the immune system. There is a vast literature on CTL escape. However, escape does not occur in all T cell epitopes. The factors that govern whether an epitope will escape are not known but are important to understand. Here we investigated whether the proliferative capacity of HIV-specific $CD8^+$ T cells drives viral evolution.
Methods: The study subject is a 44 year old white male who received a candidate canarypox vaccine (vCP205) prior to seroconversion and has controlled infection in the absence of ART for > 7 years. Epitope-specific T cells against six CTL epitopes were measured by IFN-γ ELISpot and cell proliferation by CFSE staining in six longitudinal samples between 413 and 2643 dpi. Viral evolution was monitored by sequencing specific epitopic regions.
Results: Strong epitope-specific proliferation (2.4-5.1 % $CD8^+$ $CFSE^{low}$ T cells, corresponding ELISpot values 122-294 SFU/M PBMC) was found in response to RT TI8, Nef AK9 and Gag LL8, and coincided with escape in these epitopes. We observed no escape in epitopes recognized by weakly proliferative T cells (range = 0.2-2.0 % $CD8^+$ $CFSE^{low}$ T cells against gag AK11; 0.2-0.8 % $CD8^+$ $CFSE^{low}$ T cells against gag CL9) despite comparable or higher ELISpot values (range = 43-458 SFU/M PBMC against gag AK11; 63-688 SFU/M PBMC against CL9).
Discussion: Our data suggest that HIV-specific T cells with high proliferative capacity exert selective pressure on the virus and drive escape. We propose that escape will occur if the epitope-specific T cells that target the epitope have high proliferative capacity and if escape does not have severe fitness cost for the virus. If an epitope is targeted by T cells with very low proliferative capacity there will be minimal pressure on the virus and consequently no escape. Thus, T cells with high proliferative capacity that also target constrained epitopes should be highly effective at controlling infection. Our findings suggest that the capacity of $CD8^+$ T-cells to proliferate determines their anti-viral effectiveness.

OA09-05

Donor HIV-1 gag but not nef CTL escape mutations lower viral load set point in newly infected recipients

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Background: Evolution of the HIV antigenic repertoire in human populations continues to hamper the design of effective vaccines. We hypothesized that HIV isolates with cytotoxic T-lymphocyte (CTL) escape mutations, especially those in conserved proteins, can impact viral load set point when transmitted to new hosts.

Methods: HIV sequencing (gag and nef) and human leukocyte antigen class I (HLA) genotyping were performed using plasma and PBMC DNA obtained from 88 epidemiologically-linked linked Zambian heterosexual transmission pairs. HLA-associated HIV polymorphisms were identified using Fisher's exact test and corrected by phylogenetic tree analysis. HIV polymorphisms were further restricted to those occurring within known HLA-restricted CTL epitopes. Plasma VL set point was quantified from recipient samples obtained 3-6 months post-seroconversion. Epidemiologic linkage analysis confirmed that virus was transmitted from the chronically infected partner to the newly infected recipient in all cases. The vast majority of HIV polymorphisms were identical in the donor and the recipient leaving no doubt as to the identity of the transmitting viral strain. In cases where the HIV polymorphisms were not identical, the donor virus was defined as the transmitting strain.

Results: HLA-associated CTL mutations were observed in p17 (n=4), p24 (n=8), and Nef (n=7). The majority were restricted by HLA-B alleles (2 in p17; 7 in p24; 5 in Nef). Upon transmission to a new host, only mutations in gag and p24 but not Nef were associated with lower VL (r= -0.23; p=0.03 for gag or p24; Spearman rank). Only CTL escape mutations induced by HLA-B alleles but not HLA-A or HLA-C were associated with a lower VL set point in recipients (r = -0.32; p=0.002). Remarkably, VL set point was more than 4-fold lower in recipients carrying 3 or more HLA-B associated CTL escape mutations compared to those without any of these mutations (12,300 vs 54,800 copies/ml, respectively; p=0.003; Mann-Whitney).

Conclusion: HLA-B-associated escape mutations in HIV-1 gag can lead to a reduced VL upon transmission to newly infected individuals irrespective of their HLA genotypes. Data from the MACS cohort suggest that the VL differences observed in our study are likely to be clinically significant. These findings further refine the importance of gag epitope targeting for the development of a vaccine designed to prevent HIV disease progression and identify a determinant of plasma VL set point.

OA09-06

CTL escape mutations in HIV capsid decrease viral fitness by altering cyclophilin A dependence revealing regions of gag susceptible to immune pressure

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Background: Accumulating evidence supports the importance of gag-specific CD8+ T cell responses (CTL) in containment of HIV infection. Notably HLA-B57 and -B27 are strongly associated with control of HIV and have immunodominant target epitopes in gag p24. Understanding the mechanisms of immune control is critical to the design of an effective HIV vaccine.

Objective: Determine whether mutations in HIV gag p24 induced by CTL immune pressure impair viral replication by altering the critical interaction between p24 and the host protein cyclophilin A (CypA).

Methods: Gag sequences were derived from chronically infected subjects expressing HLA-B57 or -B27. CTL escape and compensatory mutations associated with the immunodominant HLA-B57 TW10 and B27 KK10 T cell epitopes in p24 were engineered into strain NL4-3 and viral replication, reverse transcription, and capsid stability determined. CypA dependence was assessed using CypA knockout cells, RNAi knockdown, and treatment with cyclosporine A (CsA). CsA competes with capsid for the binding to CypA.

Results: Escape mutations in the B57-TW10 and B27-KK10 epitopes in p24 capsid significantly impaired viral replication and required one or more compensatory mutations to recover fitness. Moreover, these mutations altered the dependence of variants for CypA, which is normally critical for infection by HIV. In the case of B57-TW10, upstream compensatory mutations partially restored viral replication but reduced CypA binding and lowered viral sensitivity to CsA. For B27-KK10, the primary escape mutation ($R_{264}K$) dramatically impaired viral replication and was associated with a CsA-dependent phenotype, which was reversed by a naturally occurring compensatory mutation. Notably, the $R_{264}K$ virus replicated as well as NL4-3 in CypA knockout Jurkat cells, indicating that there was no major defect in capsid function apart from its severely impaired ability to replicate in the presence of CypA.

Conclusion: These data illustrate that the critical interaction between HIV capsid p24 and the target cell protein CypA can be disrupted by the process of CTL escape and compensation in gag. The structural and functional constraints on capsid may limit viable pathways for immune escape and result in a greater number of p24 variants displaying fitness defects. Identification of additional CTL-mediated escape mutations that alter the capsid:CypA interaction may provide key targets for vaccine design.

0A09-07

Probing the antigenic surface of HIV-1 gp120 in the context of the functional spike of a primary isolate by epitope insertion mutagenesis

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Background: Primary HIV-1 isolates are often resistant to antibody neutralization, particularly to antibodies in polyclonal immune sera. One often proposed explanation is that certain epitopes on the HIV envelope spike are occluded and, thus, inaccessible to antibody.
Objective: Here, we sought to gain a more comprehensive understanding of the antigenic surface topology of an HIV-1 envelope spike.
Methods: We inserted a hemagglutinin (HA) epitope into 12 different locations of the gp120 subunit from a pseudotyped primary isolate (JR-CSF) and measured virus neutralization by a panel of monoclonal and polyclonal anti-HA antibodies.
Results: Six of the 12 mutant viruses were infectious; five of the tolerated insertions were in variable regions (V1, V2, V4 (2x), V5) and one tolerated insertion was in a constant region (C2). Only the V2(HA) chimera exhibited increased sensitivity to neutralization by otherwise weakly-neutralizing antibodies, indicating that insertion of the HA epitope did not cause a measurable change in the quarternary conformation of the viral spike in most chimeras. Surprisingly, although multiple chimeric viruses could be neutralized by most anti-HA monoclonal antibodies tested here, only single chimeras could be neutralized by anti-HA polyclonal sera. This suggests that most of the inserted HA epitopes, though accessible to antibody in principal, may be in a conformation that is not compatible with broad antibody binding.
Discussion: Based on these results, we hypothesize that the relative resistance of primary HIV isolates to antibody neutralization may stem, at least in part, from conformational restriction imposed on certain antigenic epitopes in the context of the functional viral spike. These constraints, in combination perhaps with additional factors that remain to be defined, reduce the relative number of antibodies capable of binding to the spike, rather than bona fide occlusion of these epitopes.

0A09-08

Broadly neutralizing antibodies against gp41 fail to block the formation of virological synapses: a potential mechanism of HIV escape

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Background: Cellular contacts between HIV infected cells and target CD4 T cells are the main mechanism of HIV spread. Subtle differences exist between this process and the infection of CD4 T cells by cell-free virus. Indeed, cell-to-cell transmission increases effective virus concentration and activates endocytic fusion-independent capture of HIV by CD4 T cells.
Objective: Compare the ability of broadly neutralizing antibodies to inhibit cell-free HIV infection and cell-to-cell HIV transmission.
Methods: MOLT cells chronically infected with NL4-3 were used as HIV presenting cells in coculture with primary CD4 T cells. Cellular contacts (synapses) were quantified by flow cytometry and visualized by electron microscopy. HIV transfer was assessed by intracellular p24 staining. Supernatants from MOLT infected cells were used to infect TZM-bl cells
Results: Neutralizing antibodies IgGb12, 4E10 and 2F5 blocked infection of TZM-bl cells by cell-free virus with IC50s of 0.02 +/- 0.02, 1.1 +/- 0.6 and 0.5 +/-0.4 µg/ml, respectively. In parallel we tested the ability of these antibodies to block different functions of HIV envelope in cocultures of MOLT infected cells with primary CD4 T cells. IgGb12 and the anti-CD4 mAb Leu3a were able to block the formation of virological synapses; conversely 4E10 or 2F5 mAbs did not show any inhibitory effect. Consequently, IgGb12 inhibited virus transfer to CD4 T cells with an IC50 of 0.4 +/-0.2 µg/ml, whereas 4E10 and 2F5 antibodies failed to inhibit virus transfer (max. concentration tested 30 µg/ml), rather an increase of viral particles associated to CD4 T cells was observed concomitant to cell-to-cell fusion blockade. To further determine the consequences of this failure, we have analyzed electron micrographs of synaptic contacts. Images revealed a polarized virus budding towards the synaptic space in which mean virus concentration was 1.4+/-0.6 $x10^{11}$particles/ml peaking at 2.2 $x10^{11}$ particles/ml.
Conclusion: Broadly neutralizing anti gp41 antibodies failed to block the formation of stable contacts between HIV infected cells and CD4 T cells. As a result of this failure, gp41 antibodies had no inhibitory effect on HIV particle transfer from infected to uninfected cells. Given the high local concentration of HIV particles found in the synaptic space, the ability of gp41 antibodies to block HIV infection during cell-to-cell transmission may be compromised.