SYMPOSIUM 05: PROTECTION AGAINST MUCOSAL TRANSMISSION AND VIRAL DISSEMINATION

S05-01

Characterization of viral variants that initiate systemic infection after mucosal transmission.

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Understanding the early events in HIV-1 transmission and the characteristics of the viruses that establish systemic infection are critical to vaccine development. Recent studies employing single genome amplification of newly infecting viruses have demonstrated that, in a majority of new HIV infections (80-90% depending on risk group), a single genetic variant derived from the transmitting (donor) partner's quasispecies is transmitted. A comparison of the transmitted virus to the viral quasispecies in the genital compartment of the transmitting partner indicates that the transmission-associated genetic bottleneck is not the result of limited genetic diversity in this compartment. Transmission of multiple variants (2-5) from a single donor appears to occur more frequently when the mucosal barrier of the recipient is compromised by inflammation. Newly infecting viruses in the subtype-C cohort under study have a strong dependence on CD4 and CCR5 for entry into target cells, and, similar to donor-derived viruses, infect macrophages inefficiently. Analysis of the Gag, Pol and Nef sequences in both donor and recipient viruses from ~140 epidemiologically-linked transmission pairs shows that HLA-linked polymorphisms (consistent with CTL escape) are frequently transmitted. A majority of these appear to have been selected in individuals prior to the donor partner and so likely have little effect on viral fitness. Nevertheless, increasing numbers of transmitted escape mutations in Gag but not Nef, do impact the in vivo fitness of the transmitted virus and correlate with lower early set-point viral load. Preliminary experiments show that this in vivo fitness cost is associated with a reduced replicative capacity in PBMC in vitro, suggesting that targeting multiple epitopes in Gag will be important in HIV-1 vaccine design.

S05-02

Target cell availability and prevention of mucosal transmission

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I will discuss studies of vaginal transmission of HIV-1 in the SIV-rhesus macaque model that have revealed the critical importance of the availability of CD4+ T cells in local expansion and systemic dissemination. I will describe how this understanding of target cell availability can be used in designing effective microbicides and vaccines.

S05-03

Do mucosal T-cell responses contribute significantly to HIV control?

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The gastrointestinal mucosa serves as an important site of HIV transmission, active viral replication and CD4+ T-cell depletion, but is also rich in antigen-experienced CD8+ T-cells. These cells have primarily an "effector memory" phenotype, and express low levels of perforin as compared to their counterparts in blood. Mucosal T-cell responses have been described as "too little and too late" to limit HIV/SIV dissemination during acute infection. However, mucosal T-cell responses during chronic HIV infection can be surprisingly robust and polyfunctional. Notably, HIV 'controllers', individuals who durably contain plasma viremia to below 2,000 copies/ml in the absence of antiretroviral therapy, show relative preservation of CD4+ T-cells in rectal mucosa as compared to non-controllers. In a study of 28 HIV controllers, 14 non-controllers, and 10 patients on HAART, the frequency of HIV Gag-specific CD4+ and CD8+ T-cells in rectal mucosa exhibiting 3 or more functions (IFN-γ,IL-2, TNFα, MIP-1β, and CD107 for CD8+ T-cells) was significantly greater in controllers compared to non-controllers or patients on HAART($p<0.05$). Fine mapping studies revealed that CD8+ T-cells responding to immunodominant HIV epitopes are broadly distributed in blood and mucosa. Notably, in viremic individuals, mucosal tissues are rich in regulatory T-cells (Treg) and T-cells expressing markers of immune senescence. In a cross-sectional study, individuals with high viral load had significantly higher frequencies of Treg in rectal mucosa than HIV controllers ($p<0.05$). These Treg might inappropriately suppress T effector responses. Furthermore, CD4+ and CD8+ T-cells in rectal mucosa expressed higher levels of PD-1 than their counterparts in blood, particularly in patients on HAART ($p=0.002$), revealing a pre-senescent phenotype. Taken together, these findings demonstrate that the gastrointestinal mucosa remains an active site of HIV-host interactions throughout chronic infection. The overall balance between viral clearance, immune activation and disease progression may be strongly influenced by events occurring within mucosal tissues.

S05-04

Treatment as prevention

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Anti-retroviral therapy (ART) lowers viral concentration in all compartments, including genital secretions. It therefore decreases contagiousness, possibly to a degree unequalled by any other preventive measure, including condoms. Modelling suggests that expansion of treatment to most or all of the HIV-infected could, over a time span measured in decades, eliminate HIV infection from a whole population. At the present time however, this remains a theoretical proposition. Will wide-scale universal screening be acceptable? Once identified, will healthy HIV-positives accept to be treated? Having started ART, will they continue? Will compliance be good enough to ensure durable suppression of viremia, or on the contrary bad enough to select for drug resistance mutations? Treating the immunosuppressed already puts strain on available resources; in this context, is it realistic to expand treatment to those who are not ill?

Controlled trials are planned to show the feasibility of treatment as prevention. HPTN 052, in stable serodiscordant couples, has already started; the infected partner is randomized to either start treatment early, or late, and infection rates in the initially seronegative partners are monitored. To investigate infection in the general population, so-called cluster randomized trials are planned. In such trials, the unit of randomisation is not the individual, but a community, for instance a village. In intervention villages, all or most HIV-infected individuals will be treated, whereas inhabitants of control villages will receive the best available usual care and prevention. In populations with very high incidence, i.e. in East or Central Africa, the randomised clusters will have to contain approximately 10'000 individuals for a trial to be completed within 4 years. Rates of infections originating in intervention villages will be compared to rates originating in control villages. If the randomized phase shows an advantage for intervention, the expanded treatment paradigm would have to be continued for several years, in order to validate sustainability.

SYMPOSIUM 06: REFINING IMMUNOGEN DESIGN

S06-01

Structural basis of broad neutralization of HIV-1

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To develop an effective HIV-1 vaccine, it is critical to understand how to elicit broadly neutralizing antibodies against the virus. Thus far, only a handful of human antibodies have been identified that neutralize a wide-range of different HIV-1 isolates. We have analyzed crystal structures of four of the five broadly neutralizing antibodies, as well as many clade-specific antibodies, free and in complex with their viral epitopes, to determine how they neutralize and why some are more effective than others. A common theme derived from these structures is that HIV-1 has found many ways to circumvent the immune system, but the antibody repertoire is sufficiently diverse to find novel solutions for recognition of the most highly conserved, functional epitopes of gp120 and gp41. The antibodies include: b12, that recognizes the deeply recessed CD4 binding site; 2G12, that recognizes a high-mannose cluster of self-carbohydrates on the gp120 surface; and 4E10 and Z13e1 that recognize the membrane proximal region (MPER) on gp41. In addition, we have determined structures for several anti-V3 antibodies (447-52D, 2219, and F425-B4e8) that recognize this region, which is more varable, but is implicated in co-receptor binding; each antibody uses a different strategy for recognition. This structural information is now being used in a retrovaccinology approach to design immunogens that focus the immune response onto these critical epitopes and induce antibodies that have similar, broadly neutralizing properties. Crystal structures and immunological experiments with such designed antigens are in progress.

S06-02

Primary immunization influences the magnitude, quality and breadth of Gag specific T cell responses following an rAd-5 boost

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A fully effective vaccine against HIV will require broadly neutralizing antibodies to prevent infection and CD8+ T cells to control viral spread. Prime-boost immunization regimens with heterologous vaccine formulations are an effective approach for inducing such broad-based adaptive immunity. In this regard, protein-based vaccines have been used to generate antibody responses with HIV envelope proteins while viral vectors have been optimal for inducing CD8+ T cell responses to envelope and structural proteins. Recent evidence from pre-clinical mouse and non-human primate (NHP) studies show that protein vaccines are also capable of inducing potent Th1 cell responses to HIV or SIV Gag antigens. Furthermore, depending on the formulation and the type of adjuvant, Gag proteins can elicit CD8+ T cell responses through cross-presentation. Thus, since protein vaccines are not limited by pre-existing or vaccine induced immunity, they provide a flexible platform for use in heterologous prime-boost HIV vaccine regimens with viral vectors. In a series of studies, NHP were immunized with Gag protein/Poly I: C with or without formulation in a liposomal emulsion and then boosted with rAd-5 expressing Gag. We assessed the T cell responses after primary immunization and their effect on the magnitude, quality and breadth of CD4 and CD8+ Gag specific T cell responses following the rAd-5 Gag boost. Remarkably CD4 and CD8 + T cells induced following primary immunization differentially influence the CD8+ T cell response after the rAd-5 boost. These studies provide insight into the mechanism by which primary immunization enhances CD8 T cell immunity following a viral rAd-5 Gag boos. In addition the studies show how protein based vaccines may be used in HIV vaccine development.

S06-03

New applications for mosaic antigen designs

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We proposed a vaccine antigen design approach called mosaic vaccines aimed at contending with viral diversity, and this strategy has since shown some experimental promise in terms of elicited T-cell responses with greater breadth and depth than natural proteins in animal models. Mosaics vaccines are based on in silico recombinant proteins that provide a nearly optimal solution for potential epitope diversity coverage in a population for a given number of proteins to be included in a cocktail. Mosaics align well with natural proteins, do not carry unnatural breakpoints, and minimize the inclusion of rare variants. So far they have been well expressed and immunogenic. I will first briefly review how mosaics are designed, and provide comparisons that resolve common questions and misconceptions about the design strategy. Then discuss two possible novel strategies for 2nd generation mosaic vaccines. The first strategy is a simple application of mosaics to an old concept already being studied by other groups: restricting vaccines to the inclusion of only highly conserved regions. Even the most conserved regions of HIV have some diversity in T cell epitope length fragments. Mosaics simultaneously provide definitions of conserved regions and select the most common variants for inclusion in vaccines. The second idea is optimizing coverage of potential B cell epitopes defined by spatial proximity, where every amino acid is considered together with its nearest neighbors in 3-dimensional space. When viewed this way, potential B-cell eptiope diversity is vast, and highly subtype dependent. A modified version of this approach could be used to design cocktails of spatially determined regional variants for targeting specific epitopes.

S06-04

HIV-1 mosaic antigens expand cellular immune breadth and depth in Rhesus Monkeys

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Viral diversity represents an enormous challenge for the development of an HIV-1 vaccine. In particular, natural sequence antigens have been shown to elicit only limited breadth of cellular immune responses in both nonhuman primate studies and clinical trials to date. Here we explore the potential utility of HIV-1 mosaic antigens as compared with consensus antigens and natural sequence antigens expressed by Ad26 vectors in rhesus monkeys. We demonstrate that HIV-1 mosaic antigens expand celluar immune breadth, which theoretically may improve coverage of global virus diversity. We also show that HIV-1 mosaic antigens augment cellular immune depth, which we define as the capacity to elicit responses simultaneously to multiple epitope variants, and which theoretically may inhibit viral escape or exact a higher fitness cost for escape. These data suggest that further evaluation of HIV-1 mosaic antigens in clinical trials is warranted.

ORAL ABSTRACT SESSION 01: EARLY EVENTS IN TRANSMISSION AND INFECTION

OA01-01

Early events of human immunodeficiency virus type 1 (HIV-1) ex vivo penetration in the foreskin mimicking HIV-1 sexual transmission

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Background: Although male circumcision was reported to reduce male acquisition of HIV-1 by 60%, the initial mechanisms of HIV-1 transmission at the male genitals remain elusive. A chief problem in studying the early phases of foreskin HIV-1 infection is the lack of proper in-vitro model systems that reflect the in-vivo architecture.

Methods: Two novel models of the adult human foreskin mucosa were established: 1) ex-vivo polarized inner and outer foreskin explants; 2) in-vitro immuno-competent reconstructed foreskins, which include fibrous sheets produced by foreskin fibroblasts that are topped by differentiating foreskin keratinocytes (from either inner or outer foreskin) and Langerhans cells (LCs). These models permit to inoculate comparatively in a polarized manner at the mucosal pole either HIV-1-infected cells (that are present in all secretions vectorizing HIV-1) or cell-free HIV-1.

Results: Efficient HIV-1 transmission occurs following 1hr exposure of the inner, but not outer, foreskin to cells highly infected with HIV-1. Such HIV-1-infected cells form viral synapses with mucosal/apical foreskin keratinocytes, leading to polarized budding of the virus. In inner foreskin, HIV-1 is in turn rapidly internalized by LCs that migrate to the epidermal-dermal interface. There, LCs form conjugates with T-cells thereby transferring HIV-1. Cell-free HIV-1 is inefficient at foreskin penetration. To mimic the in-vivo mixture of genital fluids during woman-to-man HIV-1 sexual transmission, the effect of a mixture of seminal plasma from HIV-negative men mixed with cervico-vaginal secretions from HIV-positive women was investigated. Such mixture significantly reduced HIV-1 translocation in inner foreskin reconstructions.

Conclusion: LCs have an active role in sampling HIV-1 at the foreskin followed by transfer of virus to T-cells. This process is highly efficient in the inner foreskin and when HIV-1 originates from infected cells. This process is blocked by yet ill-defined components activated when mixing genital fluids.

OA01-02

Defining the mechanisms of HIV entry and interactions with the female genital tract

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Background: To date, little is known about the mechanisms of the sexual transmission of HIV and how the virus interacts with the female genital epithelium to gain access to underlying target cells. We illustrate that HIV is able to penetrate both intact columnar and squamous epithelium in explants and the living rhesus macaque. Through a series of approaches we were able to determine the mechanism of HIV entry into these tissues.

Methods: Human cervical explants and macaque genital tracts were exposed to PA-GFP HIV. Macaques were inoculated intravaginally and genital tissues were removed 4, 12, and 24 hours post-inoculation and dissected into relevant tissue specimens. Two separate macaques were inoculated ex vivo. Samples were snap frozen, sectioned and stained accordingly. Fluorescent BSA was added to specimens to investigate tissue permeability. Comparison of the image z-stacks before and after photoactivation reveals viral signal, accounting for background.

Results: Within 4 hours, photoactivatable virions were observed between superficial differentiated squamous epithelial cells, with penetrating virus up to depths of 50µm in macaques. Some virions remained for at least 24 hours, illustrating viral persistence within tissue samples. Fewer virions associated with the macaque endocervix compared to human explants. In contrast, macaque explants were very similar to human explants, indicating that the intact tubular cervix filled with mucus is an efficient barrier to HIV. Penetration of squamous epithelium was influenced by presence of cellular junctions and co-localized with the penetration of fluorescent BSA.

Conclusion: Together these results indicate that HIV can penetrate to depths in both squamous and columnar epithelium where they can interact with HIV target cells. Fluorescent BSA experiments reveal that viral depth is directly proportional to epithelial permeability and in areas where cellular junctions are absent, suggesting a diffusion-based mechanism for viral entry.

OA01-03

Clusterin, a natural ligand of DC-SIGN present in human semen inhibits HIV capture and transmission by dendritic cells

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Background: Although sexual transmission represents the main mode of HIV dissemination worldwide, little is known about the influence of semen on viral spread. We have shown that seminal plasma (SP) inhibits both, the attachment of HIV to dendritic cells (DC) and the ability of DC to transmit the virus to T cells. Here, we characterized the inhibitor present in SP and their functional properties.

Methods: Semen samples were collected from healthy donors. SP proteins were analyzed by 2D electrophoresis. DC-SIGN binding proteins were identified by western blot using DC-SIGNfc chimera. Selected spots were cut and clusterin was identified by MS analysis as the inhibitor present in SP. SP clusterin was then purified by affinity chromatography. In all the experiments, HIV was quantified by measurement of p24 antigen by ELISA.

Results: We found that SP clusterin markedly inhibits the attachment of HIV-1 BAL (5 ng p24 antigen) to DC in a dose-dependent mode (1-40 ug/ml), being the percentage of inhibition of 54 ± 11(n=6, p< 0.05) when used at a concentration of 15 ug/ml. Similar levels of inhibition were observed using blocking antibodies directed to DC-SIGN. In transmission experiments DC were cultured with HIV-1 BaL (5ng p24Ag) in the presence of clusterin (20 ug/ml), washed and cultured with activated peripheral blood mononuclear cells (PBMCs). Clusterin markedly prevented virus transmission to DC: % inhibition = 59 ± 17, n=5, p< 0.05). Experiments performed with THP-1-DC-SIGN+ cells showed that clusterin, at a concentration of 15 ug/ml, almost completely inhibited both, the attachment of HIV (% inhibition < 87%, n=5) and the ability of THP-1-DC-SIGN+ cells to transmit the virus to activated PBMCs (% inhibition < 82%, n=4).

Conclusion: Our results identified clusterin, as a novel ligand of DC-SIGN present in human semen able to inhibit the capture and transmission of HIV by DC

OA01-04

Striking elevations in systemic and mucosal cytokine and chemokine levels in acute HIV-1 infection

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Background: Innate immune responses can be activated rapidly in response to infection and may contribute to control of early viral replication or conversely, mediate immunopathogenic effects. To gain insight into the nature and kinetics of the earliest cytokine responses activated in acute HIV-1 infection (AHI), we analysed changes in systemic and mucosal cytokine/chemokine levels during primary infection.

Methods: Cytokine/chemokine levels were measured by Luminex and ELISA in sequential blood plasma samples collected during the eclipse and exponential viral expansion phases from US plasma donors acquiring HIV-1 infection, and in blood plasma, cervicovaginal lavage (CVL) and seminal plasma timecourses from CHAVI 001 AHI subjects (typically spanning the time from peak viraemia to early infection), plus HIV-seronegative controls.

Results: The increase in viraemia in AHI was associated with rapid activation of a striking systemic cytokine cascade, including rapid and transient elevations in IFNalpha and IL-15, rapid and more sustained increases in TNFalpha, IP-10 and MCP-1, more slowly-initiated elevations in additional pro-inflammatory factors including IL-6, IL-8, IL-18 and IFNgamma and a late-peaking increase in IL-10. Most analytes returned to baseline as viraemia declined, but low-level elevations in some factors were sustained into early infection. Multiple cytokines/chemokines were transiently elevated in CVL during AHI. Some, e.g. TNFalpha, IL-1beta and IL-8, were typically already highly elevated in CVL at the earliest timepoint studied (even prior to peak viraemia), suggestive of a local pro-inflammatory response preceding systemic immune activation. Others, e.g. IFNgamma, MIP-1beta and IL-10 were only elevated in CVL around peak viraemia. Modest elevations in seminal plasma cytokine/chemokine levels occurred in AHI in parallel with systemic analyte increases.

Conclusion: Although some individual cytokines may have beneficial effects, the intense cytokine storm in AHI may have immunopathological consequences, promoting immune activation, viral replication and CD4+ T cell loss, and could be a target for vaccine-induced down-modulation.

OA01-05

TLR-mediated pDC responses to HIV-1 ligands

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Background: Comparisons of HIV-1 disease between women and men have demonstrated that given the same viral load, measured early in HIV-1 infection, the time to AIDS progression is faster in women than in men. There is increasing consensus that faster disease progression is associated with elevated immune activation and this is consistent with our recent findings that women have higher immune activation then men given the same HIV-1 viral load. Plasmacytoid dendritic cells (pDCs) appear to play a central role in this HIV-1-induced activation of the immune system, as they can sense HIV-1 ssRNA via Toll-like receptor (TLR) 7 and produce proinflammatory cytokines.

Methods: PBMC from HIV-1-negative subjects enrolled at Massachusetts General Hospital were stimulated with HIV-1 derived TLR7/8 ligands and cytokine secretion by pDCs, mDCs and monocytes as well as T-cell activation were measured by flow cytometry. Polymorphisms of genes encoding for TLR7 and its downstream molecules were also examined to determine how these affect the resulting immune response.

Results: pDCs derived from women produce significantly more IFN-alpha in response to HIV-1 derived TLR7/8 ligand stimulation than pDCs derived from men ($p<0.05$). Furthermore, HIV-1 derived TLR7/8 ligands resulted in a significant up-regulation of CD38+ CD8+ T-cells in vitro and this TLR7/8-induced T-cell activation was also higher in women compared to men ($p<0.05$). Polymorphisms in genes encoding for TLR7 and its downstream signaling molecules modulated the IFN-alpha production by pDCs following stimulation with HIV-1 derived TLR7/8 ligands.

Conclusion: These data show that sex differences in TLR-mediated activation of pDCs might account for the described higher immune activation in HIV-1-infected women compared to men at a given viral load and subsequently faster HIV-1 disease progression in women. The data further suggest that polymorphisms within TLR genes can modulate TLR-induced cytokine production which may have implications for adjuvant use in HIV-1 vaccine design.

OA01-06 LB

HIV-1 Plasma RNA and Risk of HIV-1 transmission

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Background: Non-sterilizing HIV-1 vaccines may provide public health benefits if they significantly reduce plasma HIV-1 RNA, thus potentially reducing infectiousness. Quantification of reduction in plasma HIV-1 RNA needed to decrease HIV-1 transmission is useful for design of efficacy trials of candidate HIV-1 vaccines. We modeled the relationship between plasma HIV-1 RNA and HIV-1 transmission using data from a prospective study of African heterosexual HIV-1 serodiscordant couples.

Methods: 3408 HIV-1-infected participants with CD4 counts ≥250 cells/mm3 enrolled in the Partners in Prevention HSV/HIV Transmission Study and their partners were followed for ≤24 months. HIV-1 transmission events were assessed for viral genetic linkage within the enrolled partnership by determining HIV-1 env and gag sequences from partners. The relationship between plasma HIV-1 RNA over time and risk of genetically linked HIV-1 transmission was evaluated with a Cox model with a natural cubic spline.

Results: 84 post-enrollment linked HIV-1 transmissions were observed. HIV-1 incidence increased rapidly and non-linearly with higher plasma HIV-1: from 0.53 transmissions per 100 person-years for plasma HIV-1 RNA <10,000 copies/mL to 6.2 for HIV-1 RNA >1,000,000 copies/mL ($p<0.0001$). Baseline HIV-1 RNA in men was, on average, 0.4 log10 higher than in women; no significant difference in risk of transmission for a given HIV-1 level was observed between men and women ($p = 0.17$). Given the distribution of plasma HIV-1 RNA in this population of stable cohabiting couples, our modeling predicts that a 0.74 log10 reduction in average plasma HIV-1 RNA in the population would be required for a 50% reduction in HIV-1 transmission risk.

Conclusion: This analysis provides a detailed description of the relationship between plasma HIV-1 RNA and risk of heterosexual HIV-1 transmission. These findings suggest targets for reduction in HIV-1 RNA for use in evaluating non-sterilizing HIV-1 vaccine candidates in HIV-1 infected persons to reduce risk of heterosexual HIV-1 transmission.

ORAL ABSTRACT SESSION 02: VACCINE DESIGN

OA02-01

Construction and characterization of replication competent attenuated NYVAC-based vectors as potential HIV vaccines

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Background: Poxvirus viruses have been utilized for many years as vaccine vectors. Recent years have seen an increase in efforts to identify safer pox vectors to express heterologous antigens, in this case HIV antigens.

Safer pox vectors have included MVA, ALVAC and NYVAC, which are all replication deficient in human cells. However, the loss of replication competence has also reduced antigen expression, and therefore, immunologic response to the vaccination. We have constructed vectors in a NYVAC background that are replication competent but attenuated for virulence.

Methods: To restore replication competence in NYVAC that expresses clade C HIV gag, pol, nef and env (NYVAC-C), we inserted genes deleted in the parental vector: 1) NYVAC-C-KC includes the human host range genes K1L and C7L; 2) NYVAC-C+12 includes the entire cassette of genes from K1L to C7L that is deleted from NYVAC. To decrease virulence, we deleted the E3L gene, which is required for interferon-resistance and virulence, and replaced it with a gene from Ambystoma tigrinum virus (ATV, the new virus is NYVAC-C+12-ATV), which restores a single round of replication.

Results: In vitro characterization of the constructs demonstrates restoration of replication in primary and human cell lines. NYVAC-C+12-ATV leads to induction of pro-inflammatory signal transduction pathways. Pathogenicity studies in newborn mice demonstrate attenuation of 3-5 logs when compared to wt vaccinia virus or to NYCBH.

Conclusion: NYVAC constructs provide increased safety in the most sensitive of animal models. Because antigen expression is increased in these replication-competent constructs, they may be more immunogenic than replication-defective poxvirus vectors. Increased pro-inflammatory signal transduction in cells infected with NYVAC-C+12-ATV may also lead to an increase in immunogenicity of this highly attenuated vector. Thus, these replication competent, attenuated viral vectors may have improved immunogenicity over replication defective poxvirus vectors, while maintaining an improved safety profile.

OA02-02

Replicating measles-SHIV vaccine induces long term preservation of central memory CD4 cells in the gut of vaccinated macaques challenged with SHIV

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Background: Live attenuated vaccines are mostly appropriate for global mass immunization, controlling very efficiently global pandemics like polio or measles. Although a live attenuated HIV vaccine is not currently considered for safety reasons, a strategy based on the expression of HIV-1 particles through a live replicating viral vector might mimic the advantageous characteristics of live attenuated SIV.

Methods: With this aim, we generated a recombinant measles vaccine expressing simultaneously HIV-1 Gag and Env and evaluated its immunogenicity in mice and macaques. Measles vaccine is a live attenuated negative-stranded RNA virus proven to be one of the safest and most effective human vaccines. The efficacy of recombinant measles-HIV virus was evaluated in macaques after intrarectal SHIV challenge.

Results: In mice, the recombinant vaccine stimulated MV and HIV antibody with neutralizing activity, as well as cellular immunity composed of CD4 and CD8 T cells. In the macaque/SHIV model, the vaccine induced a 2-4 log reduction in acute viral load. More than 3 years after challenge, the macaques were sacrificed and T-cell populations were determined in different organs. We found that CD4 central memory T cells of the gut were preserved in vaccinated animals as compared to controls vaccinated with empty measles. We also demonstrated that recombinant measles-HIV infects human professional APC, such as dendritic and B cells, and induces efficient presentation of HIV-1 epitopes to autologous T-cells and subsequent activation of cytokine secretion by human HIV-1 Gag-specific T-cell clones in vitro.

Conclusion: The immunogenicity of measles-HIV virus results from its capacity to replicate in vivo after administration and to infect productively dendritic cells. This strategy, which is currently in the process of phase I human trial evaluation, provides a vaccine that might protect children and adolescents simultaneously from measles and HIV and be affordable to populations through the Expanded Program on Immunization.

OA02-03

Design and development of DNA vaccines for the co-expression of micro-RNA and HIV-1 Env

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Background: Small non-coding micro-RNAs (miRNA) are important post-transcriptional regulators of mammalian gene expression. More recently, miRNAs have been described that regulate key elements of the adaptive immune response, such as T-cell development and activation (miR-181) and antigen presentation and development in B-cells (miR-155. miR-150), and various aspects of innate immunity (miR-146). We examined whether DNA vaccine vectors co-expressing miRNA with Env antigen could influence the magnitude or quality of the immune responses to Env in mice.

Methods: Human miR-155 and flanking regions from the non-protein encoding gene microRNA host gene 2 (MIRHG2), were introduced into an artifical intron within an envelope expression vector. Expression of miR-155 and Env was examined by Northern and Western Blot respectively. Using miR-155 sequences as a scaffold, we incorporated novel miRNAs encoded to silence expression of host antiviral proteins, or alternatively, to mimic other endogenous, immunomodulatory miRNAs.

Results: The human miR-155 was efficiently expressed and correctly processed from an upstream intron within an Env-expressing DNA vaccine plasmid in human cell lines. Locating the miRNA expression sequences within the intron did not reduce Env expression. Substitution of the native miR-155 guide sequence enabled the targeting of exogenous marker genes, EGFP and ds-Red. Targeting of cellular genes thought to influence Env expression in vivo, such as PKR and SFRS1, significantly down-modulated expression of targeted genes but failed to increase Env expression in vitro. In an alternative strategy, vaccine vectors delivering immunomodulatory miRNAs such as miR-155 were used to vaccinate BALB/c mice and the generation of Env-specific T-cells and effective antibody responses was measured.

Conclusion: This study provides evidence that native and engineered miRNAs can be successfully co-expressed with HIV-1 Env antigens. The further characterisation of immunomodulatory miRNAs may enable the development of vaccine vectors better able to shape the immune responses to HIV-1 vaccines towards protective correlates of immunity.

OA02-04

HIV-1 gp41 envelope MPER mutation altered epitope conformation in lipid and increased sensitivity to 2F5 and 4E10 neutralizing antibodies

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Background: The conserved membrane-proximal external region (MPER) of HIV-1 envelope is a target for the rare broadly neutralizing 2F5, Z13 and 4E10 monoclonal antibodies (mAbs). However, MPER antibodies are rarely found in HIV-1 infected subjects nor arise following envelope immunization. A potential strategy to elicit such antibodies more frequently is to design an envelope protein with increased exposure of the 2F5 and 4E10 mAb epitopes.

Methods: Using pseudotyped virus neutralization assays and surface plasmon resonance (SPR) assays (with both peptides and peptide-liposome conjugates), we characterize an HIV-1 envelope mutant pseudovirus that is ~300 fold more sensitive to 2F5 and 4E10 neutralization than wildtype.

Results: The mutation responsible is a single leucine to serine substitution at position 669 in the gp41 Env MPER. A non-neutralizing MPER mAb, 13H11, which binds peptides with gp41 669 leucine but not 669 serine, did not interact with L669 peptide-lipid conjugates, consistent with previously observed masking of L669 when peptide is lipid-associated. In contrast, the 2F5 mAb, which recognizes an epitope C-terminal to the 13H11 mAb, bound more stably, with a slower off-rate, to MPER L669S mutant peptide-lipid conjugates than to wild-type peptide-lipid conjugates.

Conclusion: These data suggest that the L669S mutation in the gp41 MPER creates a virus that is more susceptible to neutralizing MPER antibodies because of enhanced exposure of the neutralizing epitope. Inactivated pseudotyped virus or subunit immunogens with the L669S gp41 MPER mutation may be candidates for induction of MPER neutralizing antibodies.

OA02-05

Insertion of the HIV-1 gp41 epitopes 2F5 and 4E10 into the membrane-proximal region of the vesicular stomatitis virus glycoprotein

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Background: The membrane-proximal external region (MPER) of HIV-1 gp41, which is thought to be involved in the viral fusion process, is an important target for an HIV vaccine. Two broadly neutralizing monoclonal antibodies, 2F5 and 4E10, bind to adjacent linear epitopes in the gp41 MPER. However, attempts to design immunogens that elicit a neutralizing antibody response against this region have had limited success.

Methods: We inserted the gp41-derived epitope sequences into the envelope glycoprotein G of Vesicular Stomatitis Virus (VSV). VSV G forms homotrimeric spikes on the viral surface, mediates binding of the virus to cells and promotes fusion of the viral and cellular membranes. The membrane-proximal "stem" region of VSV G, which plays a role in VSV fusion, shares sequence similarities with the HIV-1 gp41 MPER. We created chimeric polypeptides by substituting residues in the G stem with the 2F5 and 4E10 epitopes, generated pseudotyped lentiviruses or rescued VSV with the mutant envelope proteins incorporated, and tested the function and antibody reactivity of the recombinant viruses.

Results: VSV-G-2F5 and VSV-G-4E10 formed trimers and were transported to the cell surface, where they were recognized by the 2F5 and 4E10 monoclonal antibodies, respectively. Pseudotyped lentiviruses or recombinant VSV containing G-2F5 or G-4E10 on the viral surface were infectious, and the mutant viruses were neutralized by the 2F5 or 4E10 monoclonal antibodies. We are currently determining if the recombinant viruses are capable of eliciting a neutralizing antibody response in a small animal model.

Conclusion: These results suggest that the 2F5 and 4E10 epitopes inserted into the VSV G stem region, which is likely to be the functional equivalent of the HIV-1 gp41 MPER, adopt a conformation similar to the one in their native context in gp41. Our approach represents a novel strategy to develop a vaccine that induces a humoral immune response against HIV.

OA02-06 LB

Recombinant modified Vaccinia Virus Ankara expressing HIV-1 genes activates NK subset capable of controlling HIV infection in vitro

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Background: Primate innate immune mechanisms are critical for the normal development of adaptive immune responses to antigen. Part of the modern effort to design and distribute vaccines against pathogens must involve detailed descriptions of innate responses resulting from vaccine challenges. Particularly, the interaction between Natural Killer (NK) and Dendritic cells is expected to greatly impact the establishment of both innate and adaptive immune responses to vaccine. In this study, we describe responses of Natural Killer cells to recombinant Modified Vaccinia Virus Ankara expressing HIV-1 genes (rMVA) compared to wild type MVA (MVAwt).

Methods: We employed an autologous in vitro NK/DC co-culture system to explore the interaction between MVA-infected DC and Natural Killer cells.

Results: Using a fluorescent dye system, we show that MVA infected DC are phagocytosed by uninfected DC after 48h in co-culture. We also demonstrate that NK cells stimulated in co-culture with MVA-infected DC proliferate when compared to the uninfected DC stimulation; though no difference in proliferation kinetics could be identified between recombinant MVA (rMVA) and wild type MVA (MVAwt) stimulated NK. Both MVA strains were capable of inducing NK repertoire differentiation but comparative analysis demonstrated that NK stimulated with rMVA specifically expressed higher levels of CD158e and NKG2D than NK in the MVAwt condition. Finally, using HIV-GFP or HIV-Bal5 strains, we show that NK cells stimulated by rMVA are better at controlling HIV infection in vitro than NK stimulated by MVAwt.

Conclusion: These data demonstrate that innate responses to this rMVA vaccine against HIV can be detected in vitro and that this response results in the establishment of "activated/memory" Natural Killer cells capable of controlling HIV replication and infection. Future experiments will identify the specific molecular mechanisms responsible for the DC-mediated activation of NK, as well as the NK-dependent control of HIV infection.

ORAL ABSTRACT SESSION 03: MODULATION OF THE IMMUNE RESPONSE TO INFECTION

OA03-01

HIV-1 infection is characterized by early loss of CD161+ Th17 cells and gradual decline in regulatory T cells

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Background: CD4 cell depletion is central to HIV pathogenesis. However, CD4 cells present diverse lineages and the relative impact of HIV on Th17 and regulatory T cell (Treg) subsets remains unclear. CD161+ CD4 cells are a recently identified, gut-homing population with Th17 precursor potential. The balance between pro-inflammatory Th17 cells and immunoregulatory Tregs may be critical in HIV pathogenesis. This study addressed changes in CD161+, Th17 and Treg subsets during untreated HIV infection.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from 77 untreated HIV-infected and 36 HIV-uninfected subjects and stained with fluorochrome-conjugated monoclonal antibodies to characterize CD161+ CD4 cells; Th17 cells (expression of CCR6 and elaboration of IL-17A after PMA/ionomycin stimulation); Tregs (CD4+CD25hiFoxP3+ cells); and CD8 activation (CD38+/HLA-DR+ cells). In vitro infectability of CD161+ and Th17 cells was assessed by incubating activated healthy donor CD4 cells with HIV Bal for 5 days and co-staining for CD161/IL-17A and intracellular p24.

Results: Peripheral blood Th17 cells were depleted 10-fold in HIV-infected, compared to HIV-uninfected, subjects ($P<0.0001$) across a range of disease stages, accompanied by a significant reduction of CD161+ T cells ($P=0.024$). Both Th17 cells and CD161+ CD4 cells were highly targeted by HIV in vitro. The preferential and early loss of Th17 cells contrasted with a gradual decline in absolute Treg numbers during HIV disease progression in untreated subjects followed longitudinally ($R=0.71$, $P=0.003$). Loss of Tregs was associated with increased immune activation ($R=-0.33$, $P=0.03$).

Conclusion: HIV-infected subjects showed preferential loss of Th17 and CD161+ CD4 subsets, which were both highly targeted by HIV in vitro. Loss of CD161+ cells, which are Th17 cell precursors, may limit Th17 reconstitution. A gradual decline in Tregs during disease progression was associated with increased immune activation. Loss of both Th17 and Treg subsets may enhance disease progression through impaired mucosal defences, and increase in immune activation.

OA03-02

Regulatory T cells inhibit CD8 T cell proliferation in HIV-1 infection through CD39/Adenosine pathway

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Background: Generation of CD8 T cell responses following viral infection or vaccination is indispensable for infection control. Regulatory CD4+ CD25+ T cells (Treg) are a key factor for the inefficiency of CD8 responses in viral persistence. The mechansms of this suppression are not elucidated. Treg constitutively express the ectonucleotidase CD39, that generates immunosuppressive adenosine in the sites of immune activation.

Methods: HIV+ patients either treated or not with antivirals (CD4 >350 cells/mkl, n=47) were studied in parallel with HIV- controls (n=25). CD8 and Treg cells were purified by magnetic beads separation. Anti-CD3 stimulated CD8 T cell proliferation was assessed on CFSE-stained CD8 T cells cultured in the presence of either: i) Treg preincubated with anti-CD39 blocking mAb or isotype control; ii) adenosine analogue CGS 21680. Expression of the adenosine A2A Receptor (A2AR) was quantified by RT-PCR.

Results: Treg - associated CD39 expression was significantly increased in HIV+ patients as compared to HIV- controls. (mean CD39+ Treg % 2.1 vs. 0.98 and mean CD39 MFI 1011 vs. 606, $P<0.01$ for both). In the presence of Treg, proliferation of HIV+ CD8 T cells was significantly inhibited (mean inhibition 56 % vs 22.5 % for HIV- controls; $P<0.01$), while the inhibition decreased to an average of 12.3% ($P<0.01$) in the presence of anti-CD39 blocking mAb. HIV+ CD8 T cells were characterized by an elevated expression of A2AR. The inhibitory effect of Treg on HIV+ CD8 T cell proliferation was reproduced by adenosine agonist. Finally, the percentage of Treg CD39+ was correlated with plasma HIV RNA values in HIV + treatment-naïve patients and inversely with CD4 AC.

Conclusion: Treg CD39-adenosinergic axis is involved in the progression of chronic HIV-1 infection and Treg-mediated inhibition of CD8 T cell proliferation. Modulation of Treg CD39+ function might be an attractive approach for enhancing CD8 T lymphocyte responses.

OA03-03

Increased regulatory T cell frequency and HIV-1 specific suppression after therapeutic vaccination of HIV-infected patients on antiretroviral therapy

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Background: We tested the hypothesis that therapeutic vaccination against HIV-1 can lead to an increase in the frequency and suppressive function of regulatory, CD4+ T cells (Treg).

Methods: HIV-1(+) subjects on ART (n=17) were enrolled in a phase I therapeutic vaccine trial where they received 2 doses of autologous dendritic cells loaded with HIV-1 peptides. Peripheral blood mononuclear cells (PBMC) obtained from the subjects pre- and post-vaccine, and from normal controls (NC) were stained with antibodies specific for Treg (CD4+CD25hiFOXP3+), CD45RO, GITR, and CTLA4 and assayed by flow cytometry. PBMC pre and post-vaccine from 7 subjects were also evaluated for polyfunctionality using a flow cytometry-based, CD8+ T cell intracellular cytokine staining assay for 5 immune mediators after stimulation with Gag peptide, staphylococcal enterotoxin B (SEB) and medium alone. Treg were depleted in one set, and total vaccine response (post-vaccine – pre-vaccine) was compared in the Treg(+) and Treg-depleted (Treg-) sets.

Results: After vaccination, 12/17 subjects had increased Treg frequency from 0.74% to 1.2% (p=0.06); the median increase was 30%. Of the 11 patients whose CD8+ T cells did not respond to the vaccine by an increase in production of interferon γ (ELISPOT assay), 7 (64%) had increased frequencies of Treg. Although there was no significant change in CD8+ T cell polyfunctionality after vaccination, depletion of Treg resulted in increased polyfunctionality post-vaccine (p=0.029), with the percentage of CD8+ T cells producing more than 1 immune mediator increasing to more than twice the pre-vaccine levels. There was no difference in polyfunctionality in the Treg(+) and Treg(-) sets when stimulated with SEB, implying specificity of suppression to HIV-1 antigens.

Conclusion: Therapeutic immunization against HIV-1 causes a modest increase in Treg frequency and a significant increase in HIV-1-specific, Treg suppressive function. The role of Treg should be considered in immunotherapeutic trials of HIV-1 infection.

OA03-04

Impairment of HIV-1-specific CD8+ T cell function by soluble epithelial adhesion molecules

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Background: HIV-1-specific CD8+ T cell responses play an important role in the control over viral replication. Under persistent antigenic stimulation virus-specific CD8+ T cell become increasingly dysfunctional and upregulate several inhibitory molecules. The interaction and co-regulation of these molecules is largely unknown. The gastrointestinal associated lymphoid tissue (GALT) is one of the major sites of viral replication. Despite a substantial infiltration and expansion of HIV-1-specific CD8+ T cells in the GALT, viral replication appears to be more active in the GALT than in other body compartments. Here we show a distinct mechanism of inhibition of HIV-1-specific CD8+ T cells by soluble epithelial adhesion molecules with increasing viral loads in chronic HIV-1 infection.

Methods: HIV-infected individuals with chronic-progressive or chronic-controlled HIV-1 infection were analyzed. The distribution of E-cadherin in intestinal tissue was determined by immunohistochemistry. Plasma levels of soluble E-cadherin were determined using ELISA. Cytokine secretion by antigen-specific CD8+ T cells in the presence or absence of recombinant soluble E-cadherin was assessed by intracellular cytokine staining and Luminex.

Results: HIV-1 infected individuals had abnormal distribution of E-cadherin in the intestinal mucosa relative to uninfected individuals. These subjects also had significantly increased soluble E-cadherin levels in the plasma relative to HIV-negative subjects ($p<0.05$). The viral load in chronic HIV-1 infection correlated strongly with E-Cadherin levels in the plasma ($R=0.7$;$p=0.004$). HIV-1-specific CD8+ T cells in subjects with chronic-progressive HIV-1 infection showed significant elevated levels of KLRG1 expression ($p<0.05$). In the presence of soluble E-cadherin, a natural ligand for KLRG1, KLRG1hi HIV-1-specific CD8+ T cells showed reduced amounts of cytokine production upon antigenic stimulation, while KLRG1lo expressing cells were not affected.

Conclusion: Our data suggest a novel mechanism by which the disruption of the gastrointestinal epithelium leads to release of soluble E-cadherin, which specifically inhibits KLRG1hi expressing HIV-1-specific CD8+ T cells.

OA03-05

HIV escape from natural killer cytotoxicity: Nef inhibits NKp44L expression on HIV-infected CD4+ T cells

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Background: HIV infection leads to a progressive depletion of uninfected CD4 cells. We showed that NKp44L, a cellular ligand for an activating NK receptor is over-expressed during HIV infection and is correlated with both CD4 depletion and increase in viral load. NKp44L+CD4+ cells are highly sensitive to the NK lysis activity. However, HIV-infected cells are resistant to NK killing, suggesting that HIV-1 develop escape mechanisms to counteract NK cytotoxicity.

Methods: The role of Nef was determined using recombinant vaccines expressing different HIV-1 proteins and then a panel of isogenic viruses that expresses an array of established Nef mutants.

Functional NK activities were determined by 51Cr release assay, and degranulation capacities.

Results: During HIV infection, we show that NKp44L expression is restricted to uninfected cells. We demonstrate that Nef protein inhibits NKp44L expression on HIV-infected cells and their subsequent sensitivity to NK lysis. These data were confirmed with HIV-1 strains deleted for Nef, which induced an increased expression of NKp44L associated with high NK lysis sensitivity. Furthermore, our results provide evidence that Nef is required to block the translocation of NKp44L to the CD4 cell surface level.

Conclusion: We show here that Nef protein induced the dysregulation of NKp44L surface expression on CD4 cells. This novel escape mechanism could then play a key role in maintaining the HIV reservoir to ovoid recognition by NK cells. Thus, HIV induces in parallel the destruction of non-infectious cells, which over-expressed NKp44L, and the capacity for a long-term viral persistence, by the down modulation of NKp44L cell-surface translocation through Nef expression. Therapeutic intervention against Nef could be a novel strategy to destroy HIV-1 reservoir by a targeted attack mediated by NK cells.

OA03-06 LB

Unique stimulatory properties of myeloid dendritic cells in individuals with "elite" HIV-1 control

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Background: "Elite" controllers are HIV-1 infected patients who spontaneously maintain undetectable levels of HIV-1 viremia. The Identification of unique immunological characteristics in this specific patient population is a premier opportunity for determining components of immune protection against HIV-1.

Methods: Functional properties of freshly isolated myeloid dendritic cells were analyzed in mixed lymphocyte reactions. Surface expression of costimulatory molecules and myelomonocytic receptors was analyzed by multiparameter flow cytometry. Targeted si-RNA mediated gene knock-out was used to determine the impact of specific functional effects mediated by regulatory myelomonocytic receptors. Assays were performed in elite controllers (n=11) and HIV-1 progressors (n=11) or healthy HIV-1 uninfected persons (n=12) as reference cohorts.

Results: In comparison to both HIV-1 progressors or healthy persons, dendritic cells from "elite" controllers had significantly higher ability to expand allogeneic CD4+ and CD8+ T cells. Costimulatory molecules (CD80, CD86, CD40, CD83) were expressed at higher levels in HIV-1 progressors, while no significant difference was observed between controllers and HIV-1 negative persons. To determine underlying reasons for the increased functional activity of mDCs in controllers, we assessed the expression of regulatory dendritic cells receptors that can critically modulate dendritic cell function. The receptor ILT2 and ILT5 were significantly higher expressed on mDC in controllers, as opposed to either progressors of HIV-1 negative persons. Si-RNA mediated knock out of ILT2 or ILT5 led to significant reduction of the functional activity of dendritic cells, indicating that it has a critical stimulatory effect on these cells.

Conclusion: HIV-1 "elite" controllers have myeloid dendritic cells with a uniquely increased allostimulatory activity. This extraordinary functional profile is mediated by signaling through ILT2 and ILT5, which are uniquely upregulated on these cells. Manipulation of dendritic cell function might thus represent a promising strategy for immunological approaches to treatment and prevention of HIV-1 infection.

ORAL ABSTRACT SESSION 04: UPDATE ON CLINICAL TRIALS

OA04-01

Safety and immunogenicity of LIPO-5, a HIV-1 lipopeptide vaccine: results of ANRS VAC18, a phase 2, randomized, double-blind, placebo-controlled trial

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Background: ANRS HIV-LIPO-5 vaccine includes 5 long peptides, Gag17-35, 253-284, Pol325-355, Nef66-97 and 116-145, containing multiple CD8+ and CD4+ T-cell epitopes, coupled to a palmytoil tail. Phase 1 studies have shown that vaccine dosage at 500µg/lipopeptide elicits cellular immune responses. Whether HIV-LIPO5 immunogenicity varies with the dosage is unknown.

Methods: One hundred and thirty two 21- to 55-year-old HIV negative volunteers, enrolled in 6 HIV-vaccine clinical sites, were randomized to receive either the HIV-LIPO-5 vaccine at 50µg/lipopeptide (N=32;LIPO-5 50), 150µg (N=32;LIPO-5 150), 500µg (N=33;LIPO-5 500) or placebo (N=34). Vaccinations were given IM at weeks 0, 4, 12 and 24. HIV-1 specific CD8+ (IFN-gamma ELISpot on PBMC cultured 12-days) and CD4+ responses (PBMC lymphoproliferation) were assessed at baseline, two weeks after each injection, and at week 48.

Results: No adverse events attributable to vaccine were noted throughout the study. Local reactions appeared dose-dependent; no differences in systemic reactions were observed between groups. Sustained (at least on 2 separate occasions) CD8+ response rates to at least one HIV-1 pool were: 5/32 (16%) for placebo, 22/32 (69%) for LIPO-5 50, 21/33 (64%) for LIPO-5 150 and 21/34 (62%) for LIPO-5 500 groups (P≤.0001 for all comparisons to placebo). Cumulative CD4+ response rates were: placebo: 2/32 (6%), LIPO-5 50: 15/32 (47%), LIPO-5 150: 18/33 (55%) and LIPO-5 500: 15/34 (44%) (P<.0001 for all comparisons to placebo). The majority of CD4+ (75%) and CD8+ (60%) responses were directed towards Gag253-284. CD8+ responses against Nef, Pol were noted in 36% and 33% of vaccinees, respectively. At week 48, CD8+ responses persisted in 47/91 (52%) HIV-LIPO-5 recipients.

Conclusion: ANRS VAC18 shows that low and high doses of HIV-LIPO-5 vaccine elicit sustained CD8+ and CD4+ T-cell responses. According to the good tolerance of the vaccine, the lowest dose of 50 µg appears as the most appropriate to be used in further trials.

OA04-02

Strong HIV-specific CD4 and CD8 T-lymphocyte proliferation in HIV-1 DNA prime/ modified vaccinia virus Ankara (MVA) heterologous boost vaccinees

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Background: We determined HIV-1-vaccine-induced lymphoproliferative responses in vaccinees immunized with a multigene, multiclade HIV-1 plasmid DNA vaccine boosted with heterologous HIV-1 recombinant MVA in a phase I HIV safety and immunogenicity study (HIVIS01/02).

Methods: Healthy volunteers were immunized intradermally or intramuscularly (with or without adjuvant GM-CSF protein) with DNA expressing HIV-1 gag, env, rev and rt at months 0, 1 and 3 using a Biojector and were boosted at nine months with an MVA expressing heterologous inserts of HIV-1 gag, env and pol genes. Lymphoproliferative responses to AT-2 inactivated HIV-1 antigen were tested by a 3H-thymidine uptake assay and a flow-cytometric assay of specific Cell-mediated immune-response in Activated whole blood (FASCIA-WB) two weeks after HIV-1 MVA boost (n=38). A FASCIA using peripheral blood mononuclear cells (FASCIA-PBMC) was also employed during the later part of the study (n=14).

Results: Thirty-five of 38 (92%) vaccinees were reactive by the 3H-thymidine-uptake assay (SI >8). Thirty-two of 38 (84%) vaccinees were reactive by the CD3+CD4+ T-cell FASCIA-WB (%Stimulation >1.2), seven (18%) also exhibited CD8+ (CD3+ CD4-) T-cell responses. Of the 14 vaccinees analyzed using all three assays, ten (71%) and eleven (79%) demonstrated CD4+ T-cell responses in FASCIA-WB and FASCIA-PBMC, respectively. CD8+ T-cell reactivity was observed in 3 of 14 (21%) and 7 of 14 (50%) using the FASCIA-WB and FASCIA-PBMC, respectively. There was strong correlation between the proliferative responses measured by the 3H-thymidine uptake assay and the CD4+ T-cell FASCIA-WB (r=0.68; p<0.01).

Conclusion: HIV-1 specific T-lymphocyte proliferative responses were detected in a high proportion (37/38) of volunteers following HIV-1 DNA/MVA immunization. The FASCIA revealed both CD4+ and CD8+ T-cell proliferation in response to HIV-1 antigen stimulation. A standardized FASCIA-PBMC, which allows simultaneous phenotyping may be an option to the conventional 3H-thymidine uptake assay for assessment of vaccine-induced T-cell proliferation, especially in isotope-restricted settings.

OA04-03

Characterization of cell-mediated immune responses generated by recombinant modified vaccinia Ankara (rMVA)-HIV-1 in a phase I vaccine trial

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Background: Potency of the cell-mediated immune response is now the critical metric for down-selection of candidate HIV-1 vaccines. Here we characterize the potency (magnitude and quality) of cell-mediated immunity generated in response to a multigenic rMVA-based HIV-1 (CRF01_AE-derived) vaccine.

Methods: 49 healthy, vaccinia-naïve volunteers were enrolled in a Phase I randomized, double-blind, dose-escalation, route-comparison, placebo-controlled trial to assess the safety and immunogenicity of MVA-CMDR HIV-1 vaccine. The study was divided into Part A: low-dose 10^6/pfu ID versus 10^7 pfu/IM and Part B: high-dose 10^7 pfu/ID vs 10^8 pfu/IM. Vaccinations were given at months 0, 1 and 3 with an active:placebo ratio of 10:2. Chromium-release CTL, IFNg Elispot, and polyfunctional flow cytometry (IL-2/IFNg/TNFa/MIP-1b/CD107a), were performed on all volunteers. Synthetic peptide pools and GLP-grade MVA were used to assess insert (Gag/Pol/Env) and vector immunogenicity respectively.

Results: Vector-specific responses were robust (>80% response rate at high-dose), durable (maintained at least 6 months), and exhibited a dose-dependent increase in both magnitude and response rate among the 4 arms of the trial. HIV-insert-specific responses were detected using all assay platforms, but were lower than the vector-specific responses in both magnitude and response rate in all arms of the trial (~60% at high-dose by CTL, Elispot and ICS assays). Specifically, polyfunctional analysis revealed a TNFa/IL-2/IFNg bias in CD4+ T cells and a MIP-1b/CD107a/IFNg bias in CD8+ T cells, with CD4+ T cell responses more frequent than CD8+ T cell responses to the HIV inserts. Vector-specific immune responses showed a boosting effect from the 2nd to the 3rd immunization.

Conclusion: rMVA vaccination induces a dose-dependent, robust and durable polyfunctional cellular immune response as measured by IFNg Elispot, CTL and intracellular cytokine stimulation assays. Although vector-specific responses tend to dominate over insert-specific responses, the data supports further exploration of MVA as a vector modality in prime-boost vaccination strategies.

OA04-04

Perceived parental willingness for their adolescents to participate in future HIV prevention trials: a survey conducted amongst adolescents in Soweto

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Background: Following the high HIV prevalence rates in Sub-Saharan Africa, several target specific interventions have been put in place with varying degrees of success. In implementing these interventions, adolescents have become a crucial target group. This study specifically examines perceived parental willingness for their adolescent to participate in HIV prevention trials by taking parent-adolescent communication into account.

Methods: The willingness to participate in future HIV prevention trials questionnaire and the Parent-Adolescent Communication Scale (PACS) formed part of a larger adolescent survey conducted among 16-18 year olds living in Soweto, an urban setting in Johannesburg, South Africa. Adolescent participants were interviewed about communication with parents or guardians regarding their sexual health. The questionnaires were interviewer administered.

Results: In total, 303 questionnaires were administered with a sample of 176 (58.1%) females and 127 (41.9%) males (median age = 17). 184 (60.7%) participants had both parents alive, 78 (25.7%) only the mother alive and 20 (6.6%) only the father alive. Only 33 (10.9%), 24 (7.9%) and 7 (2.3%) participants indicated their mothers, fathers and guardians had post school training respectively. The PACS had a reliability of 0.77 with scores ranging between 7 and 28 with a median of 16. A score greater than 14 was categorized as representing good communication skills. 266 (88%) of the participants indicated their parents would be willing to allow them to participate in a future HIV vaccine trial. There was an association between parental willingness to participate and PACS scores ($p < 0.05$, OR=0.4085, CI 0.2-0.8) in which univariate analysis showed that willingness was dependent on good communication scores (OR=2.4, CI 1.2-5.0). The effect was particularly strong for females (OR=8.3, CI 2.2-30.7).

Conclusion: Perceived parental willingness for their adolescents to participate in future HIV vaccine trials is high and appears to be strengthened by good parent-adolescent communication.

OA04-05

Safety and viral load changes in HIV-1 infected subjects treated with autologous dendritic immune therapy following ART discontinuation (CTN#239)

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Background: We demonstrated in a phase 1 trial that an immunotherapy (AGS-004) consisting of a monocyte-derived dendritic cells (DC) and RNA encoding autologous HIV antigens (Gag, Nef, Rev, Vpr) derived from the patient's own pre-ART plasma induced immunogenicity in most patients. Based on these results a multicenter phase 2 trial was implemented to assess the safety and proportion of patients demonstrating viral load (VL) < 1000, <5000 and <10,000 copies/mL during the 12 week ART structured treatment interruption (STI).

Methods: Subjects on their initial ART regimen with VL < 50 copies/ml, CD4 > 450 cells/uL, CD4 nadir > 200 cells/uL and a pre-ART VL >10,000 to 500,000 copies/mL were eligible. The treatment consists of 4 intradermal AGS-004 doses administered monthly in combination with ART followed by two more doses during the 12 week STI. Subjects who participated in the phase 1 study were included and received a second cycle of AGS-004. Subjects may continue AGS-004 booster administration if VL remains < 10,000 copies/mL.

Results: 33 subjects were enrolled from 11 Canadian sites, and AGS-004 successfully manufactured and administered to 21 subjects. 9 subjects have successfully completed 12 weeks of STI. The immunotherapy related AEs were Grade 1 or 2 flu-like, GI symptoms, fatigue, and injection site reactions. During the STI, no reports of autoimmunity or AIDS defining events were observed. After an initial viral rebound, 4 out of 9 subjects had >2 instances of VL measures <1000 copies/mL when assessed every 2 weeks during the STI. At Week 12 of STI 5 subjects had viral loads <10,000 copies/mL with CD4 >350 cells/µL including 4 subjects with viral loads <5000 copies/mL.

Conclusion: Results from this phase 2 autologous immunotherapy trial demonstrated that this therapy is safe and induced partial control of VL when compared to pre-ART VL during the 12-week STI.

OA04-06 LB

Post-infection cellular immune responses in recipients following ALVAC-HIV® + AIDSVAX® B/E prime-boost vaccination in the Thai phase III trial.

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Background: The phase III prime-boost trial of ALVAC-HIV® (vCP1521) and AIDSVAX® B/E spanned 2003 to 2009 in Thailand. Both candidates express HIV CRF 01_AE and subtype B antigens, the predominant circulating HIV-1 subtypes in Thailand. This preliminary study assessed cell-mediated immune (CMI) responses, viral load and CD4 counts in a subset (N=47) of anti-retroviral naïve incident infections (< 270 days after estimated infection) that occurred during the trial.

Methods: Study participants were randomized 1:1, vaccine:placebo. The immunization regimen was 0, 1, 3 and 6 months for ALVAC-HIV®, with AIDSVAX® being administered at 3 and 6 months. Cytotoxic T lymphocyte (CTL) assays were conducted on freshly isolated peripheral blood mononuclear cells (PBMC) in a standard chromium release assay using autologous EBV-transformed B cells as targets (N=47). Interferon-gamma (IFN-γ) ELISpot assays were performed using cryopreserved PBMC (N=43). Target antigens for both CMI assays matched those of the vaccine candidates. Concurrent viral load and CD4 counts were measured using commercial assays. HIV genotyping was performed using the multiregion hybridization assay.

Results: Data are still blinded with respect to immunization status. The median time from infection to CMI assessment was 164 days (range: 113-265). The frequency of HIV-specific CD8+ CTL activity was 64%, with equivalent responses to Env (19/47) and Gag/Pol (18/47) antigens. HIV-specific IFN-γ ELISpot responses were measured in 47% of subjects, but the response was predominantly to Gag – 37% versus 19% for Env. Median viral load and CD4 counts were 25338 copies/ml (range: <50-272509) and 571 cells/µl (range: 220-1067), respectively. CRF01_AE was the predominant infecting subtype (82%; 37/45).

Conclusion: Robust CD8+ mediated CTL responses to HIV antigens expressed by the ALVAC-HIV® immunogen were observed in participants who became infected during the trial. Determining the relevance of these responses to viral control and/or CD4 T cell counts following un-blinding (October, 2009) constitutes a high priority.

ORAL ABSTRACT SESSION 05: NEW VACCINAL APPROACHES

OA05-01

In vivo electroporation enhances the immunogenicity of ADVAX, a DNA-based HIV-1 vaccine Candidate, in healthy volunteers

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Background: In healthy volunteers, we sought to determine the safety, tolerability, and immunogenicity of ADVAX, a subtype B'/C, DNA-based, multigenic, HIV-1 vaccine candidate, when injected intramuscularly immediately followed by in vivo electroporation (EP) using the TriGrid™ Delivery System.

Methods: Forty healthy volunteers aged 18-60 were enrolled in a double blind randomized phase-I trial. Eight volunteers each received either low dose (LD, 0.2 mg); mid dose (MD, 1.0 mg); or high dose (HD, 4.0 mg) ADVAX or saline placebo via EP. Another eight volunteers received 4.0mg ADVAX intramuscularly (IM). Vaccinations were given at weeks 0 and 8. The protocol was subsequently amended to administer a third dose of HD EP/placebo at week 36 to volunteers receiving either HD ADVAX via EP (n=8) or placebo via EP (n=3). Total study follow-up is 14 months.

Results: There have been no vaccine or device related serious adverse events to date. After two vaccinations in all subjects, the IFNg ELISPOT response rates were IM: 1/8 (13%), LD-EP: 3/8 (38%) MD-EP: 7/8 (88%) and HD-EP: 6/8 (75%). In the same order, the mean (range) response to peptide pools spanning all antigens was 72, 120 (70-193), 151 (53-440), and 141 (59-336) SFC/million PBMCs. The breadth of the response improved with EP and increasing dosage, with responses to 1, 1, 3, and 4 of the 4 ADVAX gene products. ICCS analysis of ELISPOT responders revealed a balanced CD4+/CD8+ response. There were no responses to placebo, by definition. An analysis of responses after the third vaccination in the high dosage group is ongoing.

Conclusion: This study is the first demonstration in healthy volunteers that EP in vivo is safe, tolerable, and effective in improving the magnitude and breadth of cellular immune responses to a DNA-based vaccine.

OA05-02

Analysis of DNA compared to Ad5 vaccination, as single and mixed modalities, demonstrates robust induction of cellular immune responses in macaques

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Background: We have previously reported dramatic increases in immune responses induced by DNA vaccines delivered by electroporation (EP), resulting in improved control of viral replication following a SIVmac251 challenge. We compared the immunogenicity of DNA+EP to the Ad5 vaccine, the most potent recombinant viral vector for the generation of CTL responses in macaques and humans. Futhermore, we were interested in examining the effect of prime/boost strategies using these two platforms on the magnitude and phenotype of the immune response.

Methods: Three groups of rhesus macaques (n=5) were immunized with consensus SIVmac gag, env, pol constructs with plasmid IL-12 by EP (DNA+EP), Ad5SIVgag, pol, nef (Ad5) or saline alone (Naïve). The DNA+EP group received 4 immunizations and the Ad5 group received 3 immunizations. Five months following the last immunization the DNA+EP group was boosted twice with the Ad5 vaccine and vice versa. Cellular responses were assessed by IFNγ ELISpot, CFSE proliferation, and ICS for polyfunctional populations.

Results: The Ad5 group had an early three-fold enhancement of IFNγ responses compared to the DNA+EP group (1925±610 and 666±297 SFU/106 PBMCs, respectively). Subsequent Ad5 immunizations failed to boost responses while the DNA+EP group reached a robust 9776±1589 SFU/106 PBMCs. Proliferation was five-fold better and the magnitude of the polyfunctional CD8+ T cell response was a log higher in the DNA+EP group compared to the Ad5 group (1.31% and 0.11%, respectively). When boosted with DNA, the Ad5 polyfunctional response increased six-fold (0.67%) and the DNA+EP group had a 7-fold increase (8.28%) following an Ad5 booster immunization.

Conclusion: Following the initial series of immunizations the DNA+EP group surpassed the Ad5 group in terms of magnitude, proliferation, and polyfunctionality. However both groups demonstrated a boost in all responses following crossover immunizations. These results have significant implications for HIV vaccine development and warrant further study.

OA05-03

Efficacy study of a T-cell-based DNA vaccine delivered by intradermal electrotransfer in macaques

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Background: We recently demonstrated that intradermal (ID) injection with electropororation (EP) of a new HIV DNA vaccine induced strong and persistent specific T-cells in macaques. Here, we used an equivalent vaccine encoding SIVmac251 antigens in order to study its efficacy in SIV mucosal challenge of macaques.

Methods: Eighteen macaques were vaccinated with 1 mg of the auxo-GTU-MultiSIV DNA at week 0, 4 and 12: either by the ID route only (n=6), or by the ID route combined with EP (ID+EP; n=6) or ID+EP with co-injection of a plasmid used as "genetic-adjuvant" (n=6). A control group of unvaccinated animal was included (n=6).

Results: Before challenge, all animals raised SIV-specific T-cells as evidenced by IFN-γ ELISPOT (110±42, 921±310 and 905±252 spots/106 cells in the ID only, ID+EP and ID+EP+genetic-adjuvant groups, respectively). Weak and transient antibody responses were detected. All animals were intrarectally challenged with pathogenic SIVmac251. T-cell responses increased in both ID+EP groups as early as week 1 post-challenge (3,898±395 and 3,031±893 spots, respectively), and up to 12,000 spots by week 2. Macaques immunized ID only raised delayed and lower responses remaining earlier and higher than in controls. At peak of viremia, plasma viral load was significantly reduced (p=0.0104) in the ID+EP group. Interestingly, no reduction of plasma viral load was observed by that time in the genetic-adjuvant group despite high anamnestic responses. Differences in anti-Gag responses may explain this observation. Viremia was not reduced in the ID only group. At set-point, although similar plasma viral load in all groups, reduction of SIV-DNA copies in rectal biopsies was observed in the vaccinated animals.

Conclusion: Electroporation results induced high anamnestic T-cells responses which are better associated with the control of early plasma viremia. Impact of vaccine on disease progression is currently under evaluation.

OA05-04

Gp96-Ig-SIV vaccines induce predominant immune responses at mucosal sites

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Background: We have developed a vaccine design that utilizes the unique property of the endoplasmic reticulum chaperon, heat shock protein (HSP) gp96, to bind antigenic peptides and deliver them to APCs. Cell-based gp96-Ig vaccines, by prolonged in vivo secretion of gp96-Ig peptide, imitate viral replication and provide immune stimuli comparable to attenuated viruses. In model systems in mice we have shown that gp96-Ig transfected, antigen expressing cells secrete gp96-Ig in vivo and stimulate both systemic and strong mucosal immunity. The aim of our study was to evaluate safety and systemic and mucosal SIV-immunity with secreted gp96-Ig-SIV vaccines in non-human primates.

Methods: Gp96-Ig was generated by replacing the ER retention sequence KDEL with the IgG1-Fc tag. HEK-293 cells were transfected with gp96-Ig and with the cDNAs encoding the SIV antigens gag, pol, env and retanef. Irradiated, transfected 293 cells that secrete 1, 5 or 50 micrograms gp96-Ig-SIV-peptide complexes in 24h, were injected intraperitoneally in Mamu-A*01+ macaques at 0, 4 and 25 weeks.

Results: After the third immunization the SIV-specific CD8 response was boosted to very high levels in the rectum and jejunum (30 - 50% tetramer positive cells in the CD8 gate in LPL and IEL). In vaginal IEL gag-specific CD8 responses reached 4%. Tetramer+ cells expressed appropriate functional (granzymeB) and migration markers (CD103). Control macaques immunized with 293 cells not secreting gp96, showed only background tetramer binding. The mucosal CD8+ and CD4+ T cells from lamina propria and intraepithelial compartment secrete IL-2 or IFN-gamma or both simultaneously in response to peptide stimulation.

Conclusion: We conclude that the cell secreted gp96-Ig-SIV vaccine is safe and can induce strong poly-specific, multifunctional and predominant CD8 responses in mucosal compartments that are thought to be critical for protection from SIV/HIV infection.

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OA05-05

Impact of in vivo CD4 binding during HIV-1 Env trimer immunizations of rhesus macaques

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Background: We recently reported that immunization with cleavage-defective soluble HIV-1 envelope glycoproteins (Env) trimers in monkeys, but not in rabbits, results in the elicitation of antibodies directed against the co-receptor binding site of gp120. This finding demonstrates that the high affinity interaction between Env and primate CD4 results in an alteration of Env immunogenicity. To further investigate the impact of Env-CD4 in vivo interactions during vaccination, we immunized rhesus macaques with wild type (wt) and CD4 binding defective Env trimers.

Methods: Groups of five adult rhesus macaques were immunized with wt trimers or CD4 binding defective trimers, with either retained or abrogated capacity to form the co-receptor binding-site. Plasma and PBMCs were collected 2 weeks post-immunization. Frequency of Env-specific B and T cells were assessed by ELISpot analysis and flow cytometry. Serum antibodies were measured by ELISA and neutralization assays. Four weeks following the last immunization, control and vaccinated animals were challenged with SHIVSF162P4.

Results: Antibodies against the co-receptor binding site were elicited in animals immunized with wt trimers, but not in animals immunized with the CD4-binding defective mutants. Elimination of the Env-CD4 in vivo interaction did not affect vaccine-induced Env-specific T cell responses or levels of total elicited binding antibodies. However, differences in the quality of the in vitro neutralizing antibody response were observed between the three groups. A comparable decrease in plasma viral loads compared to unvaccinated controls was also measured following challenge in all three groups.

Conclusion: These results confirm that the wt Env trimers used here interact with host CD4 in vivo affecting the quality of the elicited antibody response. As Env-based vaccines progress from small animals to primates, the effects of high affinity CD4 interaction may need to be considered in regards to immunogen design.

OA05-06 LB

First-in-human phase 1 safety and immunogenicity of an Adenovirus Serotype 26 HIV-1 vaccine vector

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Background: Adenovirus serotype 26 (Ad26) is a rare Ad serotype that differs substantially from Ad5 in terms of baseline seroprevalence, receptor usage, tropism, innate immune profile, adaptive immune phenotype, and protective efficacy in the SIV/macaque model. Here we report the initial safety and immunogenicity assessment of a prototype Ad26 vector in humans.

Methods: Ad26 expressing the VRC EnvA test antigen was manufactured by Crucell. 36 Ad26 seronegative, healthy subjects were enrolled in a randomized, double-blinded, placebo-controlled, dose-escalation phase 1 study. Groups of 12 subjects received doses of 10^9, 10^{10}, or 10^{11} vp of the Ad26-EnvA vector (N=10/group) or placebo (N=2/group) at weeks 0, 4, and 24. We performed a pre-specified blinded immunogenicity analysis after the first two immunizations. Validated IFN-gamma ELISPOT assays were performed with positivity criteria of >55 SFC/10^6 PBMC and >4-fold background.

Results: 26/36 subjects were female, and 70/72 vaccinations were administered. Some reactogenicity was observed after the initial immunization in the highest dose group but typically resolved within 24 h. No vaccine-associated AEs or SAEs occurred. In all three dose groups, 2 subjects/group exhibited no detectable vector- or insert-specific immune responses at any timepoint, whereas 10 subjects/group developed positive Ad26 NAb titers, EnvA-specific ELISA titers, and EnvA-specific ELISPOT responses following vaccination. In the 10^9 vp dose group, the median ELISA titer was 1000 (IQR 300-3,000) and the median ELISPOT response was 381 SFC/10^6 PBMC (IQR 125-545) at week 8. In the 10^{11} vp dose group, the median ELISA titer was 5,477 (IQR 3,000-10,000) and the median ELISPOT response was 365 SFC/10^6 PBMC (IQR 85-715) at week 8.

Conclusion: The Ad26 vector is safe and immunogenic in humans at all three doses. Ad26 is therefore a promising new vector for further clinical studies to evaluate novel inserts such as mosaic HIV-1 Gag, Pol, Env antigens.

ORAL ABSTRACT SESSION 06: DYNAMICS OF HIV INFECTION AND THE IMMUNE RESPONSE

OA06-01

Multiplicity of infection by HIV-1 in injection drug users, men who have sex with men and heterosexuals

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Background: We have recently shown that transmitted/founder virus(es) can be identified unambiguously using single genome amplification (SGA) (Keele PNAS 2008; Salazar JEM 2009) and that in heterosexual transmissions approximately 80% of patients are infected by a single virus or infected cell (Keele PNAS 2008; Haaland PLoS Pathogens 2009; Abrahams JVirol 2009). Here we explore the characteristics of virus transmission in men who have sex with men (MSM) and injection drug users (IDU).

Methods: Full-length env sequences were derived by SGA from plasma vRNA from 30 acutely infected MSM and 11 IDU from North America. Modes of infection were determined by self-report. Uncloned amplicons were sequenced, aligned, and analyzed by neighbor-joining phylogenies and the Highlighter tool.

Results: Maximum within-patient diversity ranged from 0.08-7.12%. In the MSM group, 19 of 30 (63%) acutely infected subjects had evidence of a single transmitted/founder (t/f) virus and 11 of the 30 had evidence of transmission by a minimum of 2-10 viruses. In the IDU group, 4 of the 11(36%) had evidence of a single t/f virus, while the remaining 7 demonstrated transmission by a minimum of 3-19 variants. In the subjects with multiple transmissions, the median number of t/f variants was 3 for MSM and 5 for IDU. Differences in single versus multiple virus transmissions among HSX, MSM and IDU were statistically significant ($p<0.006$).

Conclusion: The HIV-1 transmission event is different in HSX, MSM and IDU. We see single variant transmission in approximately 80% of HSX, 60% of MSM and 40% of IDU. When multiple transmissions occur, they tend to have a higher number of variants in IDU than in MSM or HSX. These findings indicate that risk of HIV-1 acquisition correlates with numbers of transmitted viruses. This, along with the phenotypic properties of these viruses (especially Env), may be important in vaccine design and assessment.

OA06-02

Monospecific expansion of SIVmac251 during acute infection masks multiple transmitted virus variants revealed during the chronic phase

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Background: Rhesus macaque challenge models to evaluate protection are important in the search for an AIDS vaccine. Many challenge protocols use high dose SIV to ensure infection of all control animals after a single challenge. Many virus variants are predicted to infect the animals under these conditions, which is different than the majority of human infections. It is important to identify both the infecting virus swarm in the stock, and the transmitted and replicating virus, to better evaluate vaccine candidates. It is important to develop macaque models predicting human vaccination outcome.

Methods: We performed single genome amplification (SGA) to identify the full env sequence or a fragment encompassing the highly variable V1/V2 region from naïve SIVmac251-challenged animals using plasma from the acute and chronic phase, as well as from the original SIVmac251 challenge stocks.

Results: The two closely related SIVmac251 stocks sequenced by SGA showed great diversity of env sequences. Most changes were within the V1/V2 region, known to be immunodominant for SIV antibody responses. Despite the stock diversity, only a very narrow selection of similar envs were detected during the acute phase in 7 of 9 animals infected by atraumatic mucosal application. In contrast, multiple diverse env sequences were found in the chronic phase, which can be traced back to the stock.

Conclusion: Multiple species cross the mucosal barrier and infect the host during a high dose mucosal infection. Interestingly, one or very few of these variants propagate early in the acute phase, but other transmitted variants emerge to prominence later. This may be the result of viral fitness, competition, founder effects, or innate mechanisms. These findings also suggest that estimating the number of transmitted virus variants by analysis during the acute phase is inaccurate, and evaluation of both acute and chronic virus is critical to identify the transmitted variants.

OA06-03

Dynamics of CTL epitope escape and reversion in an African subtype C cohort

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Background: HIV immune escape follows a predictable mutational path in response to the HLA alleles carried by an individual. The kinetics of CTL epitope escape and reversion in subtype B HIV-1 infected individuals have recently been reported, however, the inferences drawn from them were limited by the absence of information about the transmitted sequence. To address these issues, we examined Gag, Pol and Nef sequences in both partners of 148 epidemiologically-linked transmission pairs from an African subtype C cohort.

Methods: Cohabiting HIV-1 discordant heterosexual couples from Lusaka, Zambia were followed longitudinally. Despite counseling and condom provision, 7% of uninfected partners became infected each year. At this time and at three-month follow-up intervals, blood and PBMC samples were collected from both the donor (D) and recipient (LR). The gag, pol and nef genes were amplified and sequenced from virus in plasma obtained from both partners. CTL epitopes were identified using previously published HLA-linked polymorphisms.

Results: Analyses of the viruses in the linked transmission pair recipients indicated that a surprising fraction of those transmitted had polymorphisms relevant for the HLA of the LR. Specifically, 29% of LR had viruses with escaped Gag epitopes at the time of seroconversion and these escaped epitopes were present in the D virus at the time of transmission. Thus, we find that Gag escape occurs more slowly than previously reported, with only 5-15% of Gag epitopes exhibiting de novo escape within the first year, and that a majority of HLA-linked polymorphisms at one year were transmitted from the donor.

Conclusion: The study of epidemiologically-linked transmission pairs demonstrates the high rate of transmission of escaped epitopes and that this directly impacts the calculated rates of escape, which could not be accounted for in previous studies. The potential for these non-donor driven mutations to impact viral pathogenesis is under investigation.

OA06-04

The role of early T-cell responses in subjects with acute HIV-1 infection

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Background: Previous studies have shown that T-cells play an important role in the maintenance of virus set-point in early and chronic HIV-1 infection. This study investigates the role of the first T-cell responses in selecting virus escape and controlling the early peak of viremia.

Methods: To identify the single transmitted/founder virus full-length single genome amplification (SGA) virus sequencing was performed when patients were screened before seroconversion during peak viremia. Virus evolution was monitored by further serial SGA sequencing. Ex-vivo IFN-γ enzyme-linked immunospot assays and multi-parameter flow cytometry were used with peptides matching the founder virus sequence to comprehensively map the HIV-1 specific T cell responses in subjects at serial time points over the first 12 months post-screening.

Results: T-cell assays showed that HIV-1 specific T-cell responses selected non-synonymous sequence changes at different rates across the whole of the HIV-1 proteome. The first detectable T-cell responses induced virus escape within 18-34 days of screening. However, these T-cell responses were subsequently lost or diminished after the escape variant became fixed within the virus population. The majority of the later HIV-1 specific T-cell responses induced a slower rate of escape, whilst a minority did not select for escape variants over the study period. Selected sequence changes could also be attributable to reversion, compensatory mutations, other immune responses and linkage to other selected sites. Ongoing studies are exploring the mechanisms behind the few rapid sequence changes found where no T cell response could be identified.

Conclusion: The data shows that the first HIV-1 specific T-cell responses can induce rapid virus escape at times earlier than previously described. Appearing during the decline of viral load from peak viremia these T-cell responses provide some evidence that they contribute to this fall. The role of these first HIV-1 specific T-cell responses will have an impact on vaccine design.

OA06-05

Adaptation of HIV-1 to the human immune system at the population level is driven by protective HLA-B alleles

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Background: HIV undergoes extensive within-host adaptation to HIV-specific Cytotoxic T Lymphocyte (CTL) responses. However, the full extent and importance of CTL escape mutations in driving HIV evolution at the population level remains to be established.

Methods: We included 27 individuals from the Amsterdam Cohort Studies on HIV infection and AIDS with a known seroconversion date in 1985 or 2005/06 (12 and 15 individuals, respectively), of which HIV sequences were derived within a year after seroconversion. CTL epitopes were predicted using a proteasomal cleavage/TAP transport/MHC class I combined predictor. HLA-binding epitopes from the proteins P17, P24, Nef, Protease and RT were predicted for 5 common HLA-A and 3 common HLA-B alleles, as well as for HLA-B27 and HLA-B57, the HLA-B alleles most strongly associated with slow progression to AIDS. To avoid the possibility that observed CTL escape mutations were due to within-host evolution rather than adaptation at the population level, individuals expressing the particular HLA allele under investigation were excluded from the analyses.

Results: HIV strains isolated from recent seroconvertors were found to contain significantly less 9-mers predicted to bind to the 5 HLA-B alleles under investigation compared to historical HIV strains, which was not observed for the 5 HLA-A alleles. Remarkably, the reduction in the number of CTL epitopes during the epidemic was not due to adaptation to the most common HLA-B alleles, but instead to the alleles associated with slow disease progression, HLA-B27 and B57.

Conclusion: These data show that, over the past 20 years, HIV has adapted to the human immune system by decreasing the number of potential CTL epitopes presented via HLA-B, but not HLA-A, alleles, and that such adaptations can become fixed in the population. Adaptation was not related to the population frequency of the HLA alleles, but instead seemed driven by the immune selection pressure of the HLA alleles.

OA06-06 LB

Evidence of vaccine-induced changes in breakthrough HIV-1 strains from the Step trial

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Background: Vaccinations in the Step Study, designed to assess the efficacy of a T cell-based vaccine, were interrupted in 2007 after an interim analysis showed no evidence of efficacy.

Methods: To evaluate the impact of the vaccine on founder/breakthrough viruses, i.e., the viruses obtained from the earliest available specimen around the time of diagnosis, we obtained 0.5-14 HIV-1 whole genome sequences per individual from 40 vaccine and 28 placebo recipients who acquired HIV-1 infection as of December 2007.

Results: To assess whether the vaccine elicited T cells that exerted selective pressure on founder viruses, we identified known and likely class I MHC-restricted CTL epitopes in founder sequences based on each volunteer's HLA type. We calculated protein distances between epitopes in the vaccine and those in the founder sequences. Compared to the vaccine, epitopes from vaccine recipients exhibited more mutations than epitopes from placebo recipients ($p=0.02$). Potential epitopes were then identified across all infected individuals and compared to HXB2. The epitopic distances from the combination of Gag, Pol and Nef sequences (included in vaccine) were significantly higher among vaccine vs. placebo recipients ($p=0.004$). In contrast, there was no distinction between vaccine and placebo if epitopes from Env, Rev, Tat, Vif, Vpr, Vpu (not included in vaccine) were considered together ($p=0.90$). Lastly, ~10 AA signature sites in Gag, Nef, Pol (but none in Env) were found to classify vaccine versus placebo status.

Conclusion: Our data indicate that founder viruses from vaccinees diverged further from the vaccine than viruses from placebo recipients at potentially immune reactive sites. This suggests that the vaccine may have blocked the outgrowth of specific HIV-1 variants that were the most similar to the vaccine sequence and/or elicited immune responses that may have driven specific mutations among vaccinees' viruses post-infection.

ORAL ABSTRACT SESSION 07: T CELL RESPONSES TO VACCINE

OA07-01

HIV-Specific CD8+ T-cells of vaccinees exhibit proliferative and cytotoxic capacities comparable to those of progressors

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Background: HIV-specific CD8+ T-cells of long-term nonprogressors (LTNP) exhibit extraordinary per-cell cytotoxic capacity. To adapt cytotoxicity assays for vaccine trials, we compared HIV-specific CD8+ T-cell cytotoxic capacity with proliferation and perforin expression using cells from LTNP, viremic progressors, antiretroviral recipients with <50 HIV RNA copies/ml plasma (Rx<50) and seronegative individuals who had or had not received the Merck Ad5 trivalent vaccine.

Methods: HIV-specific CD8+ T-cell cytotoxic responses to HIV_{SF162}<-infected CD4+ T-cell targets were measured at 1 hour by flow cytometric detection of granzyme (Gr) B delivery to live targets or infected CD4 elimination (ICE). Cytotoxicity, IFN-γ production, perforin expression, and proliferation of HIV-specific CD8+ T-cells were examined following a 6-day stimulation with HIV_{SF162}-infected CD4+ T-cell targets.

Results: The HIV-specific CD8+ T-cell cytotoxic responses of vaccinees (medians 16.8% GrB activity, 37.2% ICE) were clearly distinguishable from those of seronegative controls (1.7% GrB activity, p<0.001; 0.3% ICE, p<0.001), but were comparable to those of progressors (16.6% GrB activity, p>0.5; 37.4% ICE, p>0.5). Among vaccinees, those with the protective alleles HLA B*27, B*57, or B*58 tended to have higher responses. Vaccinee responses were significantly less than those of LTNP (50.7% GrB activity, p<0.001; 82.5% ICE, p<0.001). GrB activity and ICE were strongly correlated with HIV-specific CD8+ T-cell proliferation (R=0.85, p<0.001 and R=0.87, p<0.001, respectively) and perforin expression (R=0.86, p<0.001 and R=0.9, p<0.001, respectively). When measured on a per-cell basis, cytotoxicity of most vaccinees remained at the level of progressors, even with higher effector:target ratios.

Conclusion: GrB activity and ICE correlate strongly with CD8+ T-cell proliferation and perforin expression in expanded cells suggesting these parameters are reasonable surrogate measurements of CD8+ T-cell-mediated killing requiring fewer cells. GrB activity and ICE of vaccinees were similar to those of progressors, suggesting these low responses might contribute to suboptimal control in cases of HIV infection following vaccination.

OA07-02

Adenovirus vectors induce expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1 infection

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Background: In the recently halted human immunodeficiency virus type 1 (HIV-1) vaccine STEP trial individuals that were seropositive for adenovirus serotype 5 (Ad5) showed increased rates of HIV-1 infection on vaccination with an Ad5 vaccine. We undertook a series of ex vivo strategies to address the hypothesis that immunisation of Ad5 seropositive individuals with adenoviral vectors may result in activation, expansion, and trafficking of Ad5-specific memory CD4 T cells to mucosal tissues thereby increasing the number of HIV-1 susceptible targets at the initial sites of infection.

Methods: Ad5 and Ad11 antibody titers were measured in 20 healthy volunteers. Dendritic cells (DC) were generated from these individuals, pulsed with replication defective Ad5 or Ad11 and co-cultured with autologous lymphocytes. Cytokine profiles, proliferative capacity and the migration potential of the adenovirus-stimulated memory T cells were measured. The susceptibility of re-stimulated memory Ad-specific T cells to infection with a CCR5-utilising HIV-1 was also assessed by multi-colour flow cytometric analysis and p24 ELISA assays.

Results: Stimulation of T cells from Ad5 seropositive but Ad11 seronegative individuals with Ad5, or serologically distinct Ad11 vectors induced expansion of adenovirus memory CD4 T cells expressing alpha 4 beta 7 and CCR9, indicating a mucosal-homing phenotype. CD4 T cell proliferation and IFN-gamma production in response to Ad stimulation correlated with Ad5 antibody titers. In contrast, Ad5 serostatus did not correlate with total cytokine production upon re-challenge with Ad5 or Ad11. Expanded Ad5 and Ad11 memory CD4 T cells showed an increase in CCR5 expression and higher susceptibility to infection by R5 tropic HIV-1.

Conclusion: Adenoviral-based vaccination against HIV-1 in individuals with pre-existing immunity against Ad5 may result in preferential expansion of HIV-susceptible activated CD4 T cells that home to mucosal tissues, increase the number of virus targets and lead to a higher susceptibility to HIV infection.

OA07-03

Influence of preexisting vaccinia immunity on a DNA/MVA SIV vaccine, decreased cellular immunity but enhanced control of a pathogenic SIV challenge

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Background: The influence of preexisting immunity to viral vectors is a major issue for the development of viral vectored vaccines. Here we report that for a DNA/MVA vaccine, preexisting immunity to vaccinia virus (Dryvax) decreases cellular immune responses but enhances control of an intrarectal SIV challenge.

Methods: Three groups of rhesus macaques, eight per group, were studied. The Dryvax-naive and Dryvax-immune groups received the DNA/MVA SIV vaccine (DNA at weeks 0 and 8, and rMVA at weeks 16 and 24). In addition, the Dryvax-immune group received the Dryvax vaccine 1.5 years prior to the DNA prime. The control group did not receive any vaccine. All macaques were challenged intrarectally with SIV251 at 9 months after the final MVA.

Results: Following vaccination, the frequency of SIV Gag-specific CD4 and CD8 T cells were 5-10 fold lower in the Dryvax-immune group than the Dryvax-naive group. Despite their low SIV-specific T cell responses, the Dryvax-immune macaques exhibited the best control of SIV challenge with viremia 480-fold lower at peak and 40-fold lower at set point than in the unvaccinated control animals (p=0.01). The enhanced control in the Dryvax-immune animals was not restricted to Mamu A*01+ animals and was strongly associated with reduced colorectal virus at 2 weeks post challenge. Factors that correlated with early colorectal viral control included the magnitude of vaccine-elicited CD4 T cells displaying the CCR5 viral co-receptor, which was dampened in the Dryvax-immune animals; the presence of anti-viral mucosal IgA, which was more frequent in the Dryvax-immune animals, and the avidity of the anti-Env Ab response. The frequency of anti-viral CD8 T cells did not correlate with early colorectal viral control.

Conclusion: These results highlight important roles for vaccine-elicited CCR5+ CD4 T cells in augmenting, and mucosal IgA and high avidity anti-Env IgG in restricting the early replication of a colorectal immunodeficiency virus challenge.

OA07-04 LB

Immunogenicity of ALVAC-HIV® (vCP1521) and AIDSVAX® B/E prime boost vaccination in RV144, the Thai phase III HIV vaccine trial

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Background: The Phase III trial of ALVAC-HIV®and AIDSVAX®B/E in Thailand began in October 2003 and concluded in June 2009. Both vaccine candidates express HIV-1 circulating recombinant form (CRF) 01_AE and subtype B antigens. This study assessed whether the Phase III vaccine lots show immunogenicity comparable to the previous Phase I/II study of the identical immunization regimen.

Methods: A list of blinded samples from persons completing all 4 injections with either placebo or vaccine and remained HIV negative at the end of the trial was provided. Peripheral blood mononuclear cells (PBMC) or plasma were tested to CRF 01_AE and subtype B vaccine antigens in the following validated assays: (1) Interferon-gamma (IFN-γ) ELISpot; (2) IFN-γ/interleukin-2 intracellular cytokine staining (ICS); (3) Binding antibody (BAb). ELISpot and ICS assays measured responses to Env (92TH023) and Gag (LAI) peptide pools prior to and 6 months following the completion of immunization. BAb was measured using reciprocal dilution EIA to A244 and MN gp120 and BH10 p24 prior to and at 2 weeks following the completion of immunization.

Results: Data will be un-blinded to treatment assignment by October 2009. Analyses of post-injection responses to Env and Gag by ELISpot revealed an overall frequency of 14%, with Env responses (11%) predominating over Gag (5%). The overall frequency of ICS responses to HIV peptides in samples studied to date was 35% and was greater for CD4 (26%) than CD8 (9%) T cells, with responses to Env again predominating: 26% versus 1% Gag for CD4 and 6% Env versus 2% Gag for CD8 T cells. The frequency of BAb responses to p24 was 37% and was identical for CRF01_AE and MN gp120 (70%).

Conclusion: Cellular and humoral immune responses to the ALVAC-HIV® + AIDSVAX® B/E regimen were predominantly to HIV Env and appear similar to those seen in the earlier Phase I/II study.