known about the production and role of antibodies in the human mucosal environment. Previous studies have shown an abundance of IgG+ and IgA+ plasma cells associated with penile urethral glands suggesting that this is a site of local immunoglobulin (Ig) production in men. We compared HIV-specific Ig isotypes (A, G) and subclasses (G1, G3) in blood plasma (BP), seminal plasma (SP) and urethral preejaculatory (PE) secretions from HIV-infected men and controls to determine whether a distinct population of HIV-specific antibodies is present in the genital mucosa during HIV infection.

Methods: A multiplex bead-based Luminex assay incorporating a panel of seven HIV-specific antigens was used to compare titres, isotypes, and subclasses of HIV-specific antibodies in PE, SP and BP from nine HIV-infected men and nine healthy controls. We also conducted antibody-dependent cell phagocytosis (ADCP) and cytotoxicity (ADCC) assays to evaluate antibody function in the different compartments.

Results: There was an abundance of HIV-specific IgG in all three fluids, with similar titres, subclasses and specificity profiles. Similarly, ADCC and ADCP activity did not differ between the three sample types. In contrast, a subset of PE samples from HIV-infected men had significantly higher (5 - 50X) anti-gp12 IgA titres than found in SP and BP, providing evidence for local urethral production of HIV-specific IgA during HIV infection.

Conclusions: IgG HIV antibody profiles in male genital secretions reflect those in the systemic circulation, whereas IgA HIV antibodies may be locally produced in the urethral mucosa. More research is needed on strategies to elicit urethral antibody production with vaccines, and the function of IgA antibodies in HIV prevention.

OA24.03
Targeting Mucosal Fc-Fc Receptor Interactions as Vaccine Strategy against Mucosal HIV-transmission
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Background: To ultimately end the AIDS pandemic, effective means of preventing transmission by its principal genital and rectal routes must be developed. Non-neutralizing antibody (nNab) responses, which may be easier to induce than broadly neutralizing antibodies via vaccination, have shown some protection, pointing toward a role for nNab functions, including Fc-Fc receptors (FcRs) interactions. The protective efficacy of Fc-effector function is critically dependent on innate immune cells; therefore we sought to define cell repertoires expressing FcRs in the female genital tract (FGT) and intestinal tract (IT).

Methods: Fixed tissue sections (rectum, vagina, cervix, uterus, lymph node) were stained for natural killer (NK) cells, macrophages, neutrophils and Fc receptors, FcγR: I, II, III and FcεR. HIV + biopsy samples from IT and lymph nodes were examined for changes in FcR repertoires. Flow cytometric evaluation of FcR+ cells was performed on freshly isolated cells from enzymatically digested colon and cervical tissues.

Results: NK cells were very infrequent in IT and FGT. Moreover, none of the FcR was found on NK cells, suggesting that mucosal NK cells have limited capacity to mediate antibody-driven-effector functions. In contrast, macrophages and neutrophils were present at much higher frequencies in IT and FGT and were detected close to epithelial layers. They robustly expressed FcRs with macrophages expressing FcγRI, FcγRII, FcεR but not FcγRIII and neutrophils expressing FcγRII and FcεR. Changes in expression pattern in HIV-infected subjects suggest specific antibody therapeutic opportunities for harnessing Fc-FcR interactions.

Conclusions: The ability of nNabs to provide protection at mucosal barriers is centrally linked to FcR+ cells available within vulnerable tissues. Surprisingly, our data suggest that non-ADCC mediated mechanisms, such as phagocytosis and neutrophil activation, are more abundant and potentially represent important mechanisms by which vaccine induced nNabs may offer protection.

OA24.04
DNA and Protein Co-immunization Improves the Magnitude, Longevity, and Mucosal Dissemination of Immune Responses
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Background: Determining the quality and longevity of vaccine-induced immune responses is essential for improving the prospects of AIDS vaccines. DNA and protein (inactivated viral particles) co-immunization regimen induced systemic and mucosal Ab responses, which correlated with slower virus acquisition upon challenge, and potent T cell responses providing protection against chronic viremia. We are evaluating different regimens of DNA&protein vaccines using purified SIV or HIV-1 Env to further dissect humoral and cellular responses including magnitude, breadth and mucosal dissemination.

Methods: Macaques were vaccinated using DNA&protein (inactivated viral particles) co-immunization regimen induced systemic and mucosal Ab responses, which correlated with slower virus acquisition upon challenge, and potent T cell responses providing protection against chronic viremia. We are evaluating different regimens of DNA&protein vaccines using purified SIV or HIV-1 Env to further dissect humoral and cellular responses including magnitude, breadth and mucosal dissemination.

Results: Co-immunization strategy of DNA&protein induced rapid and high humoral responses while maintaining robust cellular responses typically obtained with DNA vaccines. The vaccine induced Ab against both homologous and heterologous Env; high binding titers against scaffolded V1/V2 env region; efficient dissemination to mucosal sites; high Env-specific IgG in saliva and Env-specific IgG and IgA in rectal mucosa. Analysis of cellular responses revealed the presence of cytotoxic memory T cells against several viral proteins. These cellular responses disseminated systemically as demonstrated by their presence not only in blood and lymphoid tissues, but also in bone marrow, liver, lung (effector site) and, importantly, rectal and...
vaginal mucosa. The longevity of the cellular responses induced by this co-immunization regimen was significantly improved, with SIV-specific T cells detected > 5 yrs after the vaccination. **Conclusions:** Intramuscular DNA&protein co-delivery increases the magnitude and longevity of systemic and mucosal humoral immune responses in immunized macaques and is proposed as a practical and efficient method for human vaccination.

**OA24.05**
**Antibody Isotypes Differ in their Capacity to Bind, Capture and Aggregate HIV-1 Virions**

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**Background:** Recently, Env-specific monomeric (m)IgA was correlated with increased risk of HIV-1 infection in the RV144 vaccine trial and inhibited IgG effector functions. In contrast, mucosal dimeric (d)IgA has been associated with resistance to infection in highly exposed uninfected individuals, and viral aggregation by Env specific antibodies in mucosal secretions has been often proposed as an alternative mechanism to block HIV-1 infection at the portal of viral entry. The current study was designed to determine whether anti-HIV-1 IgG1, mIgA2 and dIgA2 with the same epitope specificity differ in their ability to bind, capture and aggregate HIV-1 BA.L.

**Methods:** Bio-Layer interferometry was used to measure kinetic parameters of the different forms of b12, CH31, 2F5 and 7B2 mAbs to soluble HIV-1 BA.L gp140 Env. Virus capture by the panel of mAbs was quantified by ELISA and antibody mediated viral aggregation (AMVA) was determined using Nanoparticle Tracking Analysis.

**Results:** Interestingly, IgGs captured more virions than both mIgAs and dIgAs with the same epitope specificity. Although capture did not correlate with binding affinity (Kd), data indicates that virions capture correlated with association rate constant (kon). No relationships was found between binding affinity and AMVA, however a significant correlation was observed between the number of binding sites and the proportion of aggregates, highlighting improved aggregation capacity of dimeric mAbs ($P=0.0108$). Further, the data demonstrated that AMVA was dependent on epitope accessibility with a classical prozone effect. No relationships was found between binding affinity and AMVA, however a significant correlation was observed between the number of binding sites and the proportion of aggregates, highlighting improved aggregation capacity of dimeric mAbs ($P=0.0108$). Further, the data demonstrated that AMVA was dependent on epitope accessibility with a classical prozone effect. No relationships was found between binding affinity and AMVA, however a significant correlation was observed between the number of binding sites and the proportion of aggregates, highlighting improved aggregation capacity of dimeric mAbs ($P=0.0108$). Further, the data demonstrated that AMVA was dependent on epitope accessibility with a classical prozone effect.

**Conclusions:** Overall, our findings indicate that antibody isotype influences effector functions, with greater capacity of IgGs to capture HIV-1 particles and Env specific dIgAs to induce viral aggregation. Thus, the ability of an antibody-based vaccine to prevent HIV-1 infection may be dependent on the isotype of the antibody, and the effector functions most relevant to the biological compartment.

**OA24.06 LB**
**Transcriptional Signatures of Viral Control in HIV-1 Infected South African Women**

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**Background:** The characterization of host genetic factors that allow small subsets of individuals to naturally suppress HIV-1 viral replication in the absence of antiretroviral treatment remains a priority, as an understanding of the immune mechanisms employed by these individuals to control viral replication could provide insights into potential therapeutic targets.

**Methods:** Therefore, in order to identify genes involved in the control of HIV-1 viral replication during chronic infection, mRNA was extracted from peripheral blood mononuclear cells isolated from 14 Black female South African HIV-1 controllers. Paired-end RNA sequencing of 100 bp fragments was performed using the HiSeq 2000 and the data obtained were processed using the GRAPE analysis pipeline. Differences in gene expression were evaluated using the R/Bioconductor package, DESeq; with genes exhibiting at least a 2-fold difference in expression and p-values of $p < 0.001$ considered to be differentially expressed.

**Results:** Genes found to be differentially expressed between the eight individuals with viral loads below 400 copies/ml included several previously implicated in the control of chronic viral infections. These include LAG3, a regulator of T cell responsiveness, and genes involved in the regulation of the interferon type I antiviral response (for example IFI6, IFIT3, IFI44 and OASL). Several of the identified genes also encoded as yet functionally uncharacterized large intergenic non-coding RNAs, whose impact on HIV-1 viral control remains to be evaluated.

**Conclusions:** Collectively, these data describe a transcriptional signature of immune activation in chronic HIV-1 infection, which suggests the involvement of innate immune mechanisms previously shown to display substantial sex-based differences. However, whether these mechanisms directly regulate HIV-1 viral replication, or rather are activated in response to increased viral loads, remains to be established.

**Adjuvants and Immunogens**

**OA25.01**
**Adjuvant Dependent Mucosal V2 Responses and RAS Activation in Vaccine Induced Protection from SIVmac251 Acquisition**


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