Investigating the effects of physical maturation on CD4 T cell subsets and epithelial junction proteins in foreskin tissue

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Introduction: Despite global reductions in HIV, incidence in adolescents continues to increase. While risk-taking behavior may contribute to heightened incidence, the effect of physical maturation, which may alter the genital microenvironment and lead to inflammation, and susceptibility to HIV. Therefore, we assessed the effect of physical maturation on HIV risk factors including CD4 T cells and epithelial junction proteins in a male adolescent cohort.

Methods: We enrolled n=200 uncircumcised adolescent males aged 15-17 with no history of sexual experience and at different stages of physical maturation, from the Rakai district of Uganda. Over three years of follow-up, we collected serum testosterone (as a measure of physical maturation), and questionnaire data every 3 months. During this time, 77 adolescents elected to undergo Voluntary Medical Male Circumcision (VMMC). Discarded foreskin tissues were collected during VMMC for quantification of HIV target cells (flow cytometry), and epithelial junction protein expression (immunofluorescence microscopy).

Results: 68 participants (88%) remained sexually naïve at the time of VMMC. In this group (containing participants at various stages of physical maturation), we observed no significant associations between serum testosterone and the proportion of T cell subsets (Th1, Th2, Th17, Th22), HIV co-receptor expression, or T cell activation (HLA-DR+/CD25+). Additionally, no significant associations were found between serum testosterone and expression of epithelial junction proteins (claudin-1 and E-cadherin) that play a critical role in foreskin barrier integrity.

Significance: Physical maturation is not associated with changes in the proportional abundance of T cell subsets or epithelial junction protein expression in foreskin tissue. Future research will use immunofluorescent microscopy to examine changes in the thickness of the epithelium, as well as the density of other immune cell types (dendritic cells, macrophages) relevant to HIV infection. Studying how physical maturation affects foreskin tissue can improve our grasp of HIV transmission in high-risk groups.

Development of an *Ex Vivo* Dual Virus Challenge Assay for Cervicovaginal Tissues to Assess HIV-1 Antibody-mediated Protection

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Blocking HIV transmission at mucosal portals of entry is critical for HIV prevention strategies. *Ex vivo* HIV challenge of cervicovaginal tissue biopsies from broadly neutralizing monoclonal antibody (mAb) recipients provides a unique opportunity to assess the antiviral function of the mAb(s) at a key mucosal transmission site. Ideally, multiple HIV strains would be tested, especially when evaluating mAb combinations. However, the few cervical and vaginal biopsies that can be collected per visit (often allocated to multiple endpoints) presents a logistical challenge. To address this, we piloted a novel dual virus challenge assay using secreted nanoluciferase (snLuc) reporter activity combined with droplet digital PCR (ddPCR). This approach allows simultaneous monitoring of HIV-1 replication and identification of replicating viruses. We utilized snLuc.HIV-$1_{1086.B2}$ and snLuc.HIV-$1_{Du422.1}$, clade C Tier 2 Env-chimeric infectious molecular clones, to discriminate between CD4 binding site and V3 glycan broadly neutralizing mAbs (e.g., 3BNC117 and 10-1074). We designed a single primer set to amplify both *env* ectodomains and specific probes to discriminate between DU422.1 and 1086.B2, ensuring specific detection of each *env* simultaneously without evidence of competition. For proof of concept, we conducted single and dual virus challenge of human vaginal explants, with and without pre-treatment with 3BNC117LS and 10-1074LS. Monitoring HIV-1 replication by snLuc activity over time, coupled with ddPCR, enabled measurement of overall HIV-1 replication and identification of virus(es) that established infection. Moreover, the ddPCR infection readout (copies/µL cDNA) for snLuc.HIV-$1_{Du422.1}$ and snLuc.HIV-$1_{1086.B2}$ correlated well with snLuc activity (RLU) at 3 weeks post-challenge ($r=0.75$, $p<0.0001$ and $r=0.67$, $p<0.001$, respectively). Future work will focus on optimizing additional virus challenge pairings, including placebo breakthrough viruses from the Antibody Mediated Prevention trials. In conclusion, our novel approach to *ex vivo* HIV challenge holds promise for facilitating more in-depth, functional assessments of HIV-1 antibodies in mucosal tissues within clinical trial contexts.

Immunoglobulin A Fc receptor (CD89) expressed by natural killer cells may restrict innate signaling and cytotoxicity within the mucosal interface

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Natural killer (NK) cells are an innate lymphoid population equipped with cytotoxic and inflammatory signaling potential that are critical for controlling viral infections. NK cells are tightly regulated by a complex balance of surface receptors including natural killer cytotoxicity receptors (NCRs), killer-cell immunoglobulin-like receptors (KIRs), and NKG2 family receptors such as inhibitory NKG2A and activating NKG2C. NK cells can also recognize immunoglobulin G (IgG) through FcRγ, or CD16, to perform antibody dependent cellular cytotoxicity (ADCC) on opsonized cells. However, in mucosal sites such as the gastrointestinal and oral mucosae, which serve as common portals of entry and replication for chronic viral infections, IgA is the predominant class of immunoglobulins. We reveal a rare subset of NK cells in the periphery but enriched in the mucosae that express the Fc receptor for IgA, or CD89, which is classically thought to be restricted to monocytes and granulocytes. We hypothesized that engagement of CD89 acts as a checkpoint regulator for NK cells in humans and macaques enriched at the mucosal interface. Indeed, CD89+ NK cells express a surface receptor profile which restrains NK cell activation and exhibit dampened Fc receptor signaling pathways distinctly unique from that of the canonical CD16 signaling cascade. Mechanistically, CD89 ligation alone restricts NK cytotoxicity, but can cooperatively enhance CD16-mediated antibody functions in an IgA subclass-dependent fashion. To this effect, CD89+ NK cells engaged in ADCC in the presence of IgA and IgG produce increased CD107a, MIP-1β, and IFNγ as compared to IgA or IgG alone. Given the tight regulation of NK cell responses exerted at the CD89-IgA axis, therapeutic targeting of CD89-expressing NK cells could provide a unique strategy to regulate innate cellular functions in the HIV-infected mucosae.

Bacteria Associated with HIV Seroconversion and Immune Cells Alter Epithelial Junctions and Increase Keratinocyte Proliferation

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Background: Specific taxa of anaerobic bacteria on the penis are associated with local inflammation and HIV acquisition risk in heterosexual males. Anaerobe-driven inflammation may increase HIV susceptibility by disrupting foreskin epithelial barrier function. We assessed whether penile anaerobe abundance is associated with disrupted epithelial integrity and explore possible mechanisms by which this may occur.

Methods: HIV-negative uncircumcised adult men were enrolled in a clinical trial in Entebbe, Uganda (n=125), and randomized to either immediate circumcision (controls) or to defer circumcision for 4 weeks and receive one of four existing antimicrobial treatments. Immunofluorescence was performed on the excised foreskin tissues for epithelial integrity analyses including epithelial thickness, keratin thickness, expression of epithelial junction proteins, and epithelial cell proliferation. Penile swabs collected immediately prior to circumcision were analyzed for (a) microbiota composition (16s rRNA gene sequencing and qPCR) and (b) soluble E-cadherin (ELISA). Epithelial integrity was similarly quantified from our lab's *in vitro* organotypic skin model after culturing tissues with HIV-associated penile anaerobes.

Results: Men with a high abundance of penile anaerobes had lower inner foreskin E-cadherin and claudin-1 expression and higher levels of soluble E-cadherin than men without these bacteria. Similar results were seen *in vitro* where tissues cultured with anaerobes had higher soluble E-cadherin levels on the tissue surface compared to control wells. Men with high anaerobe levels also had higher expression of desmoglein-1 and a significantly thicker epithelium driven by excess keratinocyte proliferation.

Discussion: These data suggest that a high abundance of specific penile anaerobes leads to alterations in the epithelial barrier by (1) altered expression of epithelial junction proteins and (2) excess keratinocyte proliferation. A compromised epithelium may leave individuals more susceptible to HIV and other pathogens. Ongoing research focuses on elucidating these mechanisms and identifying the roles of bacterial virulence factors and immune cells in these processes.

Tittle: SIV infection and ARV usage Induce Minimal Change in the Gut Microbiome of Non-Human Primates

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Abstract: The gastrointestinal (GI) system is dependent upon interactions with commensal microbiota and molecules they produce. Hence, perturbations in commensal GI bacteria and eukaryotes during heterologous infection may contribute to observed disease pathologies. Observations of the GI tract microbiome of people living with HIV (PLWH) have shown indications of dysbiosis – an outgrowth of disease-associated taxa relative to commensal taxa. Similar studies in the Simian Immunodeficiency Virus (SIV) non-Human primate (NHP) model of HIV infection, however, have yielded inconsistent results, perhaps the result of cross-sectional assessments. In this study, 16S and internal transcribed spacer (ITS) Illumina sequencing were used to assess the composition of NHP stool microbiota longitudinally, in response to SIV infection and the subsequent initiation of antiretrovirals (ARV). A comparison of the microbial composition before and during chronic infection revealed minimal differences in phylum abundance. We saw that Bacillota, Bacteriodota, Pseudomonadota and Spirochaetota bacterial phyla were moderately increased after infection. These bacterial taxa, however, diminished after ARVs but remained elevated as compared to pre infection. We further saw that the Ascomycota eukaryotic phylum was increased after infection and was not furthered influenced by ARVs. Importantly, the composition of the GI tract microbiome showed no correlation with CD4 T cell counts or viral load. These data suggest that SIV infection and ARV administration have a minimal impact on bacterial and eukaryotic relative frequencies in the NHP gut. Longitudinal assessments of the commensal metagenome are needed to determine whether commensal taxa contribute to immune dysfunction or reconstitution deficiencies in treated and untreated SIV infection. Further these data suggest the dysbiosis observed in PLWH could be attributed to confounding variables in cross-sectional studies of outbred humans.

Organotypic foreskin-microbiome co-culture recapitulates penile microbiome composition *in vitro*

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Background

Within the diverse penile microbiome, specific strict anaerobes are associated with HIV seroconversion, increased pro-inflammatory cytokines, and increased local HIV target cell density. However, observational data cannot attribute causality to these bacteria in increasing foreskin inflammation and HIV risk. To study these interactions empirically, we have developed *in vitro* foreskin mimetic tissues for co-culture with bacteria, including polymicrobial communities representative of the penile microbiome.

Objective

Compare the composition of complex penile microbial communities exposed to variable oxygen conditions (2% vs 20%) when cultured *in vitro* on organotypic foreskin tissue or enriched agar plates.

Methods

Primary foreskin fibroblasts and keratinocytes were used to generate foreskin mimetics with dermal and stratified epidermal layers. Tissues and Columbia blood agar plates were inoculated with a cryopreserved polymicrobial community derived from coronal sulcus swab eluent. Inoculated tissues and plates were incubated at 37°C in either 2% or 20% O_2 for 8 days. Bacteria were collected from previously undisturbed tissues and plates throughout co-culture and community composition was determined by 16S rRNA gene sequencing (V4).

Results

Communities recovered from organotypic tissue, under both 20% and 2% oxygen conditions, exhibited increased *Corynebacterium* relative abundance and maintained strict anaerobes observed in the inoculum (e.g., *Finegoldia*, *Mobiluncus*, *Peptoniphilus*). Conversely, communities from Columbia plates showed elevated *Staphylococcus* (2% and 20% O_2) and *Proteus* relative abundance (2% O_2 only) and greatly decreased strict anaerobe relative abundance (in both 2% and 20% O2 conditions).

Conclusions

In vitro, the penile microbiome's diverse composition is better preserved when cultured on organotypic foreskin tissue compared to enriched agar, regardless of oxygen conditions. Organotypic foreskin-microbiome co-culture provides an ideal environment for studying dynamics both within the penile microbiome and between the microbiome and foreskin tissue. We will use this approach to further investigate the influence of the penile microbiome on HIV susceptibility.

Title

Impact of Penile-Vaginal Sex on the Microbiome and Immunology of the Penile Urethra

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Abstract

Background: The penile urethra is a key HIV acquisition site in heterosexual men. In the penile coronal sulcus and the vagina, inflammation and microbiome composition play an important role in HIV exposure outcome, and both are affected substantially by penile-vaginal sex. However, the direct impact of penile-vaginal sex on these factors in the penile urethra is not known.

Methods: The Sex, Couples and Science (SECS) study was designed to examine the short-term impact of penile-vaginal sex on the genital immunology and microbiome of established couples. First-void urine was collected to characterise the penile urethra microbiome (through 16S sequencing and qPCR) and immune milieu (through a multiplex chemiluminescent immunoassay) before and after sex (immediately, 1, 7 and 72 hours after).

Results: Penile-vaginal sex induced immediate urethral inflammation that resolved within 1 hour. The urethra was also transiently enriched for the common vaginal species *Lactobacillus crispatus* and *jensenii* for up to 7 hours. Interestingly, species that are common in the vagina and that may cause vaginal inflammation and increase HIV risk – namely *L. iners* and *Gardnerella vaginalis* – were already prevalent in the urethra prior to sex, and remained unaltered. The urethral immune changes seen did not reflect cytokine transfer from vaginal secretions or semen, and were not linked to changes in the microbiome.

Conclusions: Penile-vaginal sex caused transient urethral inflammation and extensive microbiome transfer from the vagina into the urethra, with potential implications for penile HIV susceptibility. However, "non-optimal" vaginal bacteria associated with higher HIV risk in women were already present in the urethra, reflecting long term colonization and suggesting that the urethra is a reservoir for non-optimal vaginal bacteria.

Host factors in late stages of the HIV-1 life cycle

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Pr55Gag, the major structural protein of HIV-1, is translated on free cytoplasmic ribosomes and then traffics to the specific microdomains on the plasma membrane (PM) to initiate particle assembly and budding. There is growing evidence showing that host factors and pathways are involved in the transport of Gag to the assembly sites through direct or indirect interactions. Recently, ADP-ribosylation factor (Arf) proteins, including Arf1 and Arf6 proteins, were documented to function as cofactors of HIV-1 assembly and release. It is well known that Arf proteins and their regulators modulate membrane trafficking and actin remodeling along the secretory and endocytic pathways through a regulated cycle of GTP binding and hydrolysis. However, the mechanisms of action of Arf proteins on HIV-1 Gag subcellular trafficking are still not fully understood. Here, we examined the roles for Arf1 and Arf6 proteins in late states of the HIV-1 life cycle. Our results revealed that disruption of Arf1 function through a dominant-negative mutant or a constitutively active mutant of Arf1 reduced the binding of HIV-1 Gag to membranes, and inhibited HIV-1 release. Using an Arf1 GTPase activating protein and its catalytically inactive mutant, we found that Arf1 protein is involved in the targeting of HIV-1 Gag to the PM. In addition, we illustrated that dominant inhibition of Arf6 function significantly inhibited HIV-1 release by redistributing HIV-1 Gag from the PM to cellular membranes. Given the role of BST-2 post-endocytic trafficking events in its antiviral activity, we also examined the role of Arf proteins in BST-2-mediated restriction. We revealed that the disabled function of Arf1 or Arf6 proteins did not alter BST-2 subcellular distribution pattern as well as BST-2 expression levels on the cell surface. These results suggest that the modulation of HIV-1 release by Arf1 and Arf6 proteins is independent of BST-2-mediated restriction.

Mucosal effects of tenofovir 1% gel wane over longer-term use but retain pro-proliferative and type I/III interferon pathway signatures

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Background: Tenofovir 1% gel has been tested for vaginal and rectal pre-exposure prophylaxis (PrEP) against HIV transmission. Previously, we showed that after 7 days of administration, tenofovir gel had broad effects on gene expression in the rectum. Elsewhere, we also showed that 2 months of oral Truvada PrEP induces type I/III interferon-related genes in the gut. Here, we extend our results to the longer-term use of tenofovir gel in the vagina and rectum.

Methods:

We isolated mRNA from biopsies obtained before and after daily topical tenofovir gel application for 14 (MTN-014 trial) or 56 days (MTN-017), and quantified gene expression using microarrays and RNAseq as described previously (eLife 2015, CellRepMed 2020). We then compared these new data to our previous data in the rectum after 7 days of daily rectal tenofovir gel (MTN-007) and after 56 days of daily oral Truvada (oral arm, MTN-017). In MTN-014, we also compared the cross-compartmental effect of topical tenofovir (e.g., vaginal changes after rectal application).

Results:

We show that the broad-ranging changes in gene expression seen after 7 days diminish after 14 and 56 days, but that some changes, especially increased cell proliferation, remain. Most notably, we found that the induction of type I/III interferon-related genes seen with oral use also occurs with topical use at 7, 14, and 56 days. We also uncovered some unique changes in the vagina after vaginal gel application. The cross-compartmental studies showed that tenofovir has a smaller effect on rectal gene expression when applied vaginally than when applied rectally, but has an equal effect on vaginal gene expression regardless of site of application.

Conclusions:

Across these tenofovir-based HIV pre-exposure prophylaxis trials, induction of type I/III interferon-related genes is the most consistent and persistent mucosal response, occurring after both oral and topical use and at all tested time points.

Title:

A key *Lactobacillus* metabolite reduces HIV internalisation and migration through the cervicovaginal epithelial barrier

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Abstract body:

Introduction: Young women in sub-Saharan Africa are disproportionately affected by HIV. A *Lactobacillus*-dominated cervicovaginal microbiome reduces the risk of HIV acquisition by decreasing genital inflammation, which disrupts the female reproductive tract (FRT) epithelial barrier and enables the virus to infect target cells in the submucosa. We have discovered that lactic acid (LA), a key metabolite of optimal *Lactobacillus* spp. strengthens the cervicovaginal epithelial barrier. However, LA's ability to inhibit passage of cell-free virus in between epithelial cells (transmigration) or HIV uptake (internalisation) and transcellular migration through epithelial cells (transcytosis) via LFA/ICAM-1 interactions are unknown.

Methods: Immortalised ectocervical (Ect1) and vaginal (VK2) cell lines were cultured in a transwell system, treated apically for 1h with 0.3% LA (pH 3.9), lactate (pH 7.0), or acidified media (pH 3.9, HCl adjusted). At 24h post-treatment, cells were thoroughly washed, and HIV ($HIV_{Ba\text{-}L}$, 10ng p24) was added apically for 24h, after which p24 was quantified in basolateral supernatant and cell lysates. Antibodies against ICAM-1 and LFA-1 were added prior to HIV addition to block binding.

Results: LA treatment (pH 3.9), but not HCl or lactate (neutral pH) reduced HIV migration to the basolateral supernatant by 72±5.8% in Ect1 (mean ± SEM) and 89±6.8% in VK2 cells relative to untreated cells and reduced internalised virus in cell lysates by 49±7.3% in Ect1 and 67±10% in VK2 cells ($p<0.05$, n=5-11). This reduction was abrogated in the presence of antibodies to ICAM-1 and LFA-1. LA treatment reduced ICAM-1 expression in the presence of inflammatory mediators, indicating a potential protective mechanism for LA against HIV.

Conclusions: This study is the first to demonstrate a direct effect of LA on HIV migration through epithelial cells and provides novel insights into its potential mechanism of action. These findings have implications for developing novel strategies to prevent HIV transmission in women.

Title:

Harnessing vaginal microbiota metabolites for HIV prevention: elucidating the mechanisms of lactic acid signalling at the cervicovaginal epithelial barrier

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Abstract body:

Introduction: An optimal *Lactobacillus*-dominated vaginal microbiota is associated with decreased HIV risk in women. Lactic acid (LA) is a major metabolite produced by optimal *Lactobacillus* spp. that acidifies the lower female reproductive tract (FRT) to pH<4.5. LA has protective properties, as it enhances FRT epithelial barrier function and tight junction (TJ) expression, predicted to reduce HIV mucosal transmission. However, the signalling pathways by which LA elicits strengthening of the FRT epithelial barrier are unknown.

Methods: Ectocervical epithelial cells (Ect1) were untreated, treated with LA (pH 3.9 or 7) or media acidified with HCl (pH 3.9) for 20 min prior to analysis of the abundance and phosphorylation status of over 2000 kinases and kinase substrates using a commercial antibody microarray. In parallel, transcription factor binding site (TFBS) enrichment analysis was performed on differentially expressed genes (DEGs) from Ect1 cells treated with LA for 1h.

Results: LA treatment (pH 3.9) activated the MAPK/ERK signalling pathway and suppressed AKT signalling, which are collectively predicted to enhance barrier function. LA upregulated proteins associated with cell adhesion and actin cytoskeleton stabilisation such as PFN1, MAPT and TGM2 vs. untreated cells ($p<0.05$). TFBS, associated with TJ expression, were enriched by LA treatment, including Jun and CDX1.

Conclusions: LA produced by vaginal *Lactobacillus* spp.- modulates core signalling pathways in a pH-dependent manner, including MAPK/ERK and AKT pathways, potentially resulting in a strengthening of the cervicovaginal epithelial barrier. Identification of novel therapeutic targets for enhancing epithelial barrier protection may improve future HIV prevention strategies.

Resolution of CD89+ leukocyte subcompartment localization in the intestinal mucosa by imaging cytometry

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IgA is the most abundant Ig in the body and the predominant Ig class in the mucosae. The main Fc receptor for IgA, CD89, has classically been thought restricted to myeloid cells, granulocytes, and some epithelial cell subsets. In our study we have characterized multiple immune cell subsets, including monocytes, natural killer (NK) cells, and granulocytes, all that can express CD89 and their localization within the gastrointestinal mucosae. Multiplex Chip Cytometry technology allows for high dimensional imaging by utilizing repeated cycles of staining with antibody conjugated fluorophores, image acquisition, and photobleaching to quench stained fluorophores. Imaging analysis reveals the distribution and localization of CD89 expressing cells within the gastrointestinal mucosae. We present data comparing CD89+ and CD89- phenotypic, signaling, and activation/inhibitory receptor profiles of circulating and tissue resident monocytes, NK cells, and granulocytes. For example, we find that circulating CD89+ monocytes are predominantly CD16- classical monocytes, which are known for their potent phagocytic activity, pro-inflammatory cytokine production, and antibody mediated cellular cytotoxicity. Additionally, we find that CD89+ NK cells express significantly lower levels of traditional NK cell receptors, such as natural cytotoxicity receptors (NCRs), killer-cell immunoglobulin-like receptors (KIRs), and NKG2 family receptors, indicating that CD89+ NK cells may be fine-tuned for responses to IgA instead of classical NK cell activation. We find a significantly enhanced abundance of CD89+ NK cells at mucosal sites – particularly within the colon and mesenteric lymph nodes. While CD89+ and CD89- myeloid and granulocytic cells were found throughout the mucosa and lamina propria, CD89+ NK cells were particularly concentrated in Peyer's patches or other lymphoid aggregates. These data suggest that CD89 is expressed on a variety of mucosal leukocytes but that they are highly compartmentalized and these cellular distributions could be considered and exploited for immunotherapeutic interventions targeting diseases impacting the mucosae such as HIV.

Title: Microbe-binding antibodies in the female genital tract: associations with the vaginal microbiome and genital immune correlates of HIV acquisition.

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Background: Mucosal antibodies in the gut maintain homeostasis between the host and the local microbiome through the clearance of pathogenic bacteria and the development of immune tolerance to inflammatory bacteria. Whether similar bacteria-immunoglobulin interactions modulate cervicovaginal inflammation and/or bacterial colonization in the female genital tract (FGT) is not well understood. A flow cytometry-based assay was used to quantify microbe-binding antibodies (IgA and IgG) from cervicovaginal secretions in a cross-sectional cohort of 200 HIV-uninfected women from Nairobi, Kenya that was enriched for bacterial vaginosis (BV) and evaluated the associations of cervicovaginal IgA and IgG with the vaginal microbiome composition and local soluble immune factors.
Results: Total IgA and IgG were abundant in cervicovaginal secretions and demonstrated frequent and occasionally heterogenous *ex vivo* binding to key vaginal bacteria species *Gardnerella vaginalis, Prevotella bivia, Lactobacillus iners,* and *Lactobacillus crispatus*. Microbe-binding IgA and IgG were not generally associated with the detectability of the corresponding vaginal bacteria. Total bacterial abundance in the vagina was higher in women with BV and was inversely correlated with both total and microbe-binding IgA and IgG. Partial correlations demonstrated that both total and microbe-binding IgA and IgG were positively correlated with the vaginal concentration of multiple inflammatory cytokines (IL-6, TNF) and chemokines (IP-10, MIG, MIP-1α, MIP-1β, MIP-3α, MCP-1, IL-8), independent of total bacterial abundance.
Conclusions: Flow cytometry-based quantification of microbe-binding antibodies provides a platform to investigate host-microbiota interactions in the FGT. Both total and microbe-binding IgA and IgG inversely correlate with bacterial abundance and positively correlate with several cytokines and chemokines previously linked to HIV acquisition. This assay provides a method to investigate the interactions between the microbiota, inflammation and cervicovaginal antibodies in human observational and interventional studies.

Title: Spatial transcriptomic evaluation of the sites of SIV infection and rebound in NHP models reveals transcriptional patterns of host responses.

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BACKGROUND
Despite effective ART, HIV-1 persistence is the major obstacle to cure. Here, we used 10X-Visium spatial transcriptomics to understand the tissue microenvironment harboring viral reservoirs involved in viral persistence.

METHODS
Rhesus macaques (RMs) were intra-vaginally/intra-rectally challenged with SIVmac239, ART initiated at 4 days and maintained for 6 months, followed by PET/CT-guided necropsy either during ART (3-7 weeks) or after ATI (early-ATI: 4-10 days; late-ATI: 18-24 days post-ATI). ^{64}Copper-labelled probe against viral envelope efficiently detected infection sites as early as 4-days post-ATI. In parallel, we optimized a pipeline for immunoPET/CT guided bulk RNASeq and 10X-Visium spatial transcriptomics to characterize the transcriptomic environment of PET/CT+ “hot” areas of the reservoir vs non-reservoir areas in tissues.

RESULTS
RNA-seq analysis indicates activation of viral responses and cell migration in late-ATI tissues. Instead, on ART tissues catabolic processes and drug metabolism are upregulated, indicating ART effect on the tissues. ImmunoPET/CT-guided spatial transcriptomics of late-ATI tissues with detectable SIV allowed us to identify foci of viral rebound associated to differential transcriptional patterns compared to non-reservoir areas. Here, we detect the activation of genes associated to metabolic processes, cell localization, and cytokine immune signaling. The combination of both analyses indicates that active SIV rebounding foci might be characterized by immune cell migration and cytokine signaling.

CONCLUSION
Our newly developed techniques combining an immunoPET/CT-guided system with genomics and spatial transcriptomics allow us to identify with unprecedented detail possible markers of tissue reservoirs involved in maintenance and recovery of the SIV reservoir.

Genital type I interferon expression and the risk of HIV infection in women

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Interferons (IFNs) are critical mediators of inflammation and anti-viral immunity, yet their roles in HIV acquisition in the female genital tract are unclear. Here we used multiplex ELISA to estimate the effects of cervicovaginal expression of type I, II, and III IFNs prior to HIV infection on HIV acquisition risk in the CAPRISA-004 cohort and explored potential mechanisms that may explain these observations.

In cohort analyses (n=774), genital IFNa2 was one of the strongest predictors of HIV acquisition risk (aHR 3.3/log10 cytokine, 95% CI: 1.3 to 9.9). Genital IFNg showed a similar trend (aHR 2.0/log10 cytokine, 95% CI: 1.0 to 3.8). In contrast, in case-control analyses (n=90), detection of genital IFNb was associated with a reduced risk of HIV acquisition (aOR 0.23, 95% CI: 0.07 to 0.84). No associations were observed for genital type III IFNs and HIV acquisition. Genital IFNa2 clustered with pro-inflammatory cytokines and was strongly associated with TNFa, IP10, IL-8, CTACK, IL-12p40, IL-1a, MCP3, MIG, and IFNg ($p<0.001$). In contrast, genital IFNb showed weak inverse associations with chemokines including MIP1b, MCP1, CTACK, and MCP3. In a separate cohort, genital IFNa2 concentrations correlated with BV, STIs, activated CCR6/CCR5+ cells; similar trends were not observed for IFNb. Using RNAseq, we confirmed the inflammatory effect of IFNa2 in stimulated CD4+ and CD14+ immune cells sorted from the endocervix and blood. We further modeled IFNb and IFNa2 effects *in vitro* in PBMCs with or without prior exposure to inflammatory stimuli (LPS). In contrast to IFNa, IFNb upregulated ISGs even in the presence of inflammation.

Genital IFNa2 and IFNb appear to play divergent roles in predicting HIV acquisition risk in women. We hypothesize that while sustained IFNa2 signaling coincides with other pro-inflammatory pathways and HIV target cell induction, IFNb may regulate IFNa2 signaling in an anti-inflammatory fashion by competing for IFNAR binding.

CD8α+ cells suppress SIV replication without selecting for viral immune escape variants within MHC-restricted CD8+ T cell epitopes in an MCM model of PTC

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Post-treatment control of HIV is a rare phenomenon, but it provides hope for a functional cure. However, without detectable viremia, little is known about the sequences of viral populations residing within post-treatment controllers (PTCs) and whether antiviral immune responses suppressing virus replication are also selecting for new variants. To evaluate this, we sequenced plasma viruses of seven SIVmac239M-infected Mauritian cynomolgus macaques (MCM) who exhibited varying degrees of post-treatment control. All MCM received ART for eight months beginning two weeks post-infection (wpi), were rechallenged with SIVmac239 six months after ART interruption, and were depleted of CD8α+ cells two months following rechallenge. Animals were grouped as viremic (n=4) or aviremic (n=3) based on the presence or absence of detectable plasma virus between ART interruption and CD8α+ cell depletion, respectively. We found that animals that began ART early (2 wpi) after infection developed mutations within MHC-I restricted CD8+ T cell epitopes during transient viremia after stopping ART and that CD8α+ cell-mediated control of viral replication could be lost and regained during periods of PTC. We also found that all early ART animals were susceptible to rechallenge with a homologous SIV, as determined by sequencing the molecular barcode region present in plasma viruses, indicating that CD8α+ cell-mediated suppression of viremia did not protect against isogenic rechallenge. In a second cohort of SIV+ animals with delayed ART initiation (8 wpi), immune escape variants within CD8+ T cell epitopes accumulated prior to ART initiation and continued to diversify during ART treatment. Cumulatively, this study further supports the importance of early ART initiation in limiting variant accumulation which may help CD8+ cells suppress SIV replication after ART. These results may help our understanding of the virological requirements to establish post-treatment control.

Biodistribution of PGDM1400 following intravenous administration of mRNA encapsulated nanoparticle in AGM model

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The Antibody Mediated Prevention studies demonstrated that infused broadly neutralizing antibodies (bNAbs) can prevent HIV-1 acquisition. In addition to great breadth and potency, bNAbs need to persist in the body to ensure efficacious prevention of HIV. There is urgent need to answer critical questions regarding bNAb biodistribution and persistence at mucosal sites of HIV-1 exposure. Unfortunately the high demand for non-human primate models used for preclinical HIV studies has resulted in prohibitive costs hampering such research. Here we present an alternative affordable NHP, the African Green Monkey (AGM) as a model for biodistribution studies that inform HIV prophylaxis immunogen design.

The bNAb PGDM1400 mRNA was generated by in vitro transcription assays using sequences donated by IAVI Neutralizing Antibody Center and encapsulated in lipid nanoparticles (SM-102, 1,2-DSCP, cholesterol, and DMG-PEG(2000)) at the Hope laboratory, Northwestern University, Chicago. Four female AGMs were injected intravenously with 1mg of the LNP-mRNA PGDM1400 at the Institute of Primate Research, Nairobi, Kenya. Tissue, blood, cervical and rectal secretions were sampled on days 0, 3, and 7 before sacrifice on day 10. Cryopreserved tissues were immunofluorescently stained and imaged using a deconvolution microscope at KAVI-ICR, Nairobi, Kenya. Quantification of the endogenously expressed PGDM1400 in the blood was done using ELISA. Preliminary findings show that PGDM1400 reaches peak levels in the blood on day 7. We also demonstrate that PGDM1400 reaches the vaginal mucosa by days 7 and continues to saturate through to day 10.

These findings indicate that the AGM is a viable model for HIV prophylactic immunogen biodistribution and pharmacokinetics research. This study has important implications in HIV preclinical research as it presents an affordable alternative NHP model.

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Title: Advancing HIV Vaccine Discoveries through Mucosal Immunology: Integrative Approaches for Enhanced Protection

Background: Women in sub-Saharan Africa face disproportionate risks of HIV infection due to sociological, behavioural, and biological factors, including the unique anatomical and immunological characteristics of the female reproductive tract. This underscores the necessity for targeted HIV prevention strategies. Understanding these dynamics is crucial for developing targeted HIV prevention strategies that can significantly reduce infection rates among women in this region.

Objectives: The initiative aims to elucidate mucosal changes, HIV target cells, and hormonal influences on immune responses, integrating microbiome analysis and advanced imaging for comprehensive insights into mucosal immunology.

Methods: Employing innovative mucosal sampling tools (e.g., OriCol device, Merocel sponge) and preclinical models, the programme leverages cutting-edge technologies, including scRNA-seq and microbiome analysis, focusing on *Prevotella bivia* and its implications on mucosal immunity.

Results: Advanced imaging and microbiome studies have provided critical insights into the mucosal environment, identifying specific challenges in vaccine development. The research highlights the intricate dynamics of the epithelial barrier, mucus function, and initial target cells for HIV infection, laying the groundwork for novel mucosal vaccine strategies.

Discussion: The integration of comprehensive mucosal analyses elucidates the complex interplay between the microbiome, mucosal barriers, and immune responses, guiding the development of targeted HIV prevention strategies. This holistic approach addresses the multifaceted challenges of HIV vaccine research.

Conclusion: The Mucosal Programme 2023 represents a significant stride towards innovative HIV vaccine development. By combining detailed mucosal immunology research with advanced technological applications, the initiative paves the way for groundbreaking discoveries in HIV prevention, with a keen focus on enhancing mucosal protection for women in sub-Saharan Africa.

Assessing susceptibility of cervical explant tissue to HIV-1 infection following masculinizing hormone therapy.

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HIV-1 disproportionately affects minority groups, including transgender individuals, in the USA. Masculinizing hormonal therapy, which forms an integral aspect of gender-affirming care for transgender men (TGM), has immunomodulatory properties that may increase the risk of HIV acquisition. Testosterone activates both myeloid and lymphoid cells through androgen receptors (ARs), resulting in a shift in the cytokine milieu and a defective innate immune response. The cervical mucosae are enriched with HIV-1 target cells inclusive of macrophages and CD4+ T cells that co-express ARs. The extent to which this impacts HIV-1 vulnerability in TGM remains unclear.

In an ongoing study we investigate the impact of hormonal therapy on the HIV susceptibility of cervical immune cells in an *ex vivo* model of HIV infection using cervical tissue explants (CET). Cervical mucosae obtained from TGM and cisgender women undergoing medically indicated hysterectomies were sectioned into $2mm^3$ blocks. CETs were inoculated with subtype A and D transmitted founder (T/F) infectious molecular clones (IMCs) engineered to express secreted nano-luciferase (snLuc) and cultured on collagen rafts for 11 days. HIV infection kinetics were measured using snLuc. HIV infected cells were identified using intracellular p24 staining and were further characterized using a multi-parameter flow cytometry panel: CD45, CD3, CD4, CD8, CD45RA, CD45RO, CD14 and CD69, which we are extending to a comprehensive 25-parameter panel.

In preliminary findings, the Subtype D virus replicated with kinetics greater than observed for Subtype A in TGM blocks, similar to what we previously observed in cisfemale donor CETs. Infection kinetics in *cis* female CETs, were comparatively lesser for both Subtype D and Subtype A. Ecto-cervical CD45+CD3+CD4+ T cells and CD45+CD3-CD14+ macrophage-like cells were shown to permit *ex-vivo* HIV infection. Of note, over time the frequency of HIV-1 infected p24+ T cells only increased one fold, while p24+ “macrophages” increased 5-fold.

Enema use minimally impacts immunity and does not affect susceptibility to low dose intrarectal SIV.

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Men who have sex with men (MSM) are disproportionately affected by HIV. Increased susceptibility to HIV in MSM is attributed to high frequencies of susceptible CD4+ T-cells, GI barrier disruption, and dysbiosis of the intestinal microbiome. Although colorectal epithelial barrier damage is due, in part, to inflammation resulting from receptive anal intercourse, rectal douching is also a proposed contributor. To empirically assess the effects of douching on intra-rectal susceptibility, we administered enemas (Normosol-R) to rhesus macaques (Control/Enema, n=6/6) thrice-weekly, prior to repeated low-dose intra-rectal SIVmac239X challenge. Before and 28 days after enema treatment initiation (n=9 enemas) we assessed the fecal microbiome by 16S Illumina sequencing and surveyed intestinal and systemic immunity by flow cytometry and multiplex immunologic transcript quantification (NanoString). Fifty-six days after initiation (n=21) we repeatedly challenged animals with 4 TCID50 SIVmac239X. Susceptibility to SIV infection in our animals was assessed by time to infection and number of acquired transmitter-founder (T/F) variants. Prior to challenge, we observed that as compared to baseline, repeated enema administration was associated with reduced jejunal CD4+ T-cells and rectal memory CD4+ T-cells. Jejunal CD4+ T-cells exhibited reduced IL-22 and increased HLA-DR and IL-17 expression – observations previously associated with increased susceptibility to infection. Enema administration was associated with perturbations in the fecal microbiome, including increased *Prevotellaceae* - also previously associated with susceptibility. Despite these differences, few changes were observed in the intestinal immunologic transcriptome in either longitudinal or cross-sectional analyses. Of animals infected thus far (n=3/6 each group), there are no differences in the number of challenges that resulted in infection nor in the number of acquired T/F variants. Our analyses provide a detailed assessment of how the microbiome and intestinal immunity change in response to repeated enema usage. Insight gained from our study will inform the causes and consequences of repeated enema usage.

PET/CT guided 3D brain-mapping model to observe viral presence in reservoirs and rebound

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Despite the advancements in combined antiretroviral therapy (cART) for HIV-1 infection, viral reservoirs persist continuously under treatment, constituting the principal burden for an effective HIV-1 cure, since they lead to a rapid viremia rebound upon treatment failure or after analytic treatment interruption (ATI). Gaining insights into the characteristics and behavior of rebound phenomena will advance our quest to determine the reservoir associated with persistent and rapid rebound after ATI.

The PET/CT guided necropsy, is based on the use of a ^{64}Cu-labeled anti-SIV env FAB2 (^{64}Cu-7D3FAB2) guiding the necropsy process to identify and collect pieces of tissues containing foci of ^{64}Cu signal, revealing sites of SIV replication; however, this antibody does not cross the blood-brain barrier, so initially it was impossible to observe signals in the brain. A pilot study was performed injecting the probe intrathecally, then after 24h intravenously. PET/CT was performed pre- and post-necropsy (day 14) and the brain signal was observed.

The PET/CT-guided necropsy allows us to perform a 3D brain-mapping model to perform a detailed identification of the cells and tissues that support rebound of persistent SIV.
The brain was collected, fixed, and dissected into 1 cm^3 blocks for vRNA/DNA analysis. The brain now was collected and kept in OCT blocks. The presence of infected cells in these “hot” tissues has been validated by viral DNA sequencing and qPCR, as well as by immunofluorescence of GFAP (astrocytes), IBA-1 (microglia), and AG3 (infected cells).

Our project's innovative methods will unveil the dynamics of viral rebound post-ATI, crucial for HIV-1 cure strategies. Understanding this phase is vital due to the persistence of viral populations.

Inverse Correlation of NK cells and Viral Levels in Vaginal Tissue 48hrs After Intravaginal Challenge With SHIV-SF162P3 In Rhesus Macaques With Suboptimal PGT121 Delivery

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Background
Rhesus macaques(RM) that get an intravenous (IV) infusion of the broadly neutralizing antibody (bNAb) PGT121, 24hrs prior to intravaginal challenge with SHIV-SF162P3, are not protected from challenge 1-3 days later. We have shown that it takes 7 days to achieve full antibody occupancy in the vaginal epithelium following IV injection, therefore we set out to understand if timing of antibody injection could alter viral kinetics following challenge.

Methods
Utilizing Cy5-labeled PGT121 and sham DEN3, we compared -7 days(n=5) and -1 days(n=5) IV infusion prior to intra-vaginal challenge with SHIV-SF162P3 in RM and measured virus 48hrs later. Tissue and plasma levels of viral RNA and DNA were detected using gag qPCR and antibody levels were measured through Cy5 fluorescence. We used RNA-Seq to probe for transcriptomic differences. We used NKG2A to measure NK cell levels in vaginal tissue.

Results
We found less viral RNA and DNA present at the site of challenge 48hrs after challenge in the -7 day group(2/5 RM) compared to the -1 day group(5/5), which correlated with less PGT121 vaginal occupancy. There was an increase in response to virus genes in the -1 day group. In -7 day group, we found increased expression of PP14, a known regulator of NK cell responses. When measuring NK cells we found an inverse correlation with virus in vaginal tissue of the -1 day group.

Conclusions
We found that by changing timing of antibody infusion, from 1 day to 7 days, that there is a significant impact on viral kinetics following challenge. In our suboptimal animal cohort(-1 day), we found a variability of viral levels, however there was an inverse correlation with NK levels. These results have the potential to not only aid in our understanding of the host response to challenge, but can also help tease out variability in RM cohorts in challenge studies.

Single-cell transcriptomic analysis of rectal mucosal immune cells from cisgender and transgender women and men.

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A substantial body of literature has described sex-specific differences in immunity, including responses to HIV, partially attributable to both sex-chromosomes and sex-hormones. Studies generally focus on characterizing binary sex differences (cisgender women vs cisgender men) in circulating immune cells. Here, we sought to expand upon these findings by performing single-cell transcriptomic analyses with CITE-seq barcoding on 26,683 immune cells isolated from the rectal mucosa, a site of critical vulnerability to HIV. Additionally, cells were isolated from cisgender women and men, as well as transgender women and men undergoing gender-affirming hormone therapy, to explore the influence of both sex-chromosomes and sex-hormones on immune cell transcriptomes in this clinically significant tissue compartment in healthy participants.

Following batch correction (BatchLR), dimensionality reduction (KnetL) identified 30 transcriptionally unique clusters (iClust) of cells. As expected, X (e.g. XIST, EIF1AX, DDX3X) and Y (e.g. USP9Y, UTY, DDX3Y) genes were differentially expressed (fold-change >1.25, q-value <0.05) between cells isolated from XX versus XY individuals, as were a number of highly relevant immune genes (e.g. CTSW, XCL2). Interestingly, immune genes we hypothesized might escape X-chromosome inactivation (e.g. CD40L, CXCR3, BTK) were not identified, here. Furthermore, the potential influence of sex-hormones on differential gene expression was apparent within B cell subsets (IgA producing B cells and HLA-DR+ B cells), antigen presenting cells, and granulocytes, but was less clear within T cell and cytotoxic cell subsets. This is potentially due to differences in ESR1, ESR2, and GPER1 expression seen between immune cell clusters in the rectal mucosa, however transcription of these receptors was relatively low. This will need to be confirmed in future studies, as a more comprehensive understanding of the diverse effects of sex-hormones and sex-chromosomes on gut-residing immune cell transcriptomics could be critical for personalized HIV prevention and treatment strategies.

Assessing changes to target cell population densities in high progesterone states as a mechanism for increased HIV acquisition risk

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This work was supported by the Third Coast Center for AIDS Research [P30 AI117943]

Over 53% of new HIV infections occur in women, with heterosexual women displaying a 2.3 times higher HIV transmission efficiency, suggesting that there may be biological factors increasing women's risk of infection. One potential mechanism may be sex steroid hormones, which influence immune cell dynamics and tissue morphology, particularly in female reproductive tract (FRT) epithelium. Specifically, progesterone reduces the thickness of the lower FRT epithelium and increases the recruitment of CD4+ T cells and CD4+ CD68+ target cells to the ectocervical and vaginal epithelium, creating an environment that may be more susceptible to infection. In addition, Thomason *et al.* (2018) found that the risk of HIV acquisition was 3-fold higher during late pregnancy, a high-progesterone state, further supporting the association between elevated progesterone levels and HIV acquisition risk. Therefore, we are investigating the role that target cells, including mast cells (MC), T helper 17 cells (Th17 cells), and macrophages, play in HIV acquisition risk. We hypothesize that changes in target cell population densities in a high progesterone environment contribute to the increased risk of HIV acquisition seen in pregnant women.

To assess the differences in target cell density and localization in high and low progesterone environments, we obtained 10 ectocervical and vaginal tissues from first- and third-trimester women from the University of North Carolina at Chapel Hill and 11 ectocervical and vaginal control tissues from nonpregnant women from the National Disease Research Interchange. All tissues were embedded in OCT and sectioned at 12μm. We stained each tissue with MC markers CD117, tryptase, and FCεR1, Th17 markers CD3, CD4, and CCR6, or the macrophage marker CD68. We then imaged the slides on a deconvolution microscope at 40x. We took at least ten stitched panel images for each donor and tissue and analyzed cell population identity, frequency, and location using SoftWorX software.

Although studies are still ongoing, current analysis of Th17 samples show an overall trend in which the cell densities increase during the first trimester and level out by the third trimester, while macrophage samples trend towards a steady increase in CD68+ cell density as the pregnancy progresses. Moreover, in MCs the single-positive cell populations showed that first- and third-trimester pregnant tissues had a statistically significant increase in tryptase-positive cells compared to non-pregnant tissues in the cervical and vaginal epithelium. In vaginal tissue, the first-trimester tissues had a greater density of tryptase-positive cells than the third-trimester tissues. For the remaining single-, double-, and triple-positive cell populations, there were no statistically significant data, except in FCεR1-only and FCεR1/CD117 populations, which showed an increase in the control groups for the cervical and vaginal tissues, respectively.

These data are an initial step towards identifying the mechanism(s) associated with increased HIV acquisition risk in women during high-progesterone states and serve as a foundation for further investigation. We also plan to evaluate differences in other target cells and other high-progesterone states, including progesterone-dominant contraceptives, post-menopause, and the luteal phase of the menstrual cycle, to further our understanding of the relationship between high progesterone states and HIV acquisition.

Defining the dynamics of SIV infection and the viral reservoir from early ART initiation to Rebound using PET/CT analysis and a multi-scale imaging approach.

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Long-term adherence to antiretroviral therapy(ART) is not curative. Human immunodeficiency virus(HIV) persistence during ART has been attributed to a population of long-lived, latently infected memory T-cells. Other models that suggest a major role for infected myeloid cells in viral persistence, and the potential involvement of other cell types. We utilize a PET/CT ^{64}Cu-FAB2 probe(7D3) for iterative whole-body imaging to quantitatively localize SIV envelope, allowing the exploration of infection dynamics in the rhesus macaque (RM) model.

RMs were challenged with a single high-dose of SIVmac239 and daily ART was initiated 4 days or 6 weeks post-challenge. Following 6-12 months, ART treatment was discontinued and RMs were necropsied as early as 4 days post-ART cessation, or after achieving viremia. During ART and ATI, PET/CT revealed sites of SIV gene expression. SIV infections sites were detected as early as four days post-ART cessation in multiple tissues. PET/CT was used to locate discreet infection sites and these results were corroborated by fluorescent imaging, revealing the infected cell population. PET signals of discreet anatomical sites of initial infection were also monitored quantitatively for the duration of the study.

We observed intriguing differences in the infected cell populations based on the route of infection; mucosal challenge versus intravenous(IV), and the length of time after infection prior to ART initiation. Strikingly, phenotyping the infected cells in post-ATI tissues revealed a mixture of myeloid and mast cells constituting the entire infected cell population, and small numbers of T-cells were only detected in tissues form viremic animals or animals where ART was initiated 6 weeks post-challenge. We also observed in early ART RMs, the sites where infections were initially established, were the origin sites of rebounding infections.

These tools and findings, are helping to further our understanding of reservoir dynamics and will prove invaluable to the HIV Cure field.

Title: Rectal mucosal injury and healing among HIV-negative men who have sex with men (MSM)

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Background: MSM who engage in receptive anal intercourse (MSM-RAI) demonstrate a unique rectal mucosal immune environment compared to men who do not engage in RAI that could impact HIV transmission. We compared the immune and microbiome response to experimentally-induced rectal mucosal (RM) injury and healing between these groups of men without HIV.

Methods: HIV-negative MSM engaging in RAI (n=19) and men who never engaged in RAI (controls; n=6) aged 18-59 years underwent collection of RM secretions via anoscopy prior to experimentally-induced injury with biopsy forceps and subsequent digital imaging. Participants then returned on days 2, 5, and 8 for repeated mucosal secretion collection adjacent to the injury and imaging. Twelve cytokine concentrations were measured by LegendPlex, and the microbiome was characterized by 16s rRNA sequencing. Linear decomposition modeling (LDM) was utilized to examine cytokine concentrations, microbiome changes, and wound healing over time.

Results: RM levels of IL-1β, IL-17A, IP-10, IL-8, IFN-γ, and a composite cytokine score were significantly higher among MSM-RAI compared to controls at baseline and overall across study visits. Levels of IL-6 and IP-10 increased significantly after injury in both groups. Healing time after injury was faster among MSM-RAI, although not statistically significant. While the microbiome composition of MSM-RAI was significantly different from controls, we did not detect any microbiome changes after injury. RM inflammation was associated with microbiome composition, and distinct inflammation-associated bacterial networks, or guilds, were identified in both MSM-RAI and controls.

Conclusions: MSM engaging in RAI demonstrated higher baseline RM inflammation, faster wound healing, and a distinct rectal microbiome compared with men who never engaged in RAI. IL-6 and IP-10 may be important mediators in the mucosal injury response, and distinct bacterial guilds are associated with both high and low inflammation. Future research should explore how these factors facilitate or hinder mucosal HIV transmission.

Durable barrier to virus infection at the mucosa via mRNA transduction

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While PrEP has made great progress in preventing HIV acquisition, adherence and access remain an issue, especially for people unwilling to use extended preventative drug treatments. An ideal alternative would be the use of microbicides effective at the portal of entry, yet their development has been marred with recurrent failures. Along the same concept, broadly neutralizing antibodies (bnAbs) delivered at mucosal interfaces - have been shown to protect monkeys from infection, providing proof of concept that drug free protection may be achieved, though such protection is very short lived, as the bnAbs are rapidly excreted. In efforts to improve such antibody mediated protection, we tested the administration of mRNA encoding bnAbs anchored to the epithelium via a glycosylphosphatidylinositol (GPI) anchor. Vaginal delivery of water formulated mRNA encoding PGT121 and the camelid J3 using a Teleflex atomizer led to the production and durable presence of bnAbs in this compartment. Analysis of vaginal fluids and biopsies demonstrated the extended presence of bnAbs even after 4 weeks of administration, suggesting durable protection afforded even by single treatments. In fact, mucosal explants from mRNA induced bnAbs were protected from infection challenged *in vitro* with clade A, B and C SHIVs. Moreover, unlike systemic delivery, mucosal delivery of proteins allows for the design and transduction of synthetic bnAbs with enhanced bioactivity and affinity, without the risk of ADA, offering a highly flexible platform to design effective barriers to infection and even contraception devoid of hormonal treatment. While such optimization is ongoing, the next step will be to test our approach in vivo measuring protection of female rhesus macaques from viral acquisition upon vaginal challenges.

Role of cGAS-STING pathway in shaping mucosal lymphoid environment and viral transmission

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Most new HIV-1 infections are initiated by a single variant out of a quasi-species deposited at a mucosal site, and transmission is determined by the host-virus interface. Recent studies in the Rhesus model found that immature dendritic cells (DC) in the mucosa become infected, and it remains to be determined how their innate responses influence viral spread. HIV-1 infection of DCs triggers expression of antiviral factors and type I interferons as well as proinflammatory cytokines through the cyclic GAMP synthase (cGAS)-STING pathway. Paradoxically, while induction of antiviral genes by cGAS inhibits replication, proinflammatory cytokines can activate resting CD4+ T cells and may promote infection. In attempt to understand impact of cGAS-STING activation on viral transmission, we treated a non-human primate (NHP) with the STING agonist diABZI to induce the pathway prior to a single high dose, intra-rectal SIVmac 293 challenge. NHP were necropsied 3 days later and longitudinal cytokine profiling in the blood confirms that the agonist treatment induced systemic inflammation as well as changes in mucosal immune environment. Characterization of the mucosal tissues of the animals for the viral transmission status as well as lymphoid environment are on-going currently. Through this study, we hope to have better understanding of relationship between immune microenvironment and viral transmission during the mucosal challenge