In situ image analysis and protein profiling of the genital mucosa reveal epithelial barrier defects in women using DMPA

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Background
Clinical studies in HIV-endemic areas have shown that the injectable hormonal contraceptive (HC) depot medroxyprogesterone acetate (DMPA) significantly affects the risk of HIV-infection. The detailed molecular mechanisms as assessed in situ in genital tissue samples, for how these translate to increased risk of HIV infection is not known.

Methods
To unravel underlying molecular mechanisms for how DMPA can change a woman’s risk of HIV-infection we have collected paired cervicovaginal lavage and ectocervical tissue samples from Kenyan women at high risk of HIV infection. The women were divided into two study groups based on DMPA use (DMPA use, no-HC use). Identification of molecular markers in the samples included protein profiling of the cervicovaginal lavages and in situ analysis of epithelial junction proteins and HIV-target cells in the tissue samples.

Results
DMPA use was associated with over-expression of proteins representing inflammation in the secretions. While the total thickness of the ectocervical epithelium was similar between the study groups, the DMPA users had a thinner superficial layer and a less intense E-cadherin expression as compared to the controls. Furthermore, DMPA users had a higher proportion of CD4+CCR5+ cells and higher levels of activated T-cells (CD4+CD69+) in the ectocervical epithelium. In both study groups, the majority of CD4+CCR5+ cells were localized within the parabasal layer while the CD4+Langerin+ cells were primarily found in the intermediate layer of the epithelium.

Conclusion
Women using DMPA displayed a less robust ectocervical epithelium, inflammatory signals and a higher proportion of activated HIV target cells. This “mucosal signature” may be associated
with a higher risk of HIV acquisition. Further characterization of the ectocervical tissues will be performed by RNA-seq and the resulting mucosal signature will be validated in a human genital explants model.

**Cross-Species Translation of Biological Information via Statistical and Machine Learning Computational Frameworks**

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A vital challenge that the vast majority of biological research must address is how to translate observations from one physiological context to another—most commonly from experimental animals (e.g., rodents, primates) or technological constructs (e.g., organ-on-chip platforms) to human subjects. This is typically required for understanding human biology because of the strong constraints on measurements and perturbations in human *in vivo* contexts. Direct translation of observations from experimental animals to human subjects is generally unsatisfactory because of significant differences among organisms at all levels of molecular properties from genome to transcriptome to proteome and so forth. Accordingly, addressing inter-species translation requires an integrated experimental/computational approach for mapping comparable but not identical molecule-to-phenotype relationships. This presentation will describe methods we have developed for a variety of cross-species translation examples, which may be envisioned to be readily applicable to problems in pathogen infection responses and treatments.

**Bio-behavioral correlates of rectal inflammation in HIV-negative sexual minority men**  

**Background:** An estimated 70% of HIV infections in gay, bisexual, and other men who have sex with men (referred to here as sexual minority men) occur in the rectal mucosa during receptive condomless anal sex (CAS). Although oral pre-exposure prophylaxis (PrEP) is highly effective at preventing HIV acquisition, scant research has examined bio-behavioral correlates of rectal inflammation to catalyze the development of novel approaches to prevent HIV and other sexually transmitted infections (STIs).

**Methods:** HIV-negative sexual minority men were recruited in four STI clinics in South Florida to complete a brief survey that assessed rectal douching and screened for depression using the 10-item Centers for the Epidemiologic Study of Depression (CES-D) measure (i.e., total scores ≥ 10). Rectal swabs from 38 participants (19 on PrEP and 19 who had never taken PrEP) were selected for rectal cytokine measurements because these participants reported the following in the past three months: 1) receptive CAS; 2) rectal douching; and 3) no prescription for antibiotics. The LEGENDplex Human Inflammation Panel (BioLegend/Cat. No. 740118) was used to detect 13 human inflammatory cytokines/chemokines from rectal swabs.
Results: The sample was predominantly non-Hispanic white (72%), middle-aged (Mean = 39.3, SD = 15.7), and gay-identified (90%). The median number of times participants had engaged in rectal douching in the past three months was five (Interquartile Range = 4-15), and 29% screened positive for depression. PrEP was not significantly associated with any measure of rectal inflammation (p’s > 0.12). However, participants who reported douching more than five times in the past three months displayed significantly higher levels of IL-8 (Cohen’s d = 0.79; p = 0.019). Those who screened positive for depression also displayed higher levels of MCP-1 (Cohen’s d = 0.64; p = 0.047), IL-17A (Cohen’s d = 0.73; p = 0.018), and IL-33 (Cohen’s d = 0.71; p = 0.026).

Conclusions: Douching and depression are key factors that could contribute to rectal inflammation in this high priority population. Further longitudinal research is needed to examine the bio-behavioral determinants of rectal inflammation, which could amplify biological vulnerability to STIs as well as HIV.

Adding DIMENSIONS and a COMPLEMENT-ary view on HIV-1 transmission

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The growing spread of resistant HIV strains indicates the need to speed up research on innovative compounds that re-activate the immune system and to reinvent HIV vaccine development using human ex vivo models. Clinical trials only illustrated modest protective antiviral immune responses which led to the conclusions that (i) an effective vaccine must induce both humoral and cellular responses against HIV-1, (ii) novel alternative approaches are needed to design improved HIV-1 vaccines and (iii) improved methods for vaccine delivery have to be tested.

My work group recently illustrated the power of complement to boost efficient antiviral humoral and cellular immune responses via DCs - these data were generated in simple 2-dimensional (2D) cell culture models which provide principles in single cellular targets, but they do not reflect the complex interplay in vivo during HIV-1 transfer and spread. Novel and rapid developments in high content/high throughput imaging analyses as well as organotypic cultures provide groundbreaking new tools to study HIV-1 transfer at entry sites or to test novel vaccination strategies. Therefore, we design optimized intelligent human barrier models combined with infection-relevant immune cells (DCs, naïve T cells or HIV-specific T cell clones) in order to characterize HIV-1 entry and initial transmission steps within a 3D system. We, too, found within the 3D models, that the opsonization pattern of the virus or pathogenic fungi within a respiratory 3D model markedly modified DC and macrophage function compared to non-opsonized pathogens.

These human systems taking into account innate immune mechanisms, such as complement opsonization, offer improved power to test delivery methods, adjuvants, shock-and-kill compounds or novel vaccination approaches and will be an important challenge with broad interest.

Hormonal contraception and HIV transmission: Guiding clinical research with animal models

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The debate to resolve if women using depot-medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN), and levonorgestrel (LNG) are at greater risk for HIV is fueled by incongruent results from clinical studies. As most of these studies were not designed to define links between progestins and HIV susceptibility, methodological limitations likely contributed to result variability, including inadequate sample size, inability to control for higher frequencies of unprotected sex in couples not using hormonal contraception, and inability to define separate HIV risk estimates for women using different progestins. Also fueling this debate is the unspecified mechanism by which progestins may enhance HIV susceptibility, and our lab, among others, uses animal models to define these mechanisms. In mouse models, we discovered in vivo treatment with DMPA, LNG, or NET-EN reduces genital expression of the cell-cell adhesion molecule desmoglein-1α (Dsg1α). This effect weakened genital mucosal barrier function, evidenced by increases in genital mucosal permeability and susceptibility to genital infection with cell-associated HIV-1. Guided by these data, we compared ectocervical DSG1 expression and mucosal permeability in women before and 30 days after initiating Depo-Provera® or Mirena® and saw these contraceptives induced changes in ectocervical DSG1 expression and genital mucosal barrier function analogous to progestin-treated mice. By revealing progestins comparably weaken mucosal barrier function in the mouse and human genital tract, we identified mice as a valuable model for exploring effects of exogenous progestins on this vital anti-virus host defense mechanism. Returning to the mouse models, we learned combined treatment of humanized mice with DMPA and estrogen reduced genital mucosal permeability and fully reversed the HIV-1 susceptibility induced by DMPA alone. In ongoing work, we are using relevant preclinical models to define how estrogen reverses progestin-mediated effects on genital mucosal permeability, enhances genital mucosal barrier function, and reduces SIV transmission efficiency.

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CD4+ TISSUE RESIDENT CELLS IN RECTAL MUCOSA OF MSM: HIV CO-RECEPTOR EXPRESSION, ACTIVATION STATUS, AND ASSOCIATIONS WITH HIV-1 INFECTIVITY

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BACKGROUND: The rectal mucosa (RM) is a major site for HIV acquisition and replication as the gut harbors the majority of the body’s CD4+ T cells, most with high levels of HIV co-receptor (CCR5) expression. However, our understanding of mucosal CD4 cell subsets and their roles in HIV transmission and pathogenesis is still evolving.
METHODS: Utilizing multi-color flow cytometry, the percentages of CD4+ tissue-resident memory (TRM, CD69+103+, CD69+103-) and non-TRM (CD69-103-) and their expression of HIV co-receptor (CCR5), proliferation/activation marker (Ki67), and gut-homing (α4β7) were measured and compared in rectal mucosal (RM) biopsies from 66 HIV-negative men who have sex with men (MSM). Associations between the various cell subsets and HIV-1 (BAL) infectivity in ex vivo RM explant challenge experiments were evaluated.

RESULTS: In RM biopsies, CD69+103- and CD69-103- cells were most abundant while CD69+103+ comprised the minority of memory CD4 T cells (median 52.1% vs. 37% vs. 3.1%; p<0.001). Among CD4+TRM and non-TRM, CCR5 expression was highest on CD69+103+ compared to CD69+103- and CD69-103- cells (median 63.6% vs. 41.6% vs. 24.4%; p<0.0001 for all comparisons). Contrastingly, both α4β7 and Ki67 were minimally expressed by CD69+103+ CD4+TRM cells (median 1.11% and 0.27% respectively), and highest expression was seen on CD69-103- non-TRM cells (median 6.74% and 1.34% respectively; p<0.01 for all comparisons). In RM explant HIV-1 challenge experiments, expression of Ki67 strongly correlated with HIV infectivity for both TRM (r=0.55, p<0.0001) and non-TRM (r=0.43, p=0.0009) CD4 cell subsets, while CCR5 and α4β7 expression were not significantly associated with HIV infectivity.

CONCLUSIONS: RM CD4+TRM and non-TRM subsets display significant heterogeneity in expression of traditional markers of HIV susceptibility, including CCR5. Increased activation/proliferation of TRM and non-TRM CD4+ cells positively correlated with HIV replication in the explant challenge model, demonstrating its probable importance as a phenotypic marker of increased RM HIV susceptibility.

Whole organ and mucosal tissue distribution of I.V. injected Cu64, Zr89, and fluorescently labeled VRC01 and VRC01-LS in the in vivo Rhesus Macaque Model
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Although antibody mediated protection has been illustrated in non-human primates, it is still unknown how such antibodies are anatomically distributed. Using the living rhesus macaque (RM) model, correlative positron emission tomography (PET) and deconvolution microscopy (DVM), we are able to observe unique antibody distributions amongst organs and within tissues. These studies have allowed us to elucidate antibody distribution over time and provide insight into the mechanism(s) of antibody delivery to those mucosal tissues and organs important in HIV transmission and pathogenesis.

For correlative PET studies, adult RMs were IV administered Cu64- or Zr89-tagged VRC01 or VRC01-LS and followed by PET for up to 72hours or 3weeks, respectively. To assess labeled antibodies with DVM, RMs were IV administered fluorescently-tagged VRC01 and/or VRC01-LS. Following, necropsy tissues were collected. These tissues were frozen and stained with an anti-FcRn for DV microscopy.

In our PET studies, we visualize differences in VRC01 and VRC01-LS antibody distribution between whole organs, across multiple time points. Additionally, using DVM,
we are able to identify pronounced differences of antibody distribution amongst various types of mucosal tissues. For example, in squamous tissues, we find antibodies to be delivered with epithelial differentiation. Alternatively, in columnar and brain tissues we visualize antibody distribution through the vascular system, with antibodies interacting with FcRn. Lastly, we also show that VRC01-LS is able to persist in tissues at longer times than VRC01 due to endosomal recycling within endothelial cells.

Here, our data illustrates a variety of methodologies for antibody delivery to anatomical sites. Using correlative PET, we find that whole organ antibody distribution varies between VRC01 and VRC01-LS. Using DVM and anti-FcRn antibodies, we are able to further understand the intricacies of these distribution differences. These studies start to unravel the complexities of how antibodies are delivered to sites involved in HIV transmission and pathogenesis.

**Utilizing the infant SIV model to assess earliest events after mother-to-child HIV transmission**

Donald Sodora

Over 160,000 infants continue to be infected every year via mother to infant HIV transmission. We are interested in identifying risk factors that influence HIV transmission and contribute to progression to AIDS in HIV infected infants. Bacillus Calmette-Guérin (BCG) vaccination is administered at birth to infants worldwide, and has been demonstrated to increase levels of activated CD4+ T cells (target cells for HIV). BCG risk for infant transmission was evaluated in newborn rhesus macaques, with the vaccination occurring shortly after birth and three weeks prior to oral SIV challenges (SIVmac251). We observed elevated levels of activated peripheral CD4+ T cells (HLA-DR+/CD38+/CCR5+) post-BCG vaccination, however there was no difference in the rate of SIV oral transmission. Our findings therefore do not support the hypothesis that BCG is a risk factor for postnatal HIV transmission. While carrying out this study we observed that, independent of BCG vaccination, roughly half of infants progressed rapidly to disease with high viral loads. Furthermore, the analysis of secondary lymphoid tissues indicated that Rapid Progressing infants displayed germinal center dysfunction, a loss of T follicular helper cells, and a lack of an SIV-specific humoral immune response. Our findings provide evidence that higher levels of Type 1 interferon production are associated with the rapid progression phenotype in these infants. These studies build on our understanding of factors that influence acquisition of pediatric HIV infections as well as the underlying immunologic changes associated with rapid disease progression observed in some HIV+ infants.

**Vaginal microbiota metabolites and HIV susceptibility**

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Abstract:
HIV transmission is modulated by the vaginal microbiota. Women colonised with optimal vaginal bacterial communities, typically dominated by a specific *Lactobacillus* spp., have a decreased risk of acquiring and transmitting HIV compared to women colonised with “non-optimal” microbiota (e.g. bacterial vaginosis, BV) that promote subclinical genital inflammation driving increased HIV risk. BV is characterised by a depletion of *Lactobacillus* spp. and a high relative abundance or load of facultative and/or obligate anaerobes. While studies have focused on the association between vaginal microbiota and/or specific taxa and HIV risk, relatively little is known about the role of microbiota metabolites in modifying the vaginal environment to help protect against HIV. Major distinguishing features between women colonised with optimal vaginal microbiota compared to women with BV include lower vaginal pH (pH<4.5 vs pH≥4.5) and dramatically increased vaginal levels of lactic acid (~120 mM vs ≤20 mM). Accordingly, our working hypothesis is that lactic acid, an organic acid metabolite, is an effector molecule produced by optimal microbiota that plays a role in helping to prevent HIV transmission. Here, data will be presented demonstrating that lactic acid at physiological levels and pH has bactericidal activity against BV-associated bacteria but not lactobacilli, in vivo and ex vivo HIV virucidal activity, and immune modulatory properties on cervicovaginal epithelium that prevents and reduces genital inflammation that could potentially decrease HIV susceptibility. In addition, RNAseq studies suggest a role for lactic acid in directly promoting epithelial barrier function. These findings highlight the multifaceted properties of a major vaginal microbiota metabolite and suggest potential use of lactic acid and/or lactic acid producing bacteria in enhancing the vaginal environment and microbiota (EVE-M) to promote sexual and reproductive health including their use as adjuncts to reduce HIV acquisition.

**Is all genital inflammation the same, and is it all bad?**

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**Background:** Mucosal immunology is a key determinant of HIV susceptibility, and genital inflammation by a variety of definitions has been correlated with genital coinfections and increased HIV acquisition. However, more nuanced and/or standardized definitions of inflammation may be useful as the field moves forward.

**Methods:** Cervico-vaginal cytokine/chemokine levels and cervical cytobrush-derived CD4+ T cell subsets and HIV entry were assessed in several clinical contexts, based in cohorts for HIV-uninfected women from Canada, Kenya and Uganda.

**Results:** Genital inflammation, defined based on elevated levels of various cervico-vaginal cytokines/chemokines and antimicrobial peptides (AMPs), was associated with elevated numbers of cervical CD4+ T cells and with HIV acquisition. However, important bacterial components of the genital microbiome, itself an important driver of HIV risk, had divergent associations with cytokines/chemokines that are frequently grouped together within definitions of inflammation. Type 1 interferons, part of the prototypic inflammatory response, were associated with reduced HIV/SIV susceptibility *in vitro*, *ex vivo* and *in vivo* despite mucosal CD4+ T cell recruitment and other markers of inflammation.
**Conclusion:** As the field gains a more nuanced understanding of the genital immune environment and HIV risk, our definitions of genital inflammation and its implications will also need to evolve.

**Associations between gut microbiota and physical function in older people living with HIV: the gut-muscle axis.**

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Older people living with HIV (PLWH) have impaired physical function compared to controls. We previously identified an association between inflammation and impaired physical function in PLWH, and associations with inflammatory biomarkers and stool microbiome that differed by HIV-1 serostatus and age. Here we investigate relationships between microbiota and microbial metabolites with physical function in older PLWH.

Stool samples were collected from 14 PLWH receiving effective antiretroviral therapy and 22 uninfected controls, 50-75 years old. Bacterial profiles were generated using 16S rRNA sequencing and short chain fatty acids levels (SCFA; butyrate, propionate and acetate) measured by gas chromatography.

Associations between relative abundance (RA) of the 25 most abundant genera (among all participants) or stool SCFA levels with physical function (short physical performance battery [SPPB], chair rise, stair climb, grip) and lean body mass (LBM)/ht² were explored. Linear regression models provided estimated physical function or LBM change, β[95% CI], per RA increase of 1.

In older PLWH, greater RA of *Escherichia, Alstipes* and *Bacteroides* were significantly (p<0.05) associated with longer chair rise time (β=1.5 [0.4,2.5], 3.4 [0.9,5.8] and 0.24 [0.02,0.5] sec) and lower SPPB score (β= -0.3 [-0.4,-0.2], -0.3 [-0.7,-0.001] and -0.04 [-0.06,-0.009] points), respectively; *Subdoligranulum* with lower SPPB (β= -0.3 [-6.0,-0.01], p=0.04), and *Megasphaera* and *Prevotella* with longer stair climb time (β= 0.2 [0.04,0.4] and 0.03 [0.005,0.06] sec; p<0.02). Increasing RA of *Blautia, Catenibacterium* and *Dorea* associated with shorter chair rise time (β= -1.2 [-2.4,-0.02], -1.1 [-2.1,-0.1], and -2.5 [-4.3,-0.6] sec, respectively, p<0.05) and *Pseudobutyribrio* with higher LBM/ht² (β= 0.4 [0.04,0.8] kg/m², p=0.03).

Increasing stool butyrate levels significantly associated with greater grip strength (β= 1.1 [0.2,1.9] kg, p=0.02).

In older PLWH, both negative and positive relationships exist between stool microbiota abundance and physical function. Understanding the gut-muscle axis may provide alternative treatments to improve physical function and quality of life.

**Innate Lymphoid Cells Contribute to Inflammation in Colon During Acute SIV-infection**

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The gastrointestinal (GI) tract is one of the first targets of HIV infection (and pathogenic SIV infection in animal models). Hallmarks of HIV/SIV infection include massive loss of CD4+ T-cells, breakdown of GI barrier, and microbial translocation (MT) within weeks of transmission. It has been posited that HIV-infection of CD4+ Th17/Th22 cells may account for disruption of the epithelial layer and increased MT from the lumen into the lamina propria of the GI tract due to loss of Interleukin (IL)-22. Innate lymphoid cells (ILCs) also produce the homeostatic cytokines IL-22 and IL-17 and may compensate for the loss of Th17 and Th22 cells. ILCs are also capable of producing inflammatory cytokines (IFN-γ/TNF-α) that impact epithelial barrier integrity of the intestine. Inflammatory cytokines are also produced by CD8+ T-cells during HIV/SIV infection but wane as the infection progresses. We initially demonstrated that ILCs,
and not T-cells, from chronically SIV-infected and uninfected rhesus macaques (RM) secrete both IL-22 and IFN-γ in the absence of in vitro stimulation. In this study, we determined if ILCs as well as T-cells express IL-22 and IFN-γ during acute SIV-infection. We hypothesize that ILCs, like T-cells, are an important source of IL-22 and IFN-γ expression in SIV-infected colons during the early phases of SIV infection. We isolated lamina propria mononuclear cells (LPMCs) from RM colon and immediately stained them using surface antibodies to identify ILCs and intracellular antibodies to identify IFN-γ and IL-22 using flow cytometry. We found that IFN-γ and IL-22 are secreted by not only T-cells but also group 1 and 3 ILCs during acute SIV-infection in RM. However, during chronic SIV-infection only ILCs contributed to cytokine production. In uninfected colon ILCs expressed only IL-22. In future studies, we will focus on the role that IFN-γ and TNF-α have on gut barrier dysfunction.

Impact of treatment interruption on colonic mucosa in acutely treated participants

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Despite antiretroviral therapy (ART) initiation during acute HIV infection and viral suppression for years, most individuals experience viral rebound shortly after treatment interruption. The major obstacles to a cure are the cellular and tissue reservoirs that are established during the early stage in the disease. We analyzed mucosal tissue from sigmoid colon biopsies prior to and during analytical treatment interruption (ATI) from participants who initiated treatment during acute stage and were treated for a median of 3.4 years.

Thirteen virally-suppressed individuals underwent colon biopsies while on ART, after ATI at the time of viral rebound (n=6) and 6 months after ART resumption (n=3). We performed immunohistochemistry (IHC) and flow cytometry to assess immune cell numbers and frequency, quantify activation and inflammation markers and localization and phenotype of vRNA+ cells by RNAscope.

While under ART, 3 of 13 participants (23%) had detectable vRNA+ cells within lamina propria (LP) or lymphoid aggregates despite undetectable plasma viral load (<20cp/ml). At rebound, all participants (n=6) had CD4+T cells harboring vRNA in colon biopsies after a median of 30 days post-ATI. We observed an increase of CD4+T cells (51.9 to 56.55; P=0.03), which was confirmed within the LP using IHC (1.35 to 2.02 %cell/Area). The number of myeloid cells was increased within the LP at the time of rebound (5.09 to 7.82 %cell/Area). Mx1 and Ki67 markers increased at rebound then returned to pre-ATI levels after 6 months of ART resumption. No evidence of mucosal damage was detected before or at rebound.

We present here the impact of viral rebound within the colonic mucosa. We observed a significant increase in CD4+T cells within the LP at the time of rebound. The presence of vRNA+ before and at rebound is reinforcing the importance of the GALT as essential site of viral replication during treatment interruption.
Defining the viral properties of *in vivo* adapted, lineage-related, mucosally transmissible SHIVs

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HIV-1 does not persistently infect macaques due to restriction of HIV-1 by several type-I interferon (IFN)-induced macaque-specific host factors. Therefore, chimeric SIV/HIV-1 viruses (SHIVs) encoding the SIV antagonists of the known restriction have been developed for use in the macaque models of HIV-1 infection. The initial chimeric SHIVs generated *in vitro* typically replicate poorly in macaques, but a subset has been successfully adapted for high-level replication through serial passage in macaques. The resulting adapted SHIVs cause persistent infection in macaques that mimics many aspects of pathogenic HIV-1 infection.

In this study, we hypothesized that the host IFN response serves as a strong selective pressure during the process of adaption of SHIV to macaques. In order to test this hypothesis, we compared the ability of a parental, unpassaged molecular clone and three sequential macaque-passaged SHIVs to replicate in the presence of IFN in macaque lymphocytes. Importantly, these SHIVs are lineage-related and the passaged SHIV stocks displayed higher mucosal transmissibility and pathogenicity. We found that the unpassaged, parental SHIV is potently inhibited by IFN (mean IC₅₀ 1.76 U/ml), whereas the sequential macaque-adapted SHIVs are resistant to IFN inhibition (mean IC₅₀ range 350 to >5000 U/ml), suggesting that serial macaque-passaging resulted in adaptation to IFN. Our results also showed that the process of serial macaque-passage increases the replication capacity and virion-associated Env content of SHIVs. Comparative sequence analysis of the *env* from the parental and sequential macaque-passaged SHIVs showed an overall *env* sequence identity of >95% among the isolates.

In conclusion, our results indicate that serial macaque-passage selects for IFN-resistant SHIV variants that have higher replication kinetics and increased Env content. Thus, *in vivo* adaptation of SHIVs contributes to high replication capacity and IFN resistance, potentially leading to increase fitness and adaptation in the host.

The Last Gift: Whole Body Donation as a Means to Reveal HIV Reservoirs

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To cure HIV, we need a better understanding of the distribution and characterization of HIV reservoirs throughout the body and how these reservoirs contribute to viral rebound after discontinuation of antiretroviral therapy (ART). Extensive investigations in humans have been unable to fully characterize the large and complex HIV reservoirs that must be eradicated to achieve a cure because biological sampling in difficult-to-access tissues is understandably limited during life. To take the next steps in understanding and eradicating HIV reservoirs in tissues, we developed a “peri-mortem translational research model” (http://lastgift.ucsd.edu/), similar to existing models in cancer research. In this model, altruistic individuals living with HIV and approaching the end of life due to advanced, non-AIDS-related diseases are participating in HIV cure research. These altruistic individuals provide: (i) detailed clinical, pharmaceutical, and socio-demographic information before their death; (ii) regular, frequent blood
collections while they are alive and; (iii) their entire bodies after death for a rapid autopsy (<6h from the time of death). To date, we have enrolled 11 individuals and we have successfully performed rapid autopsies on 6 participants. We have been able to measure and sequence HIV DNA in most tissues sampled and have also detected cellular HIV RNA. Using this extensive sampling, we are planning to characterize populations of HIV in blood (ante-mortem) and compare these populations to various anatomic compartments (at autopsy) in both patients who maintain and those who discontinue ART to better understand dynamics of viral rebound and populations in the blood and throughout the body. These altruistic individuals will provide a wealth of clinical information and biological specimens that can be used to answer important, unanswered questions in search of the quest to cure HIV.

Impact of Menopause on Mucosal Immune Protection in the Human Female Reproductive Tract

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Problem: The elderly population (>60 years old) is increasing rapidly and projected to grow to 1.4 billion by 2030. Postmenopausal women face unique health challenges, including increased susceptibility to urinary tract infections (UTI) and sexually-transmitted infections (STI). Epidemiological studies indicate that UTI and STI rates are increasing in elderly women, thereby presenting a public health challenge that needs to be addressed. Despite ongoing interest in immune protection in the FRT of premenopausal women, little information exists as to the impact of menopause on mucosal immunity in the FRT.

Method of study: FRT tissues obtained from hysterectomies were enzymatically digested to recover epithelial and immune cells by filtration and magnetic bead selection. Epithelial secretions were measured for antimicrobials and cytokines by ELISA and pathogen killing. Immune cell distribution in FRT tissues was determined by confocal microscopy, and cell surface receptor expression by flow cytometry. Cytotoxic activity was measured by co-culture with allogeneic target cells using the IncuCyte ZOOM Live-cell Analysis System.

Results: Analysis of FRT cells and tissues of premenopausal and postmenopausal women indicate that a number of mucosal immune parameters change with menopause. After menopause: Epithelial cell secretion of antimicrobials and killing of bacterial, viral and fungal pathogens are suppressed along with barrier protection; CD4+T cells in the endometrium are reduced with increased proportions of Th17 CD4+T cells; CD103+CD8+ tissue resident memory T cells decline in the CX and ECX but remain constant in the EM; DC numbers decrease throughout the FRT; CD8+T cell cytolytic activity is increased along with the loss of uterine lymphoid aggregates in the FRT.

Conclusions: Our studies indicate that menopause significantly alters multiple aspects of immune functions throughout the FRT, possibly leading to increased susceptibility to infection. These findings are relevant to therapeutic interventions essential for maintaining the health of postmenopausal women.

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Temporal and spatial characterization of SIV infection dynamics in rhesus macaque mucosal tissues.

Danijela Maric
Our preliminary work revealed that Th17 T helper cells and immature dendritic cells are the most predominant initial targets after rectal challenge with an SIV-based replication defective reporter virus. These cell types are infected at a rate that is several-fold higher than their relative abundance predicts, indicating that they are preferentially targeted in the early time of infection.

Here, we challenged twelve female macaques with a mixture of replication defective luciferase reporter and wild-type SIVmac239 and we sacrificed the animals 48-, 72- or 96h later. We used luciferase signal to home in on small regions within the tissue to increase our chances of identifying cells infected by wild-type virus. Infected cells were identified microscopically by staining for SIV viral proteins and were validated by spectral imaging and nested PCR.

Foci of infected cells are visible as early as 48-hour post challenge and expand in size by 96 hours, stretching over several 10-micron tissue sections at times. Analysis of SIV infected cells revealed expected virus induced changes in CD4 expression, including CD4 receptor internalization and down-regulation. Comprehensive phenotypic profiling of nearly 2,000 SIV infected cells revealed that the Th17 infection rate does not vary much over the first 96h. However, from 48h to 96h, there is a pronounced decrease in iDCs infection rate and an increase in infection of other T cell subtypes, suggesting immune cell recruitment to the site of infection.

Using the wild-type SIVmac239 virus we were able to study the early infection events at the rectal mucosa and we observed very dynamic changes in respect to infected cell phenotype and immune cell recruitment in response to infection. In our future work we hope to paint the full picture of the HIV/SIV sexual transmission in time and in space and hence aid development of more effective HIV prevention strategies.

**Microbiome-inflammation interactions in the female genital tract**

**Lyle McKinnon**
University of Manitoba

Defining mucosal determinants of HIV acquisition are important for understanding transmission risk and design of novel HIV prevention strategies. We assessed HIV risk associated with a panel of 48 cytokines measured in cervicovaginal fluid collected from 774 South African women at >2,000 study visits. In adjusted Cox regression analysis, several cytokines were associated with both increased and decreased HIV risk. Similar results were obtained in analyses that accounted for variation in cytokine levels over time. Of the functional groupings of cytokines, chemokines were prominent predictors of risk; the number of chemokines in the upper quartile was associated with an 15% increased risk per chemokine (p=0.001). These data are relevant given potential mechanisms involving recruitment of mucosal immune cells to the mucosa, which could favour HIV establishment. Interestingly, this chemokine association was vaginal microbiota-dependent. While most women with non-*Lactobaccillus*-dominant microflora upregulate inflammatory cytokines and down-regulate chemokines, and subset of ~15% who have increased chemokines are at ~4-fold increased risk of HIV infection. Similar results were obtained with the number of elevated chemokines as an ordinal variable (HR 1.27, 95% CI: 1.12 to 1.43). Conversely, chemokines were not associated with increased HIV risk in *Lactobaccillus*-dominant women (HR 1.04, 95% CI: 0.91 to 1.18). The most significant chemokine-HIV effect was observed in women with Gardnerella dominance (CST-III). These data suggest that immune-commensal interactions, and not merely either alone, may be important for understanding HIV transmission and prevention.

**Characterising Chlamydia trachomatis**-specific CD4+ T cells in young South African women at risk of HIV
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Chlamydia trachomatis is a leading cause of poor reproductive health outcomes in women, including infertility and pelvic inflammatory disease. Infections are often asymptomatic and undiagnosed in developing countries, like South Africa, where syndromic management is standard of care. The success of HIV treatment and prevention has resulted in increased STI prevalence, suggestive of behavioural disinhibition and lower condom use. As such, infections have increased in key HIV risk groups in South Africa, with a prevalence of up to 40% in adolescent females. Of note, chlamydia may increase HIV risk by contributing to genital tract inflammation and HIV target cell infiltration into the genital tract. There is no vaccine for chlamydia currently available. Although there is evidence for partial protection after natural infection, immunity to chlamydia remains poorly understood. Thus, we aimed to characterise chlamydia-specific CD4+ T cells in terms of cytokine production, genital inflammatory potential and HIV infectivity potential. To achieve this, in vitro culture of C. trachomatis was optimised. Serovar E was chosen in particular, since it is one of the most common serovars circulating in South Africa. McCoy cells were infected with C. trachomatis and the infected cells were blind passaged onto fresh monolayers every 72h. Elementary bodies were isolated by renografin density gradient purification and used to stimulate PBMC from chlamydia-infected young women. Following stimulation, cells were phenotyped and intracellular IFN-γ, TNF-α and IL-17 measured by multiparameter flow cytometry. Chlamydia-specific CD4+ T cells primarily produced the pro-inflammatory Th1 cytokines, IFN-γ and TNF-α. These cells expressed CCR6 and the integrin α4β7, suggesting potential for both genital tract homing and HIV infectivity. Understanding immunity against chlamydia and its link to HIV is vital in developing effective intervention strategies with the ultimate goal of lowering HIV risk in young women.

CD8 T cell immunosurveillance and elimination of HIV target cells in the female genital tract

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The HVTN 052 vaccine trial, also known as the STEP trial employed a CD8 T cell based vaccine that while promising in animal studies, did not protect vaccines from HIV acquisition in a phase II clinical trial. While several mechanisms of vaccine failure have been proposed, we hypothesize that the quantity of mucosal HIV-specific CD8 T cells was insufficient to adequately survey and eliminate HIV target cells after HIV exposure. While HIV infection is thought to establish in a single founder cell, an effective CD8 T cell based vaccine must enable efficient surveillance of all potential CD4 targets. To test our hypothesis, we applied intravital mucosal imaging in a murine model of CD8-CD4 interaction to visualize how
Regulatory T cell contraction in early pregnancy is associated with HIV-1 infection and preterm birth

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Background: Maternal HIV-1 infection and its treatment are associated with increased risk of preterm birth (PTB). Historically, an expansion of regulatory T cells (Tregs) during the 2nd trimester was thought to be essential for maternal tolerance of the fetal allograft. However, recent studies applying more stringent immunophenotyping methods specific for suppressive Tregs demonstrate that this population remains stable and does not expand in peripheral circulation during pregnancy. We assessed early pregnancy peripheral Treg frequencies in HIV-infected (HIV+) and uninfected (HIV-) women with term or preterm births.

Methods: Peripheral blood specimens were collected from 61 1st trimester (HIV-: 52; HIV+: 9), 87 early 2nd trimester (HIV-: 70; HIV+: 17), and 179 late 2nd trimester (HIV-: 148; HIV+: 31) women enrolled in the Zambian Preterm Birth Prevention Study (ZAPPS), a prospective antenatal cohort in Lusaka. Tregs, defined as CD4+CD25+CD127lowFoxP3+, were quantified by flow cytometry. Log-transformed Treg frequencies were compared by t-test between gestational age groups by HIV serostatus. Paired Treg specimens were defined as expanding or contracting and assessed by HIV serostatus and PTB groups using chi-square.

Results: We did not observe significant differences in Treg frequencies dependent on HIV serostatus in any gestational age grouping. Furthermore, a Treg expansion was not observed either HIV- or HIV+ women. In contrast, Treg frequencies contracted from the 1st trimester to early 2nd trimester in HIV+ women (p=0.01), but not HIV- women. Using paired specimens of HIV+ women, we confirmed a decrease in Tregs from 1st trimester to late 2nd trimester in HIV+ women (p=0.03) that was associated with PTB (p=0.01).

Conclusions: Exploratory data from our African cohort, established specifically to study PTB, suggest a Treg contraction between the 1st and 2nd trimesters in HIV+ women that is associated with PTB, implying a systemic role of Tregs in HIV-associated PTB that warrants further studies applying more stringent immunophenotyping methods specific for suppressive Tregs.
Distributions and localization of IgA antibodies and virus following acute rectal HIV exposure


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Vaccines utilize antibodies to form immune complexes with antigens to protect hosts from pathogens. IgA is found at the highest concentration in the gastrointestinal tract, making it a prime candidate for HIV therapeutics. However, how IgA protects these tissues from HIV infection remains unknown. We sought to determine potential sites of virus localization after rectal challenge and how IgA1/2 influence viral penetration in the rectum.

Twenty rhesus macaques received rectal application of PBS, Cy5-labeled HGN194 IgA1 or IgA2. Thirty minutes post-antibody/PBS inoculation, macaques were rectally challenged with a radioactive 64Cu-labeled R9-PA-GFP-BaL. PET scans were obtained at zero and two-hours after viral challenge; necropsy directly followed. Tissues were immediately dissected, snap frozen in OCT, sectioned, and stained. Deconvolution microscopy was utilized to identify antibody and virion distribution and viral penetration depth.

Penetrating virions were identified in the rectum, colon and mesenteric lymph nodes (mesLNs) two hours after challenge. Overall, the descending colon was most susceptible to viral penetration. IgA1 and IgA2 were found throughout the rectum, colon, and mesLNs. Animals given IgA2 had more virions in the mesLNs and more penetrating virions in the rectum and descending colon. Animals given either IgA1 or IgA2 had more penetrating virions in the transcending colon than PBS. In all samples, the majority of virions were associated with antibody.

Our data show that HIV not only penetrates the gut mucosa rapidly after exposure, but also enter the mesLNs, suggesting viral dissemination occurs immediately after viral exposure. Our results also show that IgA1/2 co-localize with virions, suggesting IgA-virion immune complexes form after rectal challenge. We also noted that rectally applied IgA co-localizes with Muc2 staining, implying that mucus and IgA may form immune complexes. Future experiments will aim to determine cells types responsible for trafficking virions to the mesLNs and whether mucin-antibody interactions influence virion-antibody binding.

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Foreskin Migration Assay: A Technique to Characterize Migratory and Tissue-Resident HIV Target CD4+ T Cells in the Foreskin Epidermis

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Medical male circumcision (MMC) provides protection from male HIV acquisition and reduces the incidence of other sexually transmitted infections (STI). This suggests that the foreskin plays a prominent role in HIV transmission events and a better understanding of the foreskin immune compartment is required. However, current methods to isolate foreskin immune cells often rely on harsh enzymatic digestion of tissues, with variable consequences on cell phenotype, viability and marker expression. Consequently, we sought to develop a minimally invasive technique that would maintain physiological conditions and provide a means to characterize and phenotype HIV target cells in the foreskin.

We recruited 14 HIV-negative males (>18 yrs) who elected to have voluntary MMC at clinics in the Cape Town metropole. Inner and outer foreskin tissue was dissected into 1cm² pieces and the dermis and epidermis were separated following overnight incubation with dispase (5 mg/ml). Foreskin epidermal sheets were incubated in complete medium for 48 hours, allowing cells to migrate out of the tissue and into the culture medium. The remaining tissue was digested with liberase (25 μg/ml) for 2-3 hours to isolate the remaining tissue-resident cells. Multi-colour flow cytometry was then used to phenotype putative CD4+ HIV target T cells. The LSRII panel used had the following antibody markers: CD3, CD4, CCR5, CCR4, CCR6, CCR10 and CD69 to characterize foreskin T cells.

After 48h incubation, total cell yields of between 150-200 x10⁶ cells migrated out of the foreskin tissue similar to the yields obtained when foreskin tissue was digested with liberase. We showed that almost all CD3+CD4+ cells migrated out of the foreskin epidermal sheets, with less than 1% remaining as tissue-resident.

Of those that migrated out of the tissue, between 80-90% of CD4+ T cells expressed the HIV coreceptor, CCR5. A proportion of these cells also co-expressed CCR4, CCR6 and CCR10, suggesting that Th22 cells were enriched in foreskin tissue. These cells also co-expressed significantly higher levels of CD69, relative to those that remained in the tissue (p=0.0156), suggesting that almost all foreskin T cells that spontaneously migrate are tissue-resident cells. These findings indicate that the foreskin migration assay is a feasible method to isolate foreskin immune cells for downstream characterization and phenotyping. Our data show that human foreskin tissue is enriched for CD4+ CCR5+ Th22 cells putative HIV target cells. Finally, identification of differences between the cells that are stationary versus migratory may represent a useful method to understand HIV susceptibility and represents a unique advantage over enzymatic digestion of tissue.

The impact of cervical cytobrush sampling on immune factors and microbiota in the female genital tract

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Avid Mohammadi will be the presenting author and she is an early career investigator.

Background: The immunology and microbiota of the female genital tract (FGT) mucosa, the initial site of HIV infection during penile-vaginal sex, are key determinants of HIV susceptibility. Specifically, HIV acquisition is associated with inflammation, and with a vaginal microbiota with a low abundance of Lactobacillus species and high abundance of gram negative anaerobes. Proposed mechanisms include
recruitment of HIV target cells, particularly activated CD4+ T cells and antigen presenting cells (APCs). Cervical cytobrushes are often used as a non-invasive method to study endocervical immune cell populations, and can be collected in a longitudinal fashion. However, the effect of cytobrush sampling itself on FGT immunology is unknown.

**Methods:** STI free women were recruited (N=22); blood, cervicovaginal secretions and cervical cytobrushes were collected at baseline, with repeat sampling after 6 hr (N=11) and 48 hr (N=11). Cytobrush-derived immune cell subsets were assessed by flow cytometry. The density of *Lactobacillus* species and 4 key BV-associated anaerobic bacteria were determined by qPCR. Paired changes were assessed by Wilcoxon signed-rank test.

**Results:** The number of CD4+ T cells, CD14+ monocyte/ macrophages and neutrophils (CD15+CD66b+) were significantly increased 6 hr after cytobrush collection (Median difference= +268; P=0.042, Median difference= +8,160; p= 0.001, Median difference= +101,313; P= 0.0068, respectively). While neutrophil and CD4+ T cell numbers were not altered 48 hr after cytobrush collection (both P>0.1), the number of CD14+ cells remained elevated (Median of difference= +8,021; P= 0.001). There was no change in the abundance of *Lactobacillus* species or indicator BV-associated bacteria at either time point. The impact on soluble immune factors is currently being assessed.

**Conclusion:** Cytobrush collection causes a mucosal influx of various immune cell subsets at 6h, which was mostly resolved by 48h after sample collection, although CD14+ cell number remained elevated. These findings have important implications for longitudinal studies of FGT immunology.

Influence of GI tract microbiome dysbiosis on inflammation and SIV disease progression

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Alteration to the composition of the intestinal microbiome has been described in HIV-1-infected individuals and has been hypothesized to contribute to persistent inflammation in chronic infection. However, recent work has demonstrated that much of the microbial dysbiosis observed in HIV-infected individuals can be attributed to risk factors underlying acquisition of HIV rather than HIV infection, itself. Thus, the degree to which GI tract microbial dysbiosis contributes to HIV acquisition, inflammation, and progression to AIDS are unclear. Here, we studied how particular antibiotics alter the composition of the GI tract microbiome and influence inflammation in the absence of SIV. We then induced an HIV-1-like intestinal dysbiosis in rhesus macaques by repeated vancomycin treatment and assessed vancomycin-induced dysbiosis, damage to the structural barrier of the GI tract, inflammation, and the contribution of dysbiosis on SIV disease progression. While dysbiotic animals had elevated inflammation and damage to the structural barrier of the GI tract, dysbiotic and control animals exhibited comparable viremia, target-cell depletion, immune activation, microbial translocation, and survival rates, indicating that intestinal microbial dysbiosis does not necessarily contribute to untreated lentiviral disease progression. These data suggest that microbial dysbiosis can induce low level of inflammation and can result in damage to the structural barrier of the GI tract and could influence susceptibility to HIV acquisition. This could point to novel therapeutic interventions to decrease HIV transmission.

Molecular mechanisms underlying microbial associated epithelial dysfunction in the vaginal tract

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The female genital tract (FGT) is a complex mucosal environment that is composed of multiple host and microbial factors maintaining a homeostasis to help resist pathogenic infections. Bacterial vaginosis (BV) is characterized by the displacement of *Lactobacillus* bacterial species with anaerobic bacteria and is
associated with numerous adverse reproductive health outcomes, including increased risk of STIs. Epithelial dysfunction and immune activation are related to bacterial vaginosis, but the molecular pathways governing these processes are unclear. Here we utilized an integrated ‘omics approach (proteomics, transcriptomics, metabolomics) to characterize host molecular differences in the vaginal mucosa associated with vaginal microbial diversity, from 405 women living in Kenya and Uganda, and explored functional mechanisms using epithelial-bacterial co-cultures and mouse models. Five major microbiome groups were identified in women; a Lactobacillus dominant profile not species specific, Lactobacillus crispatus dominant, Lactobacillus iners dominant, Gardnerella vaginalis dominant, and polymicrobial communities, where the latter two profiles associated with clinical BV diagnosis. Molecular profiling of vaginal swab and cervical tissue samples showed a common cell signaling pathway linking cell growth, differentiation, energy metabolism and wound healing responses in women with anaerobic and polymicrobial bacterial communities, which were strongly tied to bacterial functional pathways. Epithelial co-culture using anaerobic bacteria products, or drugs that target these pathways, recapitulated epithelial wound-healing responses, and intravaginal challenge of mice with these bacteria showed similar epithelial dysfunction. Here I will discuss these results showing that BV-associated epithelial dysfunction may be mediated through key molecular pathways and how understanding these drivers and mechanisms may provide novel therapeutic targets for BV treatment.

**HIV Infection Alters the Gut Mucosal Microenvironment by Changing Stem Cells Signaling Pathways**

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HIV-associated systemic immune activation is linked to an early, potentially irreversible breach of gut epithelial integrity, allowing microbial pathogens to translocate into the lamina propria and blood where resident immune cells become chronically activated. What causes the damage to the gut epithelium in response to HIV infection is not fully understood. Here we used adult stem cell-derived organoid technology to test whether HIV infection impairs the regenerative capacity of intestinal stem cells. We show that colonoids from individuals with or without HIV infection maintain a stem-like character and can undergo differentiation into the different cell types in the gut epithelium. Real time PCR analysis showed ~25-fold, ~10-fold and 5-fold induction in the transcription of ALPI (enterocyte marker), MUC2 (goblet cell marker), and CHGA and SYP (enteroendocrine cell markers) respectively, in differentiated organoids relative to non-differentiated organoids, irrespective of HIV status. However, single cell RNA sequencing analysis revealed distinct differences in gene expression based on HIV status. For example, the tight junction protein claudin 3 (CLDN3) was expressed at lower levels in organoids from HIV+ as compared to HIV− individuals, indicating a propensity for reduced barrier function. ADAM10, a proteinase responsible for the initial cleavage of the extracellular Notch receptor domain, and TGF-β receptor type 2 known to regulate intestinal stem cells differentiation were both significantly upregulated in differentiating organoids grown from HIV+ individuals, indicating hyperactivity of critical Notch and TGF-β pathways in intestinal stem cells during HIV infection. These results point to long-term alterations in signal response and gene expression in intestinal stem cells during HIV infection, which could explain persistent impaired regenerative capacities of the gut epithelium and barrier function.
Assessment of SIV susceptibility, immunity and microbiome in the female genital tract of adolescent versus adult pigtail macaques

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Background
In southern Africa, young women aged 15-24 contribute to nearly 30% of all new HIV infections. Although social, high-risk behavioral, access to healthcare services, and economic factors all contribute to increased HIV susceptibility in young women, biological factors likely play a significant role. Here, we use the pigtail macaque (PTM) model to investigate signatures of susceptibility in the female genital tract (FGT) of adolescent versus adults, in hopes to develop an adolescent NHP model to test altered susceptibility to HIV infection.

Methods
Comprehensive FGT samples were collected across the menstrual cycle over a two-month period in 10 adult and 10 adolescent PTMs, followed by multiple low-dose (LD) intravaginal SIV challenges. Flow cytometry was performed on vaginal biopsies to assess immunity. Inflammatory markers were measured from cervicovaginal cytobrush supernatant via Luminex. 16S sequencing was performed on vaginal swabs. Vaginal biopsies were profiled by tandem mass spectrometry for proteomic analysis.

Results
Our model identified no significant differences in SIV susceptibility to LD intravaginal challenges between adolescent and adults PTMs. No significant differences in HIV target cells were detected in the vagina. Significantly more HLA-DR+ CD20+ B cells in the vagina were detected in adolescent PTMs (p=0.0435), while CD8+ T cells were increased in the vagina of adult PTMs (p=0.0369). Inflammatory markers assessed from cervicovaginal cytobrush supernatant were higher in the adult PTMs compared to adolescents such as, sCD40L and MCP-1 (p=0.0220; p=0.0041). No significant vaginal microbiome alterations were detected between adolescent and adult PTMs. Proteomic analysis identified 1687 proteins in vaginal biopsy samples. Euclidean clustering of proteins showed no relationship with age, and differential protein analysis did not identify any significant differences at a 5% FDR between age groups.

Conclusions
Our PTM model did not reveal the enhanced HIV susceptibility that is seen in adolescent females, which is supported by our negative data of any mucosal characteristics of increased SIV susceptibility in adolescent PTMs. A validated SIV NHP model of HIV infection in adolescents is currently absent and is critically needed in order to better understand and prevent increased HIV infection in adolescent girls.
Metronidazole Treatment Failure of Bacterial Vaginosis Not Linked to Community Structure, Individual Taxa, or Host Immune Profile

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Background: Bacterial Vaginosis (BV) is a polymicrobial dysbiosis without a single causative agent. Pathogenesis stems from the collective interactions between a diversified microbial community and the human host, who has lost the homeostatic protections associated with *Lactobacillus* dominance. Antibiotic treatment frequently fails to return affected women to a Lactobacillus dominated microbiota, leading to persistent dysbiosis and suggesting that specific community structures or the presence of keystone taxa could facilitate resistance.

Methods: Baseline cervicovaginal lavage (CVL) samples were collected from 33 BV+ (Nugent score 7 – 10) women enrolled in an open-label oral metronidazole study; 2 subsequent samples were collected at one week and one-month post-treatment. DNA isolated from CVL was amplified following Earth Microbiome Project protocols and sequenced using a paired-end, 2x150 MiSeq run of the V4 region; 16S rRNA analyses were performed in QIIME2 and R.

Results: Samples were categorized into cervicotypes (CTs) by dominant organism as previously described: CT1, *Lactobacillus crispatus*; CT2, *Lactobacillus iners*; CT3, *Gardnerella vaginalis*; and CT4, dominated by a number of diverse anaerobes. Of the 30 women classified as CT4 at visit 1, we observed 4 clearance patterns defined by the occurrence and duration of a cervicotype shift to CT2: no clearance (20%), transient clearance (37%), delayed clearance (10%), and sustained clearance (33%). These groups were indistinguishable at visit 1 when considering community structure, presence and abundance of individual taxa, and host immune profile. While immunological analysis demonstrated distinct host profiles in relation to CTs, we saw no significant variation within a given CT when considering the 4 clearance profiles.

Conclusions: We demonstrate that successful treatment of BV was not dependent on community structure, presence or absence of individual taxa, or host immune profile prior to antibiotic administration. Observed CTs were associated with distinct soluble immune mediator profiles in CV fluid, with no significant variation when stratified by clearance profile, suggesting changes in taxa are more deterministic of local immune response than host genetic variation. Taken together, these findings suggest that the function of these communities may be the most important determinant of BV treatment outcome. This necessitates future studies utilizing shotgun metagenomic approaches to better understand the functional and strain-level determinants of persistent BV in women.
A Liquid Chromatophy-Mass Spectrometry based investigation into the effect of asymptomatic sexually transmitted diseases on the foreskin metaproteome.

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Entry of HIV into the male genital tract involves penetrating the epithelial barrier subsequently infecting host target cells. Globally, up to 75% of HIV-infected males acquired HIV via the penis. Very little is known about the transmission events in males. The foreskin is known to act as a viral barrier in the male genital tract. Symptomatic STI's have been shown to increase the risk of HIV acquisition and transmission due to the high levels of inflammation and the formation of lesions. Asymptomatic STI's (aSTI) such as chlamydia can cause other reproductive health diseases. The presence of (aSTI's) may contribute to increased risk of HIV acquisition as well. We investigated how host and microbial proteins are associated with aSTI's.

150 HIV negative adolescent males (13-24 years old) were recruited for a study investigating the effects of aSTI's on the density and localisation of HIV target cells. An in-house shotgun LC-MS workflow was developed and peptides run on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer. Bioinformatic analysis using the Maxquant package was used to analyse spectra using the MetaNovo pipeline. Statistical analysis using MSstats R package for the relative quantification of proteins. For Gene Ontology (GO) analysis, we utilized the topGO package. Unipept lowest common ancestor analysis was performed on de novo sequences of spectra from the FS samples.

1841 proteins were identified, with 400 showing a differential expression between aSTI and controls with an adjusted p ≤0.05. GO terms with various immune related function were significantly different between the differentially enriched ontologies between the aSTI and control samples. The dominant type of immune responses in the aSTI samples were proteins involved in the regulation of humoral immune function (GO:0019730 Fischer’s exact test p=0.00031) compared to the cell mediated immune responses enriched from proteins expressed by men without aSTI. Foreskins from men without any aSTI’s detected had enriched functions involving Myeloid and neutrophil activation (e.g GO:0002275). We looked at all the GO functions associated with epithelial barrier function focusing on cell adhesion and cell junctions. More GO functions in this group were enriched in the control samples 15 exclusive terms in the control and 2 exclusive in the aSTI with 4 being common. We also showed that the microbial proteins of the aSTI samples were enriched for proteins from the Firmicute and Protobacteria phyla (p value= 0.001).

Increased Epithelial Density of Foreskin T cells in Men with Penile Anaerobes Associated with HIV-Risk

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Background: Circumcision reduces HIV-risk in heterosexual men by an unknown mechanism. We have previously shown that specific taxa of anaerobic bacteria in the uncircumcised penile microbiome are associated with chemokine production and HIV-risk. Penile anaerobes may increase HIV-risk by triggering local inflammation resulting in recruitment of HIV-susceptible immune cells to the tissue
surface. We thus evaluated the association between T cell density/location in the foreskins and penile anaerobes.

Methods: Foreskin T cell density was compared between men with high abundance of seroconversion-associated anaerobes (n=6) and low overall bacterial load (n=6). This analysis was nested in a previous cross-sectional study of 88 HIV-negative heterosexual men undergoing elective circumcision in Rakai, Uganda. DNA from sub-preputial swabs and absolute abundances of penile bacteria were estimated as the log_{10} 16S rRNA gene copies/swab by pan-bacterial real-time PCR and sequencing of the 16S rRNA V3V6 region. T cell densities in the epidermis and dermis of frozen tissue sections from both the inner and outer foreskin were quantified using immunohistochemistry (CD3) and ImageJ.

Results: Men with seroconversion-associated anaerobes had a localized increased density of T cells in their inner foreskin, with no increase in the outer aspect. T cells were near absent in men with low bacterial load. While increased T cell density was observed in both the epidermis (495.8 vs 40.1 cells/mm², p=0.09) and dermis (103.5 vs 2.2 cells/mm², p=0.03), the increase was 4.5-fold greater in the epidermis (Figure 1).

Discussion and Conclusions: High abundance of seroconversion-associated bacteria on the penis was associated with a localized increase of T cells in the inner foreskin, driven predominantly by infiltration into the epithelium. The presence of HIV-susceptible cells close to the tissue surface may explain increased HIV-risk. Further studies are warranted to determine the phenotype of infiltrating T cells.

**Monoclonal Antibody-based Multipurpose Prevention Technology: Preclinical and Ex vivo Efficacy Studies**

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We are developing human monoclonal antibody (mAb)-releasing vaginal films and rings as a multipurpose prevention technology (MPT) platform to provide women protection against sexually transmitted infections (STIs) and unintended pregnancy. Our first product, MB66, is a vaginal film that releases mAbs that neutralize HIV-1 (VRCo1) and Herpes Simplex Viruses 1 and 2 (HSV-8). The antibodies were manufactured to GMP standards in *nicotiana* (*N*), i.e. tobacco plants. Preclinical testing demonstrated that the VRCo1 mAb-N: 1) neutralized several strains of HIV-1 and SHIV, 2) retained activity following exposure to low pH (> 3.5) and to human vaginal secretions and semen, 3) protected macaques from vaginal low dose SHIV infection, and 4) did not elicit inflammation in macaques and in a vaginal tissue model. The HSV mAb-N neutralized HSV *in vitro* under the physiological conditions listed above, and protected mice against vaginal HSV infection.

We recently completed a Phase 1 clinical trial with MB66 film: Segment A entailed administration of one vaginal film containing 10mg @ of VRCo1 and HSV mAb-N, and Segment B entailed administration mAb-N or placebo films on 7 consecutive days. We tested cervical vaginal lavages (CVLs) from the women before and after film use for *in vitro* neutralization of HIV and HSV-2, and proinflammatory cytokines. Data from Segment A revealed HIV and HSV neutralizing activity in CVLs 24 hrs after film insertion, and no increase in proinflammatory cytokines. Data from Segment B have not been unblinded but indicate no increase in proinflammatory cytokines after film use and strong antiviral activity in CVLs from a subset of subjects.
We are currently working with a mAb-N (anti-CD52g) with strong sperm agglutination activity to potentially provide women with an MPT option that delivers contraception as well as protection against STIs.

**Antibody inhibition of HIV infection/transmission in DC and mucosal tissues**

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The understanding of the first steps of HIV interactions after sexual transmission are crucial in the vaccine field as antibodies induced by vaccination should impair these early events. In particular, the role of dendritic cells (DC) at the mucosa sites in capturing HIV, replicating the virus and/or directly transferring it to surrounding T cells in the tissue microenvironment need further investigations. The aim of our study is to identify DC infection/transmission in various conditions including mucosa DC infection and to decipher the potential inhibitory activity of antibodies in these early infection events.

Either DC generated from blood monocytes and cocultured with autologous CD4 T cells or cells dissociated from tissue explants of female reproductive tract (FRT) were infected with transmitted founder HIV-1 for 2 days, with or without HIV-specific antibodies. The phenotype and distribution of T cells, DCs and macrophages (Mf) subsets was analyzed by multicolor flow cytometry. The percentage of the different cell population infected and the inhibition of these infected cells by antibodies were determined. In addition, GFP and mCherry labelled HIV-1 were tracked in polarized FRT tissues infected by immunofluorescence and confocal videomicroscopy to define the first HIV infected cells in FRT.

We found that DC generated from blood and DC dissociated from FRTs were infected and replicate HIV when co-cultured with other immune cells. This DC infection was inhibited following dissociation of DC/CD4 T cell cross talk and in the presence of broadly neutralizing antibodies. In cells dissociated from FRT tissues, the percentage of DCs (CD11c+CD64-) infected was similar to the percentage of T cells infected although DC represent only 6 % of total CD45+ cells. Moreover, fluorescent-HIV is tracked in polarized FRT explant to identify the first HIV target at mucosa site.

These results strengthen the role of DC in the mucosa environment as strategic cells involved in the early events of HIV infection and replication and suggest that future HIV prophylactic vaccine design should develop new strategies to prevent infection of DC present at the mucosa site.

**Mucosal delivery of TFV via hypo-osmolar douche as an efficacious on demand alternative to oral daily PrEP to prevent the acquisition of HIV.**

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Oral daily pre-exposure prophylaxis (PrEP) with Truvada\textsuperscript{8} (TFV/FTC) has been approved and has shown >95% efficacy in preventing HIV acquisition in people at risk of infection. However, its efficacy is tied to good adherence to treatment and its use suffers from high costs in developed countries. While long acting formulas are being developed solving the former issue, many individuals worry about continuous treatment and would prefer an on demand strategy protection. We have tested and optimized such behaviorally congruent on demand approach for MSMs using hypo-osmolar (HOsm) formulated TFV rectal douches and shown that such approach achieve the highest levels of active drugs in the mucosal tissue, while allowing for a peak of circulating TFV levels comparable to a single oral PrEP dose. Explant cultures collected at various times post douche showed good protection from infection ex vivo. Vaginal douching, while more limited in volume also achieved mucosal tissue levels commensurate with protection from SHIV acquisition. Single rectal douches with 30 ml of HOsm 5.28 mg/ml TFV were compared side by side for efficacy with daily oral Prep with TFV alone (22 mg/Kg) or TFV/FTC (22mg/20mg/Kg) in a series of repeated rectal challenges with SHIV162p3. On demand HOsm rectal douching provided equal or better protection for up to 24 hours post douche, when compared to daily oral PrEP with one or 2 drugs in this stringent challenge protocol. These results demonstrate the efficacy of on demand topical ART delivery in preventing SHIV acquisition at relatively low cost and behaviorally congruent approach.

**Piecing Together the Mechanism of Transcytosis, One Endosome at a Time**

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In 2017, 1.8 million new cases of Human Immunodeficiency Virus (HIV) were reported worldwide, accompanied by 940,000 deaths caused by AIDS-related illnesses. In the United States the majority of new cases belong to the at-risk group, Men who have sex with men (MSM). Research to kill the virus, reverse latency, and prevent further spread is paramount to curing HIV. In MSM a major, yet largely uncharacterized route of HIV entry into the host, is via transcytosis - the transport of virus across a cell, through the colonic epithelium. We hypothesize that transcytosis of HIV is a multi-step process involving (i) virion binding to the apical surface of the colonic epithelium, (ii) viral endocytosis, (iii) transport of the virion across the cell, and (iv) HIV release from the basolateral membrane. Our project characterizes the mechanism of transport using human Caco-2 colonic epithelial cells plated as an intact monolayer in transwells, focusing on 1) The mechanism and timing of viral release from the epithelial cell. 2) The location of virus during transport. 3) The mechanism and type of endocytosis. We incubated GFP-labeled HIV on the apical surface of a polarized epithelial monolayer for 2 hours, washed away the virus, and then harvested the basolateral media to measure viral particle release from 2 to 24 h. RT-qPCR of the viral RT gene revealed transcytosis is a dose dependent process that shows cooperativity, with released virus first detectable at 18 h. Deconvolution microscopy revealed HIV within the confines of the epithelial cells, with no virus detectable between cells, indicating that viral transport is transcellular. Treatment of the monolayer with the endocytosis inhibitors, Dynasore, Pitstop 2, and Nystatin, revealed transcytosis is predominantly mediated by caveolin-coated endosomes. Our findings suggest that...
transcytosis is a dose and time dependent process, mediated by caveolin-based endocytosis and microtubules.

**Extracellular vesicles from human semen induce tolerance in antigen-presenting cells and decrease antigen-specific T cell responses**

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Exposure to semen is the primary route of transmission for many sexually transmitted infections. Accumulating evidence suggests that components in semen directly impair leukocytes, which could compromise mucosal immunity against infections. Human semen contains trillions of extracellular vesicles (SEV) (average 2.2 x 10¹³ particles per ejaculate n = 18). Using a new click chemistry technique, we labeled SEV with fluorescent quantum dots and showed that they penetrated the cervicovaginal barrier of mice and reached the local lymphatics. SEV entered human antigen-presenting cells (APC) and reduced downstream memory T cell function, impairing the production of cytokines in response to viral antigens an average of 40% for CD4⁺ T cells and 41.3% for CD8⁺ T cells. SEV exposure to APCs alone markedly reduced antigen-specific cytokine production, degranulation and cytotoxicity by CD8⁺ T cells. CD4⁺ T cell inhibition required simultaneous exposure of APCs and T cells to SEV. SEV did not alter MHC or co-stimulatory receptor expression on APCs, but impaired glycolysis and induced upregulation of indoleamine 2,3 deoxygenase, an enzyme known to inhibit T cells. SEV-mediated T cell inhibition varied between, but was consistent across different functional assays within, recipients. Thus, SEV reduce the ability of APCs to stimulate T cells and thereby tune immunological tolerance versus pathogen defenses in the recipient mucosa. Understanding how programmed immune responses in the mucosa are altered by the presence of semen is important to develop better vaccines and improve fertility outcomes.

**The role of cannabis use in mucosal immunity and bacterial fermentation in HIV-infected individuals**

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**Background:** Despite viral suppression with antiretroviral therapy (ART), HIV-infected individuals still have increased gastrointestinal (GI) dysfunction, which is highly associated with morbidities and mortality. In the United States, medicinal cannabis and self-medicated use are common to treat GI symptoms. Additionally, several studies have highlighted the ability of cannabinoids to alter immune responses and decrease inflammation. The impact of cannabis use on HIV-associated inflammation and
immune activation is not well understood. We hypothesized that during HIV infection, cannabis use can decrease inflammation and immune activation through improving gastrointestinal immunity and health.

**Methods:** We assessed peripheral and colon immune cell frequency, activation and function in 28 HIV+ adults using flow cytometry. 13 of the HIV+ individuals were cannabis users after measuring plasma cannabinoid metabolomes using LC-MS/MS. Short-chain fatty acids (SCFA) concentrations in stool were assessed by GC-MS.

**Results:** We observed that overall T-cell frequencies in blood and colon were similar in cannabis users when compared with non-cannabis using adults. Within the T cell population, we observed decreased frequencies of activated CD4+ and CD8+ T cells as measured by HLA-DR+CD38+ expression, in the blood (p=0.0027, p=0.0087) and colon (p=0.0080, p=0.0023), of cannabis users as compared to non-cannabis using individuals. Cannabis users also demonstrated decreased frequencies of intermediate and nonclassical monocyte subsets (p=0.0007, p=0.0080) and decreased frequencies of TNFα-producing antigen presenting cells in blood and colon (p=0.0013, p=0.0003). Cannabis users also had increased butyrate levels (p=0.0001), as well as total SCFA production (p=0.0001) in stool when compared with non-cannabis using individuals.

**Conclusions:** We demonstrate that cannabis use can decrease systemic inflammation and immune activation amongst HIV+ adults through improved GI function and decreased immune activation in the gut. Cannabis use can potentially alleviate gut dysfunction through increased fermentation and available energy via SCFAs. With a large population of HIV+ individuals reporting cannabis use, more information is needed regarding the impact on human health.

**The neovaginal microbiome and mucosal proteome of transgender women post-gender reassignment surgery**

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Transgender women represent a population that are 49 times more likely to acquire HIV than the general population. Polymicrobial communities in the vagina have been linked to increased genital inflammation and HIV acquisition risk. Limited data regarding the composition and function of the neovaginal microbiome represents a gap in knowledge that could help explain this population’s increased risk for HIV infection.

Metaproteomics was performed on secretions collected from neovaginas (n=5) and rectums (n=7) of male-to-female transgender women, and compared with vaginas of cis women (n=32). Bacterial protein information obtained was used to map and characterize the composition and function of each individual’s microflora. Protein functions were inferred from the KEGG Pathway, ConsensusPathDB and Uniprot databases.

We identified 541 unique bacterial proteins from 38 taxa. The most abundant taxa in the neovaginal samples were *Porphyromonas* (30.2%), *Peptostreptococcus* (9.2%), *Prevotella*
(9.0%), Mobiluncus (8.0%), and Jonquettella (7.2%). Rectal samples (71.4%) and natal vaginal samples (87.5%) were mainly composed of Prevotella and Roseburia, and Lactobacillus and Gardnerella, respectively. Neovaginas (median Shannon’s H index=1.33) had more diverse bacterial communities compared to natal vaginas (H=0.35, p=7.2E-3, Mann-Whitney U-test). Shannon H indices positively correlated with neutrophil degranulation (n=12, p=1.45E-6), fatty acid degradation (n=4, p=2.8E-5) and cellular response to stress (n=6, p=4.46E-3), and negatively correlated with the humoral immune response (n=6, p=2.06E-7) and cornification proteins (n=6, p=5.12E-8). Cornification and keratinization proteins unrelated to bacterial diversity were also found to be decreased in the neovagina with possible links to estrogen levels.

Neovaginas have polymicrobial communities that elicit similar host responses to cis vaginas with bacterial vaginosis including decreased cornification and increased protection against oxidative stress. The combination of polymicrobial communities and epithelial barrier disruption in the neovagina may contribute to the increased risk this population experiences for HIV infection. Follow up studies are required to further validate these findings.

**Analysis of the effect of HIV-1 infection and antibody virus depletion on viral particle mobility in mucus.**

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The majority of new HIV-1 infections are acquired via sexual transmission across mucosal barriers. To initiate the infection in the female reproductive track, the virus must first cross a layer of mucus found covering the cervical and vaginal epithelia to infect the susceptible immune cells protected by these anatomical barriers. This mucus layer contains various innate and adaptive immunity components, including physical barrier properties, comprising the first defensive mechanism against HIV-1 infection. Thus, it is critical to study how the virions move through mucus and which factors can alter this mobility, to understand HIV-1 transmission across the mucosal barrier.

In the present study, we have analyzed the mobility of HIV-1 in cervicovaginal mucus (CVM) ex vivo using fluorescent microscopy. We used state-of-the-art technology that digitally tracks virions and 200-nm PEGylated beads to calculate their mean-squared displacement. To examine the effect of HIV-1 infection on virus mobility in mucus, we compared the displacement of both virions and beads in CVM collected from HIV-1 infected (n=51) and un-infected women (n=18). To gain a deeper understanding of viral mobility across mucus, in which the viral envelope plays a key role, we tracked wild-type HIV-1 and Δenv virus and analyzed the effect on mobility of viral depletion by antibodies. We used 3D6 and VRC01 to target wild-type env, and HIV-Ig for the Δenv virus.
The results obtained showed an overall lower particle mobility in mucus from HIV-1 infected participants. When testing the effect of antibody depletion in the HIV-1 infected participants, we observed a very significant decrease in virion mobility caused by VRC01 and, unexpectedly, by 3D6. Instead, the depletion with HIV-Ig increased the mobility of both Δenv virions and beads. This analysis depicts a complex system of interactions occurring between virus and mucus that cannot be just explained by simple binary interactions.

An ODE model of Bacterial Growth Dynamics and MNZ resistance in the context of bacterial vaginosis

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Submitted by: Christina Lee (1st year Ph.D. student)
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The treatment of bacterial vaginosis (BV) primarily depends on the use of broad-spectrum antibiotics like metronidazole (MNZ). These antibiotic treatments are highly variable and recurrence rates remain high. The pathogenesis behind this recurrence is unknown, though proposed mechanisms include Gardnerella vaginalis resistance to MNZ treatment. We developed an ordinary differential equation (ODE)-based model to give quantitative insight into the relationship between varying MNZ levels and the effect on in-vitro cultures of G. vaginalis and Lactobacillus iners. The model was parameterized and validated using in-vitro data generated by our collaborators in the Klatt Lab. The model assumes inhibition of G. vaginalis growth is a function of internalized MNZ. We designed the model to capture how the concentration of internalized MNZ fluctuates dependent on bacterial growth rates, MNZ internalization rate, and MNZ conversion rate to metabolites. These rates were determined by nonlinear least squares curve-fitting of the ODE model to the experimental data. Our model demonstrates how bacterial-mediated interactions of MNZ could account for MNZ resistance in G. vaginalis. Additionally, we demonstrate in silico that the efficacy of MNZ on decreasing G. vaginalis in a mixed bacterial population is dependent on the initial ratio of G. vaginalis and L. iners. Variability in MNZ efficacy occurred between 200 g/ml-700 g/ml, with concentrations less than 200 g/ml ineffectual. Interestingly, MNZ was most efficacious at inhibiting G. vaginalis growth between 315-750 g/ml when G. vaginalis is 10-102.5-fold greater than L. iners. At ratios of G. vaginalis to L. iners higher than 102.5, G. vaginalis could survive in up to 1833 g/ml MNZ. Further analysis and validation from co-cultures of G. vaginalis and L. iners will provide additional insight on the complexities behind MNZ treatment of BV. Moreover, this model potentially provides a quantitative framework for predicting complex polymicrobial growth dynamics given personalized differences in microbial communities and varying concentrations of therapeutics.
2019 HIV Mucosal Systems Meeting Abstracts

POSTER SESSSION (In alphabetical order)

Investigating the Role of Cysteine Domains of Gel Forming Mucins Against Viral Pathogens in Conjunction with Antibodies

Authors: Koree W. Ahn, Thomas J. Hope

Abstract:

Nearly two-million individuals become infected with HIV yearly. Despite significant efforts, current vaccine efforts are unable to elicit an appreciable protective effect. To address this continuing need for an effective vaccine, alternative approaches that to enhance efficacy must be explored. One such avenue is to study HIV-1 targeting antibodies at the most common site of transmission, the mucus membrane of the female reproductive tract (FRT). The cells of the FRT mucus membrane are shielded by mucosal secretions composed in a large part by gel forming mucin protein, MUC5AC. MUC5AC is a large protein and its heavily glycosylated bottlebrush like structure gives mucus its viscous characteristics that in turn lubricate the FRT and physically protect cells from pathogens. We have recently shown that MUC5AC can bind tightly to IgG constant regions and perform a novel effector function. In this effector function, the presence of anti-HIV envelope antibodies in mucus drastically decrease the motility of viral pathogens multiple orders of magnitude in comparison to mucus with no anti-envelope antibodies. Characterizing this effector function and subsequently optimizing antibody-based treatments in the mucosal environment could lead to the development of superior therapeutics and possibly a vaccine. Here we show that the Cys domain (CysD) of MUC5AC plays a critical role in the mechanism of this effector function. This small cysteine rich domain has been attributed with the oligomerization of mucins due to its non-glycosylated state in comparison to the rest of the MUC5AC structure. When a recombinant CysD was introduced to our mucins it enhanced the effector function by further decreasing the motility of viral particles in the presence of anti-Envelope antibodies. To delineate the mechanism of this effector function and study the nature of MUC5AC oligomerization, we are currently investigating the interaction of CysD with the other domains and regions of MUC5AC.

Th17 cells are early targets of SIV infection at 72hr post intravaginal challenge

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Identification of infected cells immediately after mucosal HIV/SIV transmission is important for the development of effective prevention strategies. Previous studies have identified CD4+ T-cells, especially, Th17s, as early targets of HIV/SIV in the female reproductive tract (FRT). Using the rhesus macaque (RM) SIV vaginal challenge model, we sought to identify early targets of infection and how target cell phenotypes change over time during acute infection.
12 female RMs were challenged intravaginally with a non-replicative luciferase reporter, LiCH, and wildtype SIVmac239 mixture. Animals were sacrificed either 48-, 72-, or 96-hours post-challenge and FRTs were removed. Macroscopic luciferase signal detected by in vivo imaging system (IVIS) allowed us to identify tissue regions likely containing infected cells. IVIS positive and negative tissues were serially cryosectioned for immunofluorescence staining and RNA isolation. Infected cells were phenotyped by microscopy to identify Th17s (CD3+CCR6+), other T-cells (CD3+), immature dendritic cells (iDCs)(CCR6+), and other cells (CD3-CCR6-). RNA was extracted from infected and non-infected adjacent tissue sections for RNA-Seq.

Here, we are reporting phenotypic analysis of SIV-infected cells in FRT of 4 RMs sacrificed at 72hr post-challenge. Infection foci were identified throughout FRT in 3/4 of the animals illustrating a vaginal squamous mucosa preference. Phenotypic profiling of 1,164 infected cells in FRT tissues revealed that Th17s accounts for 70.8% of cells infected early after infection followed by iDCs (27.3%). These findings corroborate previous data identifying the entire FRT susceptible for infection with Th17s as early targets. We are also performing analysis on FRT tissues from animals sacrificed at 48hr and 96hr post-challenge. Also, comparing the transcriptome profiles between infected and non-infected tissues at different time points will help to understand early cascades of host immune responses. Combined, these data will present a clearer picture of virus transmission at FRT mucosa and hence, aid in developing effective prevention approaches.

Unraveling mechanistic complexities of antibody-Fc receptor interactions with a quantitative systems approach
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Surprisingly, the first and only human HIV vaccine trial (RV144) that showed modest (31.2%) but significant efficacy did not induce broadly neutralizing Ab responses, but instead induced Abs with the capacity to activate Fc-mediated functions via engagement of the Ab Fc (Fragment crystalizable) portions with cellular Fc receptors (FcRs). Mechanistic understanding of how Ab Fc-FcR interactions activate antiviral cellular functions is a major focus of the HIV vaccine field, however a major challenge lies in deconvoluting the relative contribution of multiple parallel Ab and FcR features that have the capacity to alter protective Ab complex formation and associated cellular functions. Concentration and binding properties of Ab-FcR systems can be altered by host genetic factors (Ab allotypes and FcR polymorphisms), post-translational modifications (glycosylation), vaccination regimens, differential expression of FcRs on innate cells, and in systemic vs. mucosal tissue environments. Experimental evaluation of all possible changes in HIV-Ab-FcR interactions in isolation is extremely laborious and in some cases not feasible. Furthermore, experiments alone do not give insight into unexpected synergisms that may arise from multiple parallel alterations. Here we will describe a transformative approach using ordinary differential
equation (ODE)-based models to gain new quantitative insight into the relative importance of Ab modifications driving protective Fc-mediated Ab complex formation in HIV. Overall we illustrate how these models are able to 1) determine the relative importance of genetic background in protective complex formation; 2) assess mucosal responses vs. systemic responses; and 3) predict personalized differences in individuals based on unexpected synergies between genetic and post-translational antibody features.

IL-18 producing NK-B cells contribute to HIV/SIV-associated chronic inflammation in the colon through increased IFN\(_{\gamma}/\text{TNF}\alpha\) production by ILCs

\(^1\)Andrew Cogswell, \(^2\)Stephanie Dillon, \(^1\)Natasha Ferguson \(^2\)Moriah Castleman, \(^2\)Cara Wilson and \(^1\)Edward Barker

Chronic systemic and mucosal inflammation is responsible for increased morbidity and poor clinical outcomes among patients with HIV. These long-term complications are linked to the disruption of gastrointestinal (GI) tract epithelial barrier integrity and subsequent microbial translocation. However, the mechanisms responsible for these downstream effects of infection are unknown. Here we link infection-related changes in inflammatory cytokines released by innate lymphoid cells (ILCs) to the release of ILC-triggering cytokines from natural killer B (NK-B) cells ectopically located in the colon secondary to SIV infection. Samples of fresh colon tissue collected from SIV-infected and uninfected rhesus macaque monkeys revealed the absolute number of ILCs producing IL-22/IL-17 was not altered during SIV infection, however, the combined number of IFN\(_{\gamma}\)+ILCs in infected colons was higher. T-cells from the same tissue did not contribute to cytokines. ILCs from infected tissue that produced IFN\(_{\gamma}\) also expressed TNF\(\alpha\). IL-18 secreted from NK-B cells in the lamina propria of infected colon can drive IFN\(_{\gamma}\) expression from the ILCs given IL-18 receptor expression on almost all ILCs. These events combined likely triggers a pathway that contributes to chronic inflammation, GI barrier breakdown, and microbial translocation within the context of SIV/HIV infection.

Interaction of HIV, Mucins, and IgG Affects Viral Motion at a Mucosal Surface

Authors: Miranda Becker, Viktoria Zaderer, Doris Wilflingseder, Thomas Hope

For most people who acquire HIV, an early step of infection is transit of the virus across a mucosal surface. The first barrier that virus encounters there is mucus. How virions interact with mucus can determine whether they are sloughed away from the epithelium or whether they get close enough to infect. In the upper female reproductive tract, the major component of mucus is the gel forming mucin MUC5AC. These epithelia also express MUC16, a cell surface mucin. The Hope lab has shown that MUC5AC and MUC16 bind tightly to IgG and that MUC16 binding is modulated by IgG glycosylation. We have also found that that IgG-MUC5AC interaction enhances HIV neutralization, and that this interaction alters the motion of particles in the gel. These results suggest a new effector function of IgG. However, the effect of antibody-mucin interactions at the mucosa is unknown. To clarify this, we have developed a mucosal model system based on bronchial epithelium. Like the columnar epithelium of the upper
female reproductive tract, this columnar epithelium produces MUC16 and MUC5AC, making it an appropriate model in which to study interactions of HIV, antibodies, and mucins. Using a fluorescent labelled anti-HIV Env IgG, we see that antibody accumulates in ciliated cells. Further studies will use fluorescent microscopy of living cultures to probe kinetics of antibody localization and effects of HIV-IgG-mucin interaction on HIV mobility. Use of virus populations depleted of particles that bind specific antibodies and CRISPR-Cas9 knockouts of MUC5AC and MUC16 will demonstrate mechanistic dependence upon binding in the previous experiments. The completion of this study will shed light on a crucial interface of innate and adaptive immunity.

**Circumcision Increases the Barrier Function of the Glans Epithelium**

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**Background:** Circumcision reduces the risk of heterosexual penile acquisition of HIV by about 60% and is recommended by the WHO as part of a comprehensive protection strategy. The foreskin has been extensively studied and shown to be rich in HIV target cells. But little is known about the effects of circumcision on the glans as it transitions from being in a moist environment to being constantly exposed to air.

**Methods:** We recruited 21 circumcised and 21 uncircumcised adult men in Chicago. The glans and shaft were evaluated for skin barrier function with noninvasive hydration and water loss meters. A shave-biopsy was obtained from the glans and shaft to be processed. A portion of each biopsy was exposed to photoactivatable GFP labelled Bal virus for 4 hrs then frozen in optimal cutting temperature compound (OCT). Cryosectioned tissues were imaged by deconvolution microscopy to measure virus penetration depths.

**Results:** The moisture meters revealed that total water content and trans-epithelial water loss in circumcised glans were significantly lower than in uncircumcised glans or shaft, a sign of improved barrier function. We also measured the penetration of paGFP-tagged virions in the tissues. The penetration profile in these tissues is similar up to a depth of 25um. However, while shaft and circumcised glans only let about 5% of penetrating virions beyond 25um, in uncircumcised glans 10% of virions were deep penetrators.

**Conclusions:** Our moisture meter data and virus penetration data suggest in two completely independent ways that circumcision increases the barrier function of glans epithelium. First, circumcised glans exhibited reduced total water content and trans-epithelial water loss, and in a manner that is detectable by simple, portable, non-invasive meters. Second, the percentage of penetrating virions that are deep penetrators is halved in circumcised glans with respect to uncircumcised tissue.
SIVmac239 infected cells persist in vaginal mucosa over the course of ART treatment in intravaginal infected rhesus macaques.


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While much of the focus on woman HIV reservoir has been on blood cells, lymphoid tissues have additional reservoirs of latent and/or persistently infected cells, including cells localized to vaginal mucosal tissues at the site of exposure when sexual transmission occurs. This mucosal reservoir is established during the earliest days of acute infection and is poorly defined.

To define if the vaginal mucosa might be a site of viral reservoir and then a potential source of viral rebound after ART cessation in women, we infected through vaginal route 4 females rhesus macaques with SIVmac239. Two of them are sacrificed right after 96h of infection while the two others are placed on ART and sent to necropsy 3 months after ART initiation. None of these macaques exhibited any systemic proviral load, before and during ART. Female reproductive tracts (FRT) are collected along with their associated draining lymph nodes, and tissues blocks surrounding the site of infection are collected. Every block is tested for DNA and RNA viral detection by q-PCR against Gag. The results show that SIVmac239 spread all around the site of injection at 96h post infection. We've been able to find Gag DNA it in different parts of the FRT like vagina, ectocervix, labia and some draining lymph nodes. Remarkably, we still find Gag DNA in different parts of FRT after 3 months of ART, making vaginal mucosa a strong candidate for a SIV reservoir in females. It's likely than rebound occurs from this mucosal reservoir in macaques experiencing rebound while not having proviral load and antiviral response at the time of ART initiation.

These results demonstrate that FRT mucosa might be a major viral reservoir established in the very first moments of infection. They give us a better understanding of reservoir localization in woman and their kinetic of formation that so far are not characterized. The identification of the viral reservoir have an immense importance in the HIV field, since these reservoirs are the main cause of viral rebound when ART is ceased, and then prevent HIV eradication.

Development of 6-color spectral imaging panel to visualize antibody-dependent cell mediated cytotoxicity (ADCC) dynamics in response to SHIV infection in the rhesus macaque model

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Background: ADCC has been proposed as a viral clearance mechanism of HIV/SIV by the adaptive immune system but has not been well characterized. The broadly neutralizing monoclonal antibodies (bNAb) VRC01 and VRC07 recognize the gp120 epitope of HIV envelope and may be good candidates for mediating ADCC. The objective of this project is to expand beyond the conventional 4-fluorophore panel to visualize markers of ADCC dynamics in vivo in rhesus macaques.

Methods: Here, we intrarectally challenged three adult male rhesus macaques with a high dose of macrophage-tropic SHIV AD8EO then intravenously infused each with 20 mg/kg of Cy5-labeled VRC01, VRC01-LS or VRC07-LS (one per animal) two days later. The animals were necropsied 48 hours post-infusion and their tissues were frozen down in OCT for microscopic
Results: We can successfully spectrally and confocally image six fluorophores simultaneously and unmix their unique signals in rhesus macaque tissue, surpassing the conventional 4-color limit. We have observed the interaction of all three bNAbs with AD8 infected cells. Large-scale phenotyping is pending; however, we have seen CD16+ cells concentrating the bNAbs on their surface in the vicinity of infected cells.

Conclusions: A 6-marker panel can successfully be imaged and unmixed using a hybrid confocal/spectral imaging protocol and has indicated that post-exposure intravenously injected VRC01, VRC01-LS, and VRC07-LS antibodies may mediate ADCC against SHIV infection at the rectal mucosa.

Mucosal proteome libraries of innate and adaptive immunity in the female genital tract

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Background: Inflammation and immunity of the female genital tract (FGT) is an important factor for HIV acquisition and prevention strategies, but the relationships between soluble components in vaginal mucosa and cellular immunity has not been well defined. Here we generated molecular libraries of proteins in cervicovaginal lavage (CVL) associated with innate and adaptive immune cells using a mass spectrometry-based approach.

Methods: Study participants included 48 women enrolled in the Vaginal Mucosal Systems (VMS) study in Winnipeg, Manitoba. Endocervical cytobrush samples were immunophenotyped by flow cytometry and CVL was analyzed by tandem mass spectrometry. Differences in immune cell types were assessed by Mann-Whitney U test, while host proteome pathways were assessed using a modified Fisher’s Exact Test (DAVID Bioinformatics) and Spearman’s correlation when appropriate.

Results: More than 20 immune cell subsets were analyzed and 925 host proteins were confidently identified. B cells (CD19+), neutrophils (CD15+CD16+CD49d-), activated CCR5+CD4+ T cells (CD3+CD4+CD38+HLA-DR+CCR5+), and double negative T cells (CD3+CD4-CD8-) showed the strongest associations with mucosal proteome differences (P<0.05). Host pathways were assessed for all participants based on the relative amount (above/below median) of these 4 immune cells. B cell levels were associated with immunoglobulin mediated immune response (P=3.8E-07) and complement activation (P=2.0E-08), while participants with above median levels of activated CCR5+CD4+ T cells had decreased expression of keratins (P=6.4E-06) and proteins for epithelium development (P=2.2E-02). Double negative T cells negatively associated with complement activation (P=7.0E-07) and serine-type endopeptidase inhibitors (P=8.5E-07). Neutrophils negatively associated with nucleic acid binding (P=3.1E-02) and oxidoreductase activity (P=4.1E-03).

Conclusion: This dataset represents a comprehensive investigation of systems immunology in the FGT. We identified unique biological pathways associated with immune cells important for vaginal mucosal
health including epithelial disruption, protease inhibition, complement activation, and oxidoreductase activity. Cervicovaginal proteome libraries could be a useful tool to assess vaginal immunity for HIV prevention.

**Exploratory, descriptive analysis of soluble immune analytes at diverse mucosal sites**

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Background
Mucosal inflammation is associated with increased risk of HIV-1 acquisition. The use of secreted biomarkers to measure mucosal inflammation is attractive, since secretions can be collected non-invasively and repeatedly, while biomarker quantification can be performed using simple ELISA-based assays. However, sample volumes limit extent of analyte assessment, and many cytokines associated with vaginal HIV acquisition are largely undetectable at other mucosal sites (i.e. foreskin), limiting cross-study comparisons and biological inference. This descriptive study quantified a large panel of soluble immune biomarkers across diverse mucosal sites to serve as a reference for future studies.

Methods: Participants were HIV-uninfected individuals from Canada and Uganda. Males from Rakai (Uganda) provided swabs from foreskin (n=16), urethra (n=4), and anus (n=4) that were re-suspended in ultra-pure PBS. Site-specific swab eluent was pooled to make sufficient volume to assess all 136 analytes without further dilution. In Toronto (Canada), cervical secretions were collected by SoftCup and diluted 1:10. Samples were assessed in duplicate using the MesoScale Discovery platform. Analyte concentrations were visualized by concentration heatmap with unsupervised hierarchical clustering (Fig1).

Results
Analyte detection (>=10-fold LLOD) varied between mucosal sites, with urethral swabs having the most detectable analytes (41.6% >=10-fold LLOD), followed by cervix (29.4%), foreskin (28.5%), and anal swabs (19.9%). Mucosal secretions contained higher concentrations of numerous analytes previously not studied in the context of genital inflammation and HIV-1, including epithelial integrity markers (e.g. YKL-40, MMP-9). Also, several cytokines previously used to score vaginal inflammation had low detection at other mucosal sites (e.g. MIP1α, MIP-1β, IP-10).

Conclusions: Several previously under-studied analytes in mucosal secretions could be useful for the assessment of mucosal inflammation and HIV acquisition risk.

**Topical or systemic TDF administration against SHIV transmission in Pigtail Macaques?**

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Globally, sexual HIV acquisition in young women is double that of men. Effective HIV prevention is under investigation. In this study we evaluate and compare the ability of the TDF- IVR and oral TDF to protect the female reproductive tract (FRT) of pigtail macaques
from early viral spread of SIVmac239, 72 hours after vaginal challenge. We demonstrate the ability to determine the drug distribution within the FRT of TDF-IVR administered macaques and TDF orally treated animals. We show how the path of drug administration impacts early viral transmission within the FRT of these animals.

Three pigtail macaques were treated with TDF-IVRs, while two macaques received placebo IVRs for 28 days. Four macaques were administered oral TDF at 22mg/kg dose once daily for 7 days. All animals were vaginally challenged with a high dose (~10^4-10^6) of a mixture of replicative SIVmac239 and single round non-replicative SIV-based vector expressing HIV envelope, Luciferase, and mCherry reporter genes. The FRTs were analyzed using IVIS, fluorescent microscopy, and real-time PCR to detect early infection events. TFV tissue concentrations were quantified using LC-MS/MS, with 13C-labeled TFV used as an internal standard.

Large foci of SIVmac239 infected cells were found in the FRT regions distal from the ring, such as in uterus, where drug levels were lower than in the tissues proximal to the ring, upper/lower cervix, where smaller foci were observed. In orally treated animals, foci of infected cells were also smaller, and tissue drug concentrations were similar to those in the IVR distal regions; however, the drug distribution was uniform throughout the FRTs in animals with oral TDF administration. Utilizing the combination of PK/PD, fluorescent microscopy, and real-time PCR analysis will help us to understand the impact of the path of drug administration, tissue drug transport, and the occurrence of immune events on early viral transmission.

HIV-1 Clade C Transmitted/Founder Viruses Replicate Efficiently in Human Rectal and Vaginal Tissue Explant Cultures


Background: Characterisation of the targets and kinetics of HIV infection within mucosae is key to the assessment of novel prevention agents. We have infected human cervical and rectal tissue explants with laboratory-adapted (BaL) and transmitted/founder (T/F) HIV-1 viruses from infected individuals to characterise infection in vitro.

Methods: 14 T/F HIV-1 molecular clones derived from the IAVI Protocol C cohort (recruited during acute infection) and BaL were expanded in PBMCs. 6 were clade C, 5 D, 2 A and 1 recombinant A/D. Cervical and rectal tissues from 118 HIV-1-negative volunteers from Chicago, USA and Nairobi, Kenya were infected and viral replication monitored (p24-ELISA) and infected cells characterised by flow cytometry.

Results: 7/14 of viruses replicated in PBMC; 5/6 clade C and 1 each of clade D and A/D. Within viral replication capacity categories (vRC), Clade C viruses replicated more rapidly than others with p24 levels ranging from 611-9361 ng/mL compared with A/D (K3388, high vRC) at 999ng/mL and clade D (K3384, low vRC) at 212ng/mL. BaL replicated to similar levels as clade C high vRC viruses with p24 levels of 13020ng/mL. p24 gag flow cytometry of tissue cells confirmed productive T/F and BaL HIV-1 infection. Among CD3+ T cells on day 15 post-inoculation, the fraction of p24 gag-expressing cells ranged between 0.7-17.3% and 0.6-10.6% for ecto and endo-cervix, respectively, which was not significant (p=0.58). No differences were observed for the proportion of lymphocytes in ecto- and endo-cervix, whereas a greater frequency of CD3+CD4+ T cells out of total lymphocytes was observed in ecto compared with endo-cervix (9.5-31.5% vs. 0.9-14.8%; p=0.0042).

Conclusion: Mucosal tissue explants can be infected with T/F viruses and BaL in vitro and the trajectory of infection varies amongst the donors and different types of tissue. The identification and characterisation
of T/F viruses that replicate efficiently in human mucosal tissue explant cultures provide an important foundation to study HIV transmission in humans.

**An ODE model of antibody-mucin complex formation against HIV in the mucosal environment of the female reproductive tract**

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Submitted by Melissa Lemke (Third Year PhD Candidate - Early Career Investigator)

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Mucosal antibodies play a critical role in protection, and recent research indicates that mucin proteins can interact with the Fc regions of antibodies, potentially increasing protection against infection under specific conditions while unexpectedly decreasing protection in others. Adolescent girls and young women remain uniquely vulnerable to HIV acquisition, with women age 15-24 years 44% more likely to be infected than men of the same age1. Susceptibility varies, and effective preventative strategies will need to account for variation in the female reproductive tract from person-to-person, and over the menstrual and reproductive life cycles.

We have created a model based on ordinary differential equations (ODEs) to generate quantitative systems-level insight into mucin-antibody complex formation against HIV. Our models assess the importance of mucin-antibody binding affinities, concentrations, and the effects of antibody glycosylation on formation of antibody complexes against HIV. Antibody-mucin binding affinity was measured using surface plasmon resonance (SPR), and antibody and mucin concentrations were set based on *in vitro* infectivity assay conditions. Stoichiometric data from isothermal titration calorimetry (ITC) assays guided model construction. Model findings were validated using a library of antibodies and mucins in cell-based infectivity assays and compared to model predictions at steady-state conditions. Interestingly, the model predicted an optimal mucin-antibody ratio, where the fraction of antibody bound to mucin is maximized, and this value varied with antibody-mucin binding properties. Experimental validation confirmed model predictions over VRC01 concentrations of 0-10⁴ nM, including an optimal mucin-antibody ratio of 1:9.8 at 245 nM of VRC01. Experimental validation also confirmed model predictions for a second antibody (VRC03) suggesting an optimum ratio of 1:6.4 at 159.0 nM of VRC03. Overall the model provides a quantitative platform for rapid and inexpensive hypothesis testing based on personalized combinations of antibody-mucin concentrations and binding properties. Insight may provide future quantitative design strategies for preventative therapeutics.

Mucin 16 is a novel Fc receptor that can enhance the neutralization potential of anti-HIV IgG at mucosal sites

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MUC16 is a cell associated mucin that is expressed by columnar epithelial cells of the female reproductive tract where it helps to form a mucus barrier that HIV must traverse during transmission. Recently, work in our lab has demonstrated that IgG can bind to MUC16 and that this binding is regulated by the glycosylation state of both the IgG and MUC16. In addition, IgG from HIV infected patients binds better to MUC16 than IgG from healthy patients due to a shift towards the G0 glycosylation state under conditions of chronic infection. Further work has shown that MUC16 bound IgG from SIV infected rhesus macaques is enriched for certain epitope specificities. Despite these results the mechanism and consequences of IgG binding to MUC16 are not known. Through the use of the HIV neutralization assay we have been able to show that binding to MUC16 can enhance the neutralization potential of anti-HIV IgG such as VRC01 and that this is dependent on the subclass as well as glycosylation state of the IgG. Through further biochemical characterization of the binding interaction we have narrowed down the IgG binding region of MUC16 to the membrane proximal SEA domains. These SEA domains contain conserved cysteine residues as well as two conserved N-linked glycosylation sites. Removal of either N-glycan site on a single SEA domain reduces binding to IgG and removal of both completely abrogates binding. With continued progress towards an HIV vaccine and interest in antibody mediated protection, understanding how antibodies function at mucosal sites is of great importance. Further characterization of the determinants of this MUC16 IgG interaction will allow us to target HIV specific IgG to the sites of transmission and further protect mucosal sites from HIV.

VRC01 helps maintain vaginal epithelium integrity following HIV-1 infection

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Over 37 million people across the world are living with HIV; more than 70% of infected people reside in sub-Saharan Africa, and young women in this region account for the majority of new infections. Despite the many advances made in the prevention and treatment of HIV, millions of women remain vulnerable to infection and would greatly benefit from a novel product to protect themselves. We are developing a topical vaginal microbicide product, a film containing broadly neutralizing HIV monoclonal antibodies, to protect women from HIV infection during intercourse. Previous work by our group and by others has shown that HIV infection can disrupt cell-cell junction integrity, enabling infection of HIV target cells within and below the epithelium. We are using in vitro models of human vaginal epithelium, VK2/E6E7 cells and MatTek EpiVaginal™ tissue models, to further study the mechanisms underlying the disruption of the tight junctions, adhesion junctions, and desmosomes following infection with HIV and to
Introduce whether topical application of HIV neutralizing antibodies can protect against epithelial disruption. Staining of VK2/E6E7 cells has shown a decrease in the density of claudin and E-cadherin four hours after infection with HIV. This disruption of tight junctions and adhesion junctions was partially blocked by pretreatment of the cells with VRC01, a broadly neutralizing anti-HIV monoclonal antibody. VRC01 was also shown to be non-inflamatory to vaginal epithelial tissue. Vaginal application of VRC01 has the potential to be a potent microbicide against HIV infection in vaginal epithelium by neutralizing the virus and protecting the integrity of intercellular junctions.

Characterization of potential HIV target myeloid cells that can migrate from the foreskin epidermis


Introduction: The human foreskin is an immunologically active tissue that is a rich source of lymphoid and myeloid cells. Within sexually active men, the foreskin is suggested to be the main portal of entry for HIV, as its removal through medical male circumcision, lowers HIV acquisition. It is thought that epithelial tissue-resident cells can serve as HIV targets with the potential to support viral replication. To better understand the process of HIV acquisition in the male genital tract, we sought to immunophenotype foreskin Langerhan’s cells (LC) and macrophages which are potential HIV targets.

Methods: Migratory foreskin cells were obtained from foreskin epidermal tissue from 17 adult South African men undergoing vMMC. Briefly, epidermal sheets were obtained from overnight dispase digestion of 1cm² foreskin pieces. Spontaneously migrating cells were collected after a 48-hour incubation in complete R10 media at 37°C. Resident cells were isolated from the subsequent enzymatic digestion by using liberase (5 mg/ml). Langerhans cells and macrophages able to migrate from the epidermis of inner and outer foreskins were identified using two antibody panels a) CD207, CD1a, CD40, CD80/86 and HLA-DR and b) CD11c, DC-SIGN and CD163.

Results: We were able to differentiate LCs and macrophages that either migrated out of foreskin tissue or remained tissue-resident. Migrating CD1a/CD207 foreskin LCs showed significantly higher levels of CD40 (p=0.01), CD80 (p=0.006) and HLA-DR (p=0.02) expression relative to LCs that remained in the tissue co-expressing these surface antigens (p=0.015). Our data suggests that migratory LCs are more activated and mature relative to tissue-resident cells. Conversely, there were no phenotypic differences seen between migratory and foreskin-resident macrophages.

Conclusions: LCs and macrophages are able to spontaneously migrate from foreskin epidermal sheets. The movement of LCs may depend on levels of cell activation and maturation, but this was not observed for macrophages. However, the identity of MI/M2 polarization with migratory or tissue-resident macrophages is required.

Norethisterone enanthate increases mouse susceptibility to genital HSV-2 and HIV-1 infection

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As more women are affected by human immunodeficiency virus (HIV) than any other life-threatening infectious agent, it is important to identify factors increasing a woman’s risk of HIV acquisition. While results are inconsistent, multiple reports suggest that using an injectable progestin for contraception
increases HIV susceptibility. The two most popular injectable progestins are norethisterone enanthate (NET-EN) and depot-medroxyprogesterone acetate (DMPA), and many research groups used animal models to explore biological mechanisms that may underlie the putative connections between DMPA and HIV. We reported previously that treating mice with DMPA lowered genital expression of the cell-cell adhesion molecule desmoglein-1α (Dsg1α) and increased genital mucosal permeability and susceptibility to genital infection with cell-associated HIV-1. Conversely, the immunomodulatory effects of NET-EN treatment in the mouse genital tract were relatively unexplored. In recently completed studies, we discovered that pharmacologically relevant serum NET levels are similarly associated with reduced genital expression of Dsg1α, increased genital mucosal permeability to activated leukocytes and LMW molecules, and increased susceptibility to genital infection with HSV-2 or cell-associated HIV-1. As compromised function of the genital mucosal barrier was detected in the genital tract of both DMPA- and NET-EN-treated mice, our findings indicate these progestins share an effect with potential to enhance genital pathogen transmission. On the other hand, compared to DMPA, NET-EN-mice displayed smaller reduction of genital DSG1α protein, reduced penetration of leukocytes into deeper layers of genital epithelial tissue, and slower onset of HSV-induced genital pathology and lower burden of systemic HIV infection. Also compared to DMPA, NET-EN-treated mice displayed lower genital expression of several kallikrein-related peptidases, proteases known to cleave DSG1α and other cell-cell adhesion molecules. Understanding these newly defined differential effects of DMPA vs. NET-EN in the mouse genital tract may have clinical relevance and is an area of active study in our laboratory.

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**HIV-specific T cell responses in the Female Genital Tract of HIV-positive women can be detected using menstrual blood**

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Despite their important role in combating HIV infection, information concerning antigen-specific T cells within the female genital tract (FGT) is sparse. To determine whether HIV-specific T cells could be detected from cells isolated in the FGT, and to determine how the frequencies of the response compared to peripheral blood mononuclear cells (PBMC), intracellular cytokine staining assays on paired samples of menstrual blood cells (MBC) and PBMC from women with chronic HIV infection was performed. In a small set of women, vaginal and cervical biopsies for comparisons were also obtained. It was determined that the frequency of HIV-specific CD4+ and CD8+ IFN + T cells was similar between MBC and PBMC. This was true irrespective of the HIV antigen tested. Using a polyclonal stimulator, we were able to discern that most of the IFN produced after stimulation resided in CD8+ T cells expressing the mucosal retention receptor E 7(CD103). This was seen for samples obtained from both HIV-positive and -negative women and remnant tissue from the FGT. The frequency of CD8+ HIV-specific T cells secreting IFN in MBC, segregated by the expression of CD103, was statistically significant (p=0.01) with an increased frequency (4.95%) of HIV-specific cells in this population compared to total CD8 T cells (0.4%) or CD8+CD103- T cells (0.5%). Interestingly, no responses to HIV antigens from vaginal and cervical biopsies from these same women were detected in an IFN ELISPOT assay, despite detectable mitogenic responses. These data suggest that T cell responses evaluated in the FGT of HIV-positive
women can be detected using MBC at an increased frequency. This information will enhance our ability to optimize the understanding of the antigen-specific CD8 T cell responses generated in the FGT after infection that could be used to facilitate the development of effective vaccines against mucosal pathogens in the FGT.

Distal Site accumulation of SHIV-SF162P3 viral DNA in lymph nodes, 48hrs after intravaginal challenge, in rhesus macaques that received PGT121 one week prior to challenge

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Background: In a recent study, rhesus macaques (R.M.) that got an intravenous (IV) infusion of PGT121, 24hrs prior to intravaginal challenge with SHIV-SF162P3, had distal site accumulation of virus 1-3 days after challenge. Using Cy5-labeled VRC01 IV-injected into R.M. we found that it takes antibodies ~1wk to achieve steady-state tissue levels. The aim of this study is to determine if giving antibodies more time to fully distribute can block distal site accumulation of virus following intravaginal challenge.

Methods: Utilizing Cy5-PGT121 and sham antibody Cy5-DEN3, we compared -1wk IV infusion and -24hrs IV infusion prior to intravaginal challenge with SHIV-SF162P3 in R.M. and measured virus 48hrs after challenge. Tissue levels of viral DNA were detected using gag qPCR and antibody levels were measured through Cy5 fluorescence using deconvolution microscopy and a fluorometer.

Results: Similar to what was reported, we found accumulation of viral DNA in the lymph nodes (LN) of R.M. given PGT121 24hrs prior to challenge. In R.M. that were given PGT121 1wk prior to challenge, however, we saw more distal site accumulation of viral DNA in the LN despite having less viral DNA at the site of challenge. Interestingly, in these distal site tissues where we observed viral DNA, PGT121 was also present. R.M. that received DEN3 had no distal site accumulation of viral DNA and was localized to the site of challenge.

Conclusions: We have found that despite giving antibodies more time to achieve steady state at the site of infection, there was still distal site accumulation of viral DNA 48 hours after challenge. Since this does not occur in DEN3-injected RM, the early distal site accumulation of virus in the LN appears to be PGT121 dependent. This hypothesis is supported by the observation that PGT121 is present in all the distal sites where we detect viral DNA.

Gastrointestinal MAIT cells in chronic HIV infection

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Mucosa-associated invariant T (MAIT) cells are innate-like T cells that are abundant in human mucosal tissues, liver and blood. MAIT cells recognize antigens displayed by the MHC-1-related protein, MR1,
which presents microbial riboflavin metabolites from a wide range of microbes. Once activated, MAIT cells can produce pro-inflammatory cytokines, kill infected cells, and/or inhibit microbial growth. We previously showed that MAIT numbers and cytokine production are severely and persistently reduced in chronic HIV infection. MAIT defects could predispose HIV-infected persons to increased risk of microbial co-infections. To further explore the role of MAIT cells in chronic HIV infection, we assessed colorectal and/or terminal ileal MAIT cell subsets from over 20 HIV-positive individuals (Controllers, Viremic, and ART-suppressed individuals), and healthy controls by multiparameter flow cytometry. MAIT cells were less abundant in PBMC of HIV-positive compared to uninfected individuals (P=0.057). However, in colorectal and ileal mucosa the percentages of MAIT cells were similar in all participant groups, suggesting either reduced MAIT loss from mucosal tissues compared to blood during HIV infection, and/or redistribution from blood to mucosal sites. The majority of MAIT cells in colorectal and ileal mucosa expressed CD8, while a minority expressed CD4 or were CD4/8 double negative. Greater than 50% of mucosal MAIT cells expressed CD45RO; 15-25% expressed CD56; and <20% expressed perforin or granzyme B. Intracellular cytokine staining of mucosal MAIT cells following ex vivo stimulation revealed robust functionality when stimulated with PMA/Ionomycin, dominated by CD107, IFNγ, IL17 and TNF. However, while blood MAIT cells responded strongly to stimulation with inactivated E. coli, mucosal MAIT cells responded weakly to this antigen. Thus, gastrointestinal MAIT cells may be partially unresponsive towards commensal microbes, while remaining responsive to other stimuli. Supported by NIH/NIDDK R01 DK108350.

ASSESSMENT OF INNATE CELLULAR SUBSETS AND ASSOCIATIONS WITH HIV-1 INFECTIVITY IN RECTAL MUCOSAL TISSUE AMONG MEN WHO HAVE SEX WITH MEN

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Background: Our understanding of the innate immune cell compartment within human rectal mucosal tissues and their relative contributions to HIV transmission and pathogenesis is limited.

Methods: We quantified the percentage of neutrophils, macrophages, dendritic cells (DC), and natural killer (NK) cells in rectal mucosal (RM) biopsies from 56 HIV-1 negative men who have sex with men (MSM) aged 18-65 by multicolor flow cytometry. For NK and DC subsets, blood and RM compartments were compared. The associations between RM innate cell subsets and HIV-1 BaL replicative capacity were determined in ex vivo RM explant challenge experiments.

Results: Innate cellular subsets were differentially distributed in RM vs. blood. In RM, CD11c-CD123+ plasmacytoid DCs (pDCs) were the least abundant cell subset analyzed (p<0.0001, all comparisons), while CD3-CD56+CD16- NK cells were most abundant (p<0.0001, all comparisons). CD11c+CD1c+ DCs outnumbered pDCs in RM (median 8:1), in contrast to blood, where CD1c+ DCs and pDCs were found at the expected 1:1 frequency (median 1:1; RM:Blood ratio, Wilcoxon p<0.0001). NK subsets were also differentially distributed, with CD56dimCD16+ cells far outnumbering the CD56+CD16- NKs in blood, while the opposite distribution was observed in RM. A low percentage of
neutrophils (CD66b+CD16+) and macrophages (CD163+) were identified among CD45+
RM cells (median 0.18% and 0.27%, respectively), however these cells were present at
>1% frequency in some individuals. Interestingly, there was an inverse correlation
between the percentage of total NK cells in rectal biopsies and the ability to propagate
HIV in RM explants (n=42, Spearman, p=0.002, r=-0.47); no other innate subset was
associated with HIV replication. This correlation was primarily driven by CD56+CD16-
NKs (p=0.008, r=-0.40).

**Conclusions:** Compared to the blood, the RM is enriched for CD1c+ DC and
CD56+CD16- NK cells. These data demonstrate, for the first time, the potential
importance of tissue-resident NK cells in human mucosal HIV-1 replication.

**Multiple pathways to avoid IFN-β sensitivity of HIV-1 by mutations in capsid**

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Type I interferons (IFN), including IFN-α and IFN-β, potently suppress HIV-1 replication by up-regulating
an array of IFN-stimulated genes (ISGs). The viral capsid protein (CA) partly determines the sensitivity of
HIV-1 to type I IFN. However, it remains to be determined whether CA-related functions including
utilization of known host factors, reverse transcription or uncoating contribute for the sensitivity of HIV-
1 to type I IFN. Recently, we identified an HIV-1 variant that is unusually sensitive to type I IFN. The
hypersensitivity of this unique variant is determined by CA, as this variant, called RGDA/Q112D, contains
multiple mutations in CA: (H87R, A88G, P90D, P93A and Q112D). To investigate how an IFN-hypersensitive
virus can evolve to overcome IFN-β-mediated blocks targeting the viral capsid, we adapted the
RGDA/Q112D virus in IFN-β-treated CD4+ T cells. We successfully isolated two distinct variants selected
for IFN-β resistance, which contain either a single Q4R substitution or the double amino acid change
G94D/G116R. These two IFN-β resistance mutations variably changed in sensitivity to MXB, truncated
CPSF6, and CypA indicating that the observed loss of sensitivity was not due to interactions with these
known host CA interacting factors. In contrast, the two IFN-β resistance mutations apparently functioned
through distinct mechanisms. The Q4R mutation dramatically accelerated the kinetics of reverse
transcription and initiation of uncoating of the RGDA/Q112D virus in the presence or absence of IFN-β.
Alternatively, the phenotype of the G94D/G116R resistance mutations were only observed in the
presence of IFN-β, most consistent with a mechanism of disrupting binding to an unknown IFN-β regulated
host factor. These results suggest that HIV-1 can exploit multiple, known host factors-independent
pathways to avoid IFN-β-mediated restriction by altering capsid sequences and subsequent biological
properties.