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Vaginal Microbial Profile and Socio-demographic Characteristics of young African American (AA) women with Asymptomatic Bacterial Vaginosis (BV) in the United States (US)

Background: Approximately 50% of cases of bacterial vaginosis (BV) are asymptomatic and as such remain untreated. Untreated BV can progress and lead to damage of the vaginal epithelium. This compromises the integrity of the vaginal mucosa, increasing the risk of HIV infection. HIV positive women co-infected with BV experience increased viral shedding.

Methods: The vaginal swabs of 10 African American (AA) women were analyzed. These swabs were obtained from a previously conducted prospective, randomized, open label trial of home screening and treatment of young women with asymptomatic BV who were also at high risk for sexually transmitted diseases. Whole genome sequencing (WGS) was conducted on the vaginal swabs and descriptive analyses of sociodemographic characteristics conducted using SPSS 23.

Results: The mean age of the sample was 21 years (range 18-25 years). The highest level of education attained in the sample was a master's or advanced degree while the lowest was high school attendance (no diploma). 80% of the population had never been treated for BV in their lifetime and 60% have had prior pregnancies. In the past year 60% of the women have had 2 or more different sexual partners and 40% of women had new sexual partners. The microbial taxa of the sample included species from the genus Annaerococcus (tetradius, prevotii, and lactolyticus), genus Actinomyces (turicensis and urogenitalis), order Lactobacillales (Facklamia genus and Peptostreptococcus stomatis) and Prevotella amnii and Atopobium vaginae. Novel co-occurrence patterns were observed through network analysis, the most significant of which includes the species from the genus Annaerococcus and Actinomyces. Maximum variance was observed among Gardnerella vaginalis, however, this was not significant.

Conclusion: The use of whole genome sequencing to analyze the vaginal microbiome can aid in identifying potential biomarkers that may be associated with increased risk for HIV and increased viral shedding.

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Candida interfaces with oral microbiota in HIV+ opiate users with “Thrush”

Oropharyngeal candidiasis ("thrush"), a fungal disease of the oral mucosa and tongue, is the most common intraoral lesion among persons infected with HIV. Oropharyngeal candidiasis results in the abrasion of the mucosa, bacterial gingivitis and periodontitis and HIV-1 positive patients are at a greater risk for periodontal disease. The dental plaque microbial community that forms on the enamel salivary film (supragingival; above the gum line) differs from the subgingival (below the gum line) community, which forms on the proteinaceous film that coats the cementum of the root. The microbiota of the dental plaque biofilm drives the inflammatory process. Both host-induced species-specific suppression and interspecies microbial competition can increase the pathogenic potential of the complex, mixed-species dental plaque community. Drug abuse is a major co-morbidity associated with HIV and drugs of abuse such as opiates can further exacerbate gum disease. Opiates can cause severe oral problems, that constitutes rampant caries, gingiva inflammation, xerostomia, enamel erosion, and other periodontal diseases. In HIV+ drug abusers’ periodontal disease is further aggravated by synergistic effects of opiates with HIV antiretroviral (ARV) drugs which cause substantial dry mouth due to reduced saliva production. The microorganisms most associated with periodontal diseases include Porphyromonas gingivalis, Tannerella forsythia, and Treponema species, that are termed the “red-complex”. The rationale for the study was the limited information available about the oral microbiome in HIV-1 patients with “thrush” particularly in the context of drug use, and no information is available on the fungal-bacterial interactions and commensal relation of Candida with the red-complex microbiota in the oral microbiome in HIV+ Drug users (DU+). We obtain oral gingival plaque samples from HIV+ patients with and without thrush and those who abuse opiates. Group 1: HIV+DU+ (with thrush – with periodontal disease); Group 2: HIV+ DU- (without thrush- no periodontal disease) (normal age & sex matched controls). Evaluation of the oral microbiota in these groups of patients will likely lead to predictive markers for dental caries- in these high risk patients and help highlight highly sensitive diagnostic markers of periodontal conditions. We used the 16S rRNA gene sequencing which is the gold standard in molecular surveys of bacterial and archaeal diversity. Our preliminary data on oral microbiome from n=134 periodontally healthy individuals compared to n=259 with periodontal disease showed that 5 bacterial species were elevated in periodontally healthy individuals and 7 in patients with periodontal disease with increased red complex bacterial species in HIV patients with “thrush”. We anticipate that thrush in HIV+ patients would directly influence oral microflora, additionally opiate abuse would impact the growth and/ or virulence potential of a key oral bacterium, the periodontal pathogen P. gingivalis, which is adept in subverting and undermining both the innate and adaptive immunity of the host to cause immune dysfunction. We believe that this can lead to the development of a dysbiotic oral microflora further exasperating periodontal disease and associated systemic manifestations in HIV+ DU+ patients with “thrush”.

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Alteration of gut microbiome of HIV infected infants in Yaounde, Cameroon

BACKGROUND-AIM: The immune system becomes compromised through a progressive depletion of CD4+ T lymphocytes (T-helper cells) which gives rise to opportunistic infections. The study aims at establishing the alteration of gut microbiota in HIV infection amongst HIV infected children aged 3-24months, in Yaoundé.
METHODS: We conducted a cross-sectional and case-control study, carried out from April to November 2013 during which fecal samples were collected from both HIV infected and uninfected children, after their parents/guardian's read and signed the assent informed form. After collection, the fecal samples were transported and cultured on different media and icubated at different conditions; aerobic, strict anaerobic, 10% CO2 and microaerophilic at the bacteriology laboratory of the Yaoundé University Teaching Hospital. Microorganism identification was done using laboratory based conventional classical gallery and BioMérieux's API identification kits.
RESULTS: Out of the 80 children enrolled for the study, 33 (41.25%) were HIV exposed infected, 15 (18.75%) HIV exposed uninfected infants and 32 (40%) non HIV exposed infants. Among the HIV infected children we mostly identified Lactobacillus spp. 32/33 (96.97%), Streptococcus spp. 28/33 (84.85%) and Bifidobacterium spp. 27/32 (81.81%), with decreased identification of Bacteriodes spp. 11/33 (33.33%). Whereas in HIV negative infants (non HIV exposed) high frequency of Lactobacillus spp. 31/32 (96.88%), E. coli 30/32 (93.75%) and Bifidobacteria spp. 27/32 (84.38%) respectively. On the other hand, potentially pathogenic bacteria like Shigella spp. (24.24%), Staphylococcus aureus (15.15), Klebsiella spp. (12.12%), Acinetobacter spp. (3.03%), Pseudomonas spp. (3.03%) and Proteus spp. (3.03%) were identified in HIV infected infants while it was absent in HIV negative children.
CONCLUSIONS: There's an imbalance in gut microbiota of HIV infected children with the presence of WHO critically considered potential pathogenic bacteria whereas none were identified in HIV non infected children.
Keywords: HIV infected infants, potential pathogenic bacteria, gut microbiota,

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Fecal transplantation from HIV-negative and HIV-positive men to gnotobiotic mice reveals immunological effects of gut microbiota associated with sexual behavior

High-risk men who have sex with men (MSM) have differences in immune activation and gut microbiome composition compared with men who have sex with women (MSW), even in the absence of HIV infection. Gut microbiome differences associated with HIV itself when controlling for MSM, as assessed by 16S rRNA sequencing, are relatively subtle. Understanding whether gut microbiome composition impacts immune activation in HIV-/+ MSM has important implications since immune activation has been associated with HIV acquisition risk and disease progression. To investigate the effects of MSM and HIV-associated gut microbiota on immune activation, we transplanted feces from HIV- MSW, HIV- MSM, and HIV+ untreated MSM to gnotobiotic mice. Following transplant, 16S rRNA gene sequencing determined that the microbiomes of MSM and MSW maintained distinct compositions in mice and that specific microbial differences between MSM and MSW were replicated. Immunologically, HIV- MSM donors had higher frequencies of blood CD38+ HLADR+ ($p=0.008$) and CD103+ T cells ($p=0.04$) and their fecal recipients had higher frequencies of gut CD69+ ($p=0.01$) and CD103+ T cells ($p=0.001$), compared with HIV- MSW donors and recipients, respectively. In contrast, no significant microbiome differences were observed between HIV- and HIV+ MSM in this small cohort of donors, with their recipients showing no significant immune differences – a larger donor cohort may be needed to detect immune-modulating microbes associated with HIV. To investigate whether our findings in mice could have implications for HIV infection, we stimulated primary human lamina propria cells with isolated fecal microbiota and found that microbiota from MSM promote higher in vitro HIV infection than microbiota from MSW. Finally, we identified several microbes that correlated with immune readouts in both fecal recipients and donors, and with in vitro HIV infection, which suggests a role for gut microbiota in immune activation and potentially HIV acquisition in MSM.

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Modeling microbial changes in HIV infected humanized mice.

In recent years, humanized mice have offered an animal-based research option for human-specific infectious diseases. An emerging humanized mouse model is the Bone Marrow-Liver-Thymus (BLT) mouse. In addition to a humanized immune system, we show that these animals exhibit a chimeric gut and bronchial epithelium. Here, we describe and validate the effectiveness of the BLT mouse model for research in HIV infection and its role in intestinal microbial dysbiosis. Using both 16s and whole genome sequencing, we show that HIV infection in this model leads to microbial dysbiosis and systemic inflammation, where we mapped the significant similarities and departure from the microbial data available from HIV patients. Finally, we use metagenomic sequencing data for metabolic reconstruction of microbial cohorts in HIV infected and uninfected BLT humanized mice.

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Analysis of gut microbiota composition in ART-treated perinatally HIV-infected patients reveal a distinct profile associated with inflammatory and cardiac biomarkers

Background: The interplay between gut microbiota (GM) and immune system during HIV infection is currently a subject of intense research. Despite effective antiretroviral therapy (ART), adult HIV-infected patients have an altered GM associated with systemic inflammation and immune activation. In addition, alteration in intestinal microbiota has been associated with the development of non-AIDS defining morbidities such as cardiovascular diseases (CVD). While there are several studies on this topic in adults, the contribution of GM alteration in inflammation and immune activation in perinatally HIV-infected patients (PHIV) has never been explored. Thus, the aim of this study was to investigate, for the first time, the GM composition in PHIV under effective ART and its association with markers of systemic inflammation, vascular endothelial activation (CVD biomarkers) and microbial translocation.
Materials and Methods: Sixty-one ART-treated PHIV (age range 3-30 years old) were investigated for GM composition and compared to age-matched healthy controls (HC). GM was analysed by 16S rRNA metagenomic sequencing from stool samples. Sequences were organized into Operational Taxonomic Units (OTUs) with a 97% of clustering threshold of pairwise identity. Alpha and beta diversity (i.e., Bray-Curtis and Unweighted UniFrac) analysis were computed through Qiime pipeline, as well as OTUs statistical significance through Kruskal-Wallis test. PERMANOVA test was applied on beta diversity metrics to compare PHIV with HC. We evaluated ICAM-1, VCAM-1 and E-selectin (sE-sel), as cardiac biomarkers, IFN-γ and IL-6 as markers of systemic inflammation by Luminex assay. sCD14, marker of microbial translocation, was measured by ELISA. Immune activation was analysed by flow cytometry on CD4+ and CD8+ T-cells expressing CD38 and HLA-DR markers. Mann-Whitney U test was used to compare levels of ICAM-1, VCAM-1, IL-6, sCD14 and percentage of CD4 and CD8 activated T-cells between group A and group B; t-test was used to compare biomarkers levels of sE-sel and IFN-γ. Dietary habits were evaluated through a questionnaire.
Results: We did not observe any correlation between the major clinical HIV-related characteristics in our cohort (age, sex, ethnicity, CDC, timing of ART initiation, type of ART treatment, dietary habits) and the type of GM. Interestingly, measurement of the species richness and evenness (alpha diversity) of GM revealed a greater variability in PHIV than HC. Moreover, through beta diversity analysis, it was possible to observe two distinct subsets among PHIV (A and B groups). Remarkably, at species level, Akkermansia Muciniphila was the dominant species in the A group, while the B group was characterized by higher biodiversity. In addition, the analysis of soluble markers revealed a significantly higher level of sE-sel ($p= 0.0296$), ICAM-1 ($p= 0.0028$), VCAM-1 ($p=0.0230$), IL-6 ($p= 0.0247$) and sCD14 ($p=0.0142$) in group A compared to group B. No statistical differences in IFN-γ production were found between the groups.
Conclusions: We found that distinctive GM profiles in PHIV are differently associated with systemic inflammation, microbial translocation and vascular endothelial activation. Future studies are needed in order to understand the role of A. muciniphila and risk to develop CVDs in this population.

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Integrated multi-Omics analyses: Altered Gut Microbiome, Metabolome and Functional Gene Contents in chronic HIV infected Women.

Background

Gut microbiota alteration has been noted in HIV-infected individuals. However, potential functional contents of alternated gut microbiome remain largely unknown in individuals with chronic HIV infection. Beside the sequencing-based microbiome / functional gene content approaches, metabolomics also plays a key role to provide insight in these microbiome-conferred functionalities associated with HIV infection. Multi-omics analyses, which integrate microbiome, metabolome, and related pathways, may help facilitate understanding the role of gut microbiota in HIV-infected individuals.

Material & Methods

Multi-omics measurements (including gut microbiota 16S rRNA sequencing, predicted microbiome functional contents, and targeted metabolomics profiling) were performed among 185 participants (128 HIV+, 57 HIV-) from the Women's Interagency HIV Study (WIHS). In short, the16S rRNA V4 region sequencing was conduct on stool samples to analyze the microbial community. OTU picking, taxonomy assignment and α, β diversity analyses were carried out using QIIME, with uclust, Greengenes Database, and R phyloseq/vegan package. Microbiome function and gene content was inferred by PICRUSt with GreenGenes Database and KEGG. A targeted metabolomics profiling was performed to measure 133 metabolites in plasma using liquid chromatography-tandem mass spectrometry. Statistical correlation-based methods, regression models and bioinformatical network-based approaches were performed on genus level gut microbiota, KEGG pathways/ ortholog groups, and metabolites, for the integrative Multi-omics analyses.

Results

Multiple bacterial genera were significantly altered in HIV+ participants compared to the HIV- group. HIV+ participants showed reduced relative abundance of Bifidobacterium, Collinsella, and Catenibacterium and increased relative abundance of Ruminococcus, Oscillospira, Clostridium compared to HIV- participants. (all $P<0.05$). PLSDA analysis also showed different gut microbiota patterns which were driven by these bacterial genera between HIV+ and HIV- participants (PLSDA VIP score: all >2). The functional analyses of gut microbiome, revealed seven significantly altered metabolism-related pathways between HIV+ and HIV- groups at the KEGG level III, including the increased lipid metabolism pathway in HIV infected participants. These results were supported by metabolomics analysis which indicated increased plasma levels of multiple lipid species in lysoPC a glycerophospholipids , PC aa glycerophospholipids , and sphingolipids were significantly higher among HIV+ individuals compared to HIV- individuals.(all $P <0.01$).Notably, integrative multi-omics analyses showed that, Ruminococcus and Oscillospira enriched in HIV infection, were major contributors to the altered lipid metabolism pathway, and positively correlated to host plasma levels of lipid metabolite. $P <0.01$). Another interesting finding was that, the reduced Bifidobacterium was the first predominant contributor of KO group "biotin carboxylase", suggesting its potential role in the altered fatty acid biosynthesis.

Conclusions

Our integrated analyses reveal altered gut microbiota and, as well as the related functional changes which may contribute to altered plasma lipid metabolites plasma in women with chronic HIV infection.

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HIV EXPOSURE ALTERS THE FECAL MICROBIOME AND EFFICACY OF ORAL POLIO VACCINE IN NIGERIAN INFANTS

Background: Microbial marker gene datasets are compositional in nature, conveying only relative information about the taxa detected and precluding statistical inference in their native state. Despite these limitations, marker gene surveys are frequently treated as independent measurements existing in Cartesian space. Here, we introduce pico, an L1 penalized transformation that converts compositional microbiome datasets into real Cartesian coordinates constructed from ratios of bacterial taxa that maximize the variance across the dataset. These ratios, termed 'balances', are used to construct an orthonormal basis within Cartesian space to evaluate hypotheses in a robust and statistically valid way. We employ pico to reveal shifts in the composition of fecal microbiota associated with altered oral polio vaccine (OPV) response in a longitudinally sampled cohort of HIV-exposed uninfected (HEU) and HIV unexposed (HU) Nigerian infants.
Material & Methods: The main driver of the pico package, which we term the ‘penalized isometric log ratio transformation’, consists of three steps. First, we transform marker gene datasets into Cartesian coordinates by performing a centered log ratio (CLR) transformation. The CLR is a transformation that centers compositional parts (counts of microbial nucleotide sequences) to the geometric mean of each sample. Second, we identify compositional balances (ratios of microbial taxa) by applying a Sparse Principal Component Analysis via an L1 Penalized Matrix Decomposition to the CLR transformed data. Third, we transform the original dataset to an orthonormal subspace by performing the isometric log ratio transform on the ratios of taxa identified in step 2. This results in the construction of a Cartesian coordinate system composed of balances that successively maximize the variance. For both the mock community and Nigerian datasets, we sequenced hypervariable regions of the bacterial 16S ribosomal rRNA gene on an Illumina platform.
Results: We validate this approach by demonstrating that pico accurately builds compositional balances that maximize the distance between known even and staggered mock community samples. Applying pico to the Nigerian cohort dataset described above, we fit penalized regression models to the pico-transformed data to identify balances that discriminate fecal communities of HEU versus HU infants during weeks 1, 4, 15, and 36. Furthermore, we report that the week 1 ratio of Staphylococcus aureus and Streptococcus pneumoniae to Propionibacterium acnes is significantly increased in HEU infants and that this ratio is a significant linear predictor of vaccine efficacy (measured by humoral Sabin2 titer responses) at week 4.
Conclusions: pico enables robust analysis and modeling of compositional microbiome data with taxon-level resolution. The orthonormal subspace built by the pico transform is composed of ratios of microbial taxa that maximize the variance across the dataset, in most cases obviating dimensionality reduction techniques and maximizing interpretability. Our data reveal shifts in the composition of infant fecal microbiota that are significantly associated with HIV exposure and highly predictive of humoral responses to OPV. Collectively, these data illustrate the consequences of HIV exposure on the infant fecal microbiota and vaccine response, which are highly germane to clinical outcomes.

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HIV COPD: could differential host recognition of the lung microbiome be contributing to pulmonary disease?

Rationale: Greater than 70% of HIV+ outpatients manifest at least one lung function abnormality, with many developing COPD; however, the mechanisms are poorly understood. Alterations in the lung microbiome that occur in HIV may increase risk of COPD, as taxonomic differences in lung bacterial communities between HIV+ and HIV- individuals detected by 16S rRNA gene sequencing can be seen in untreated individuals and those with lower CD4 counts. Given the significant alterations in adaptive immunity that occur in HIV infection, there may be differences in bacterial recognition by the host that contribute to lung dysfunction. Using magnetic activated cell sorting (MACS), immunoglobulin-bound bacteria can be sorted from bronchoalveolar lavage (BAL) fluid and analyzed using flow cytometry and 16S rRNA gene sequencing to elucidate the amount and type of Ig-bound bacteria in the lungs.

Methods: BAL fluid was obtained from individuals with and without HIV and with varying lung function. Bacterial pellets were stained with SYTO BC and IgG PE, then incubated with iron-containing anti-PE micro beads, creating a magnetic label on Ig-bound bacteria. The fluid was then run through MACS columns composed of ferromagnetic spheres, embedded within a super magnet (MACS sorter). Unbound bacteria freely flowed through the column while immunoglobulin-bound material, including bacteria, were held in suspension within the column until being manually expelled and collected in separate tubes. The immunoglobulin-bound and unbound material was then analyzed using flow cytometry. DNA extraction was performed on both sorted and unsorted bacteria. PCR was used to amplify the V4 unit of the 16S rRNA gene, with subsequent purification and pooling. 16S rRNA sequencing was performed on all samples, identifying which bacterial species are bound by IgG.

Results: BAL samples from 65 individuals (43 HIV+, 22 HIV-) were sorted with MACS. Approximately 70% of individuals were male and average age was 51 years in both groups. HIV+ individuals had significantly more IgG-bound bacteria than HIV- individuals based upon flow cytometry results (Figure 1). HIV+ individuals not taking antiretroviral therapy tended to have more Ig-bound bacteria than those on appropriate therapy. 38 sorted samples have been sequenced thus far. IgG+ and IgG- fractions from the same individual have different bacterial communities, indicating that different bacteria are being recognized by the host. Interestingly, HIV+ individuals and those with COPD recognized Pseudomonas taxa in the lungs far more than HIV- individuals in terms of relative abundance. In this preliminary analysis, differences between groups based upon HIV or COPD status were not significant.

Conclusions: MACS is able to separate immunoglobulin-bound bacteria from un-bound in BAL samples. HIV+ individuals have a greater number of Ig-bound bacteria in BAL than HIV- individuals. Preliminary 16S rRNA gene sequencing suggests that there are differences in Ig-bound and unbound bacteria in individuals, and the specific bacteria that are Ig-bound in the lungs may be different between HIV+ and HIV- individuals. The relationship of alterations in amount of Ig-bound bacteria, correlation with CD4 count, and impact of ART remains to be determined.

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Distinct cervical microbial diversity and community structure by human papilloma-virus and cervical disease status in HIV-positive women in South Africa

Background: Cervical cancer continues to cause significant morbidity and mortality in HIV+ women especially in resource-limited settings. Although HPV infection is a necessary cause of cervical cancer, other biomarkers which could distinguish which women with HPV infection are most likely to have cervical intraepithelial neoplasia (CIN) would be beneficial. The role of the cervical microbiome in progression of HPV-related disease, especially in HIV+ women, remains unclear. Here, we aimed to identify if cervical microbiota in HIV+ women with HPV16 predict CIN.

Methods: We enrolled HIV+ women aged 30 to 65 from Khayelitsha Day Hospital in Cape Town, South Africa. Exclusion criteria were history of anogenital cancer, treatment for cervical dysplasia, or hysterectomy. HIV and HPV testing and histological diagnosis based on biopsy and/or LEEP were performed to define three experimental categories: (1) HPV16+/CIN3 (n=17), (2) HPV16+/No CIN (n=15), and (3) negative for all high-risk HPV types with no CIN (n=19). Cervical swabs were collected during gynecological exams and stored in ThinPrep. We extracted DNA (Qiagen PowerViral) and performed 16S rRNA (V3V4) sequencing. Closed-reference operational taxonomic unit (OTU) picking against the Greengenes 97 database was performed using QIIME (minimum count cutoff: 2,500). Microbiome Chao alpha-diversity and UniFrac beta-diversity were calculated using phyloseq and differential abundance testing was performed using DESeq in R.

Results: After quality-filtering of sequencing data, we were able to analyze 42/51 cervical swabs (n=16 group 1 (HPV16+/CIN3), n=14 group 2 (HPV16+/No CIN), and n=12 group 3 (No hrHPV/No CIN)). We found that samples from women with no HPV had comparable alpha-diversity to those from women with HPV16 and CIN3. However, women with HPV16 and no CIN had significantly lower alpha-diversity (Chao index of 101 compared to 130 (group 3) and 142 (group 1), $p<0.05$). Overall microbial community structure was not significantly different across all three groups (UniFrac PERMANOVA, P=0.218). Compared to women without hrHPV, those with HPV16 and CIN3 had cervical communities significantly enriched in Lactobacillus iners and Akkermansia muciniphila (DESeq, $p<0.05$, FDR<0.05). Conversely, women without hrHPV had significantly higher levels of OTUs assigned to Prevotella, Fusobacterium, Megasphaera, Anaerococcus. Furthermore, HPV16-positive women with CIN3 were significantly enriched in L. iners and two OTUs within the Rs-045 family of the TM7 phylum. HPV16-positive women with no CIN were instead enriched in Prevotella.

Conclusions: Our results support distinct microbiota by HPV and CIN status in HIV+ women. Further studies are needed to evaluate their role in the pathogenesis of cervical cancer in HIV+HPV+ women. Our findings demonstrate the potential utility of the cervical microbiome and specific microbiota as adjunct diagnostic tools to improve screening.

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Rapid gut epithelial repair at the enteroendocrine-immune intersection through microbiota-mediated rescue of tryptophan metabolism during chronic HIV/SIV infection

Gastrointestinal complications in HIV and SIV infections are driven by impaired mucosal immunity and disrupted gut epithelium which support chronic inflammation and viral persistence. The enteric immune and endocrine systems intersect through tryptophan (TRP) metabolism, which is dysregulated during HIV and SIV disease progression. TRP in the gut is metabolized into serotonin (5-HT) or kynurenine (KYN) and KYN-IDO-Th17 axis is impaired during the viral infection. We hypothesized that rapid gut epithelial repair and protection may be mediated through the rescue of TRP metabolism by commensal bacteria. We utilized an innovative simian ligated intestinal loop model to directly introduce Lactobacillus plantarum and Bifidobacterium infantis into the intestinal lumen of SIV-infected animals and to determine the impact on the gut mucosal recovery. Rapid gut mucosal responses to the bacteria were investigated through the analysis of transcriptomic and metabolomic profiles and immunohistochemical evaluations for gut epithelial barrier. We found that chronic SIV infection is associated with CD4+ T cell depletion, gut epithelial disruption and altered TRP metabolism. Increased levels of 5-HT were detected and attributed to decreased serotonin reuptake transporter (SERT) expression and reduced 5-HT clearance. Additionally, we found increased expression of indoleamine 2,3-dioxygenase (IDO1) and accumulation of KYN metabolites. In contrast, exposure to L. plantarum and B. infantis resulted in remarkable recovery of the gut epithelial barriers within 5 hours and occurred in the absence of mucosal CD4+ T cell recovery. This correlated with a dramatic increase in the levels of indolelactate (ILA), an indolic acid derivative of TRP and was coincident with decreased IDO1 expression and lower levels of KYN metabolites. We found that ex vivo stimulation of lamina propria lymphocytes with ILA induced CD4+CD8+ T cells producing IL-22, a cytokine supporting gut epithelial repair. Our findings demonstrate that probiotic bacteria rescue the TRP metabolism from virus-mediated effects and drive towards ILA biosynthesis. This leads to rapid gut barrier repair through enteric immune-epithelial-commensal axis. Our study shows that damaged gut epithelium in SIV infection can be rapidly restored by leveraging commensal metabolites to modulate immune-epithelial interactions and provides novel targets for repair and protection of the gut immunity in HIV disease.

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Gut Microbial Diversity Is Associated with Inducible HIV Promoter Activity in an In-vitro Model of the CD4+ T-cell HIV Reservoir

Background: Interaction between the gastrointestinal microbiota and mucosal lymphoid tissue drives homeostatic CD4 T-cell maintenance. Previous work has observed a positive association between gut microbial diversity and circulating CD4 T-cell number among persons living with HIV (PLWH), suggesting a potential mechanism by which the microbiota influences CD4 T-cell activity and proliferation.

Objective: We report the results of an exploratory analysis suggesting an association between gut bacterial diversity and activation of latent HIV provirus within infected CD4 T-cells.

Methods: A sample of in-care PLWH participating in our New Orleans Alcohol and HIV (NOAH) study were tested (n = 248). Fecal DNA samples were deep-sequenced for the 16S gene V4 hypervariable region and subjected to qPCR using universal 16S-targeting primers to estimate bacterial diversity and burden, respectively. Homogenized fecal samples collected from NOAH study participants were cultured in Gifu Anaerobic Broth for 24 hours under anaerobic conditions. Live/whole organisms were removed by centrifugation and filtration, and supernatants containing microbial products were collected. Jurkat cells, containing a full-length integrated HIV-1 genome (HIV-R7/E-/GFP) that expresses GFP upon activation, were treated with fecal microbial products for 24 hrs, and re-activated HIV promoter (GFP expression) was assessed via flow cytometry. A Generalized Additive Model of Location, Scale, and Shape (GAMLSS) employing a log-normal response variable distribution was used in the regression analysis adjusting for subject age, sex, body mass index, detectable plasma HIV viral load (> 20 copies / mL), ART adherence, recent history of therapeutic antibiotics, and Alcohol Use Disorder Identification Test score. DESeq2 was used to test for significantly differentially abundant bacterial genera adjusting for the same covariates.

Results: Variable induced HIV-promoter-reporter activity (GFP+ in 5-58% of live cells) was observed following treatment with microbial products from cultures obtained from the NOAH subjects. Interestingly, fecal bacterial alpha-diversity (richness) measured before culturing was independently, positively associated with HIV-promoter-reporter activity ($p < 5x10\text{-}6$) after covariate adjustment. Pre-culture estimated bacterial burden failed to associate with HIV-promoter-reporter activity. The abundance of specific bacterial genera were positively and/or negatively associated with reporter activity (FDR q-value < 0.1).

Conclusions: These data suggest that specific gut microbial products may re-activate latent HIV provirus within the CD4 T-cell reservoir. Interventions (i.e. fecal transplant) intended to maximize gut taxonomic and/or metabolic diversity in PLWH may lead to unintended proliferation of HIV virus and undermine clinical viral suppression.

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Comparative Analysis of Rectal Mucosal Collection Methods for Microbiome Studies

Background: Gut microbiome sampling methods are potential sources of variability, and can limit the interpretation of microbiome studies. In this study, we compare multiple rectal mucosal sampling collection methods within individuals to understand how these methods may influence microbiome composition analyses.
Methods: Multiple rectal samples were collected at a single visit from healthy HIV-negative participants (N=12) using varying methodologies. Specimens included: pre-enema self-collected swab, pre-enema physician collected swab and sponge via anoscopy, enema stool, post-enema self-collected swab, post-enema physician collected swab, sponge, cytobrush, and mucosal biopsy via anoscopy. Microbiome composition was analyzed using targeted sequencing of the V4 region of the 16S rRNA gene followed by exact sequence inference using DADA2.
Results: All specimen collection methods resulted in comparable numbers of amplicon sequence variants (ASV); two tissue biopsy specimens were removed from analysis due to low read counts despite additional purification prior to sequencing. Self-collected swabs yielded a significantly more diverse microbiome (p=6.3e-4, Chao1); no other differences in bacterial diversity were noted. Specimens were spatially separated by individual, rather than collection method, on principle coordinate analysis (PCoA) plots. Using linear mixed effects models, we compared specific bacterial genera among collection methods and found significantly increased Anaerococcus (p=3.6e-4), Corynebacterium (p=0.04), Finegoldia (p=3.6e-4), and Peptoniphilus (p=0.001) in pre-enema self-collected rectal swabs. We similarly found significantly decreased Bacteroides (p=0.001) in pre-enema self-collected rectal swabs. Specific genera were not significantly different among other collection methods, including when specifically comparing mucosal methods to biopsies.
Conclusions: In general, self-collected swabs showed greater variability than provider-collected specimens, but these methods may be reasonable in larger microbiome studies. The use of preparatory enema or alternative collection materials (sponges, cytobrush) did not alter microbiome recovery for rectal mucosal specimens. Additionally, there was no significant difference in microbiome composition between direct mucosal sampling methods and whole mucosal tissue biopsy; however, tissue biopsy specimens can require additional purification to reduce human DNA prior to sequencing.

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Exploring the Microbiota for the Diagnosis of Anal Precancerous Lesions in MSM

Background: The risk of anal cancer is markedly increased in HIV-infected subjects, especially among MSM. The current screening strategy is based on the detection of high-degree squamous intraepithelial lesions (HSIL), using anal cytology. While this approach is highly sensitive, the specificity is poor, leading to an excess number of invasive procedures, explaining the poor implementation of this screening strategy. We aimed to identify in MSM a set of anal-associated bacterial biomarkers obtained by anal cytobrush to improve the accuracy of anal cytology for the diagnosis of biopsy-proven HSIL.
Methodology: Cross-sectional prospective study performed in a high-resolution anoscopy clinic. We recruited HIV+ and HIV- MSM who were referred to our clinic for high-resolution anoscopy. The primary outcome was the presence of biopsy-proven HSIL (bHSIL) at the inclusion or during the previous year. We analyze fecal and mucosal microbiota to search for bacterial biomarkers predictive of the presence of bHSIL. Fecal samples were collected in specific containers, and anal mucosa specimens were obtained with an anal cytobrush. The V3-V4 region of the 16S rRNA gene was sequenced using the Illumina platform. We selected the anal-associated bacterial biomarkers based on their LDA scores and AUC-ROC in logistic regression models.
Results: We included 118 HIV+ and 33 HIV- MSM: 47 (34.6%) had bHSIL during the previous year and 12 (8.9%) at the moment of the inclusion. Bacterial diversity and richness were significantly higher in mucosa than in feces, yet the differences among patients with bHSIL vs no-bHSIL or in HIV+ vs. HIV- participants were irrelevant. The differences in beta diversity between sampling locations appeared significant, although no clustering was observed in the analysis by the presence of bHSIL or HIV status. LEfSe analysis revealed 40 biomarkers in mucosa and 53 in stools. After exploring the predictive value of the 15 taxa with greater LDA scores, we selected four OTU in anal samples based on their individual predictive performance and their concomitant predictive value in feces. In anal samples, each 25% increase in the abundance of the Ruminococcaceae NK4A214 group and Alloprevotella genus were associated with a 17% (p=0.041) and 8% (p=0.016) increased risk of HSIL, respectively. In contrast, the absence of Prevotella melanonigenica and Ruminococcaceae UCG-014 were protective of bHSIL (OR 0.16, P=0.018 and OR 0.31, P=0.026, respectively). The combination of these four biomarkers correctly classified 72% of the population as having bHSIL and yielded the following predictive values: AUC 0.737, sensitivity 37%, specificity 90%. While the presence of LSIL or HSIL as predictive of bHSIL in anal citology had an AUC 0.645), the combination of these four biomarkers with the anal citology significantly improved the prognostic value) to AUC 0.805.
Conclusions: We found anal and fecal-associated bacterial biomarkers able to identify subjects with present or recent history of precancerous anal lesions, which combination was highly specific. If confirmed after external validation, these biomarkers could be exploited as diagnostic tools with concomitant cytologic examination to overcome the low specificity of current screening strategies for anal cancer.

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Alterations in oral and gut microbiota in HIV-infected individuals related to pulmonary function

BACKGROUND: HIV infection is a risk factor for chronic obstructive pulmonary disease (COPD), but the mechanisms involved are poorly understood. Because HIV infection impacts the immune system, alterations in the microbiome have been proposed as a possible mechanism of disease development or progression. Recent studies have demonstrated that HIV infection is associated with dysbiosis in saliva. Much of the lung microbiome is derived from the oral microbiome via aspiration, but whether the oral dysbiosis associated with HIV correlates with COPD has yet to be described. In addition, the gut microbiome may influence lung disease, but this relationship has not been investigated in HIV. We collected oral and gut samples from individuals with and without HIV infection and determined relationships of bacterial communities to lung function.

METHODS: 84 HIV-infected and 108 HIV-uninfected men participating in the Pittsburgh site of the Multicenter AIDS cohort study (MACS) performed pre- and post-bronchodilator spirometry and diffusing capacity for carbon monoxide (DLCO) per guidelines and had oral and stool samples collected. Sequencing of the 16S rRNA gene (V4 subunit) on the Illumina MiSeq platform was performed to characterize the composition of oral and gut microbiota. Taxonomic analyses were performed using mothur.

RESULTS: In saliva samples, the oral microbiome composition of the HIV-infected individuals was significantly different compared to HIV-uninfected individuals (Permanova for Bray-Curtis distance, p-value=0.0002). HIV-infected individuals had reduced alpha diversity (by Shannon index, t-test p-value<0.05) compared to HIV-uninfected. 45% of HIV-infected participants were diagnosed with COPD, while 37% of the HIV-uninfected individuals had COPD (defined as a post-bronchodilator forced expiratory volume in one second/forced vital capacity [FEV1/FVC] ratio < 0.7, or predicted FEV1 < 80%, or predicted diffusing capacity of the lung for carbon monoxide[DLCO] < 70%). Microbiome profile alterations (Permanova for Bray-Curtis distance, p-value<0.05) and lower alpha diversity (by Shannon index, t-test p-value < 0.05) were seen in HIV-infected individuals with COPD compared to HIV-infected individuals without COPD. There was also a significant difference in microbiome composition between HIV-infected individuals with normal DLCO (>= 80% predicted) versus low DLCO (<80% predicted) (Permanova for Bray-Curtis distance, p-value=0.008). However, among HIV-uninfected individuals, microbiome communities were taxonomically overlapping between groups with/without COPD or an abnormal DLCO. For stool samples, the gut microbiome communities of HIV-infected individuals were different from those of HIV-uninfected (Permanova for Bray-Curtis distance, p-value=0.005), but there was no relationship to lung function in either group.

CONCLUSIONS: Next-generation sequencing analysis identifies oral and gut microbiota alterations in HIV-infected individuals. We also found that the oral microbiome was altered in HIV-infected individuals with COPD or abnormal DLCO, but not in HIV-uninfected individuals, suggesting a potential unique relationship of the microbiome to lung disease in HIV.

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Impact of reproductive aging on the vaginal microbiome and soluble immune mediators in women living with and at-risk for HIV infection

Background: Reproductive aging may impact the vaginal microbiome and genital tract mucosal immune environment and contribute to genital tract health in women living with and at risk for HIV infection.

Material & Methods: A cross-sectional study of 102 HIV+ (51 premenopausal, 51 postmenopausal) and 39 HIV-uninfected (HIV-) (20 premenopausal, 19 postmenopausal) women was performed in Bronx and Brooklyn, NY. Cervicovaginal lavage (CVL) was collected in 10 ml sterile water for quantification of innate antimicrobial activity against E. coli, HSV-2 and HIV and soluble immune mediators by Luminex and ELISA. Vaginal swabs were obtained for microbiome studies by qPCR and 16S rRNA sequencing. Categorical variables were compared between groups by Fisher exact test and continuous variables were compared by ANOVA. Spearman correlation coefficients were calculated to assess for associations between variables. Analysis of sequencing data was performed using QIIME v1.9.1 and the Greengenes database v13.8. Sample diversity was evaluated using weighted and unweighted Unifrac metrics and visualized by PCoA to observe clustering by groups. ANOVA test with post-hoc Tukey was employed to test for significant differences in alpha diversity metrics. LefSe analysis was used to identify microbial biomarkers for the different subpopulations of samples, with linear discriminant analysis (LDA) score threshold of 2.0.

Results: HIV+ postmenopausal compared to HIV+ premenopausal participants had significantly lower median E. coli inhibition, lower proportions of Lactobacillus sp. and median gene copies of L. crispatus and L. iners, significantly higher proportions of Gardnerella and Atopobium vaginae and lower mean log10 concentrations of human beta defensins (HBD-2, HBD-3) and secretory leukocyte protease inhibitor (SLPI). In contrast, HSV-2 inhibitory activity was higher in HIV+ postmenopausal compared to HIV+ premenopausal participants and correlated with the proinflammatory molecules interleukin (IL) 6, IL-8, human neutrophil peptide (HNP) 1-3, lactoferrin and fibronectin. Similar trends were observed in HIV- postmenopausal compared to HIV-premenopausal participants. The HIV inhibitory activity was variable, but higher in HIV-postmenopausal compared to premenopausal participants with no differences by reproductive status in the HIV+ participants. HIV inhibitory activity was also higher in participants with suppressed plasma viral load, and inversely correlated with G. vaginalis and BVAB2. A significant proportion of HIV+ participants on antiretroviral therapy exhibited HIV enhancing activity.

Conclusions: HIV+ postmenopausal compared to premenopausal women have less CVL E. coli inhibitory activity, reflecting a lower proportion of lactobacilli species and a greater proportion of Gardnerella and A. vaginae, and more HSV-2 inhibitory activity, reflecting increased mucosal inflammation. The effect of menopause on mucosal immunity was greater in HIV+ than in HIV- participants, suggesting a synergistic impact. It is possible that promotion of a lactobacillus dominant vaginal microbiome and reduced mucosal inflammation in HIV+ menopausal women may improve vaginal health and reduce risk for shedding of HIV and potential for HIV transmission.

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HIV-associated gut microbiome differences and concurrent immune activation are dependent on host context

Background: In HIV-infected individuals, markers of inflammation predict greater mortality, in part due to death from inflammation-related non-communicable diseases such as cardiovascular disease, but the causes of this inflammation are not fully understood. Recent studies found HIV-associated gut microbiome differences, but few of these data were from sub-Saharan Africa where HIV burden is greatest. We hypothesized that HIV-associated gut microbiome differences vary by geography, influencing their relationship with elevated host inflammation during chronic HIV.

Methods: We performed 16S rRNA gene amplicon sequencing on nucleic acid extracted from stool samples from 570 subjects: 145 (77 HIV-infected, ART-treated and 68 HIV-uninfected) in rural Mbarara, Uganda; 194 (41 HIV-infected, ART-untreated; 73 HIV-infected, ART-treated; and 80 HIV-uninfected) in urban Gaborone, Botswana; and a high-income country comparison group of 231 (43 HIV-infected, ART-untreated controllers; 53 viremic HIV-infected, ART-untreated; 55 HIV-infected, ART-treated; and 80 HIV-uninfected) in urban Boston, MA, USA. We analyzed sequencing data with Quantitative Insights Into Microbial Ecology (QIIME) and the R packages Divisive Amplicon Denoising Algorithm 2 (dada2) and phyloseq. Community difference significance was measured by permutational multivariate analysis of variance (PERMANOVA). Differentially abundant taxa were identified using the DEseq2 package in R. Immunoassays were used to quantify serum inflammatory markers sCD14, sCD163, IL-6, and I-FABP.

Results: The gut microbiomes of HIV-uninfected subjects were significantly different ($p<0.001$) across all geographic locations. Within each geographic cohort, the microbiomes of HIV-infected and -uninfected individuals were significantly different ($p<0.01$). In Uganda, HIV-uninfected individuals had communities dominated by Prevotella, while HIV-infected individuals had a greater abundance of many taxa including Bifidobacterium and Akkermansia. Gut communities in Boston subjects exhibited contrasting associations from those in Uganda, with Prevotella enriched in subjects with HIV and Bifidobacterium, Akkermansia and other taxa increased in uninfected individuals. Compared to each of the other cohorts, the Botswana cohort had both common and distinct HIV-associated differences. Inflammatory markers were elevated in HIV-infected individuals in all cohorts and associated with unique bacterial taxa in each cohort. Studies of HIV-associated gut microbiome changes should include populations in multiple geographic locations, including in low-income countries and rural areas, as these microbial and inflammation changes are highly context dependent.

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Altered bacterial carbohydrate metabolism predicts increased HIV target cells in the female genital tract

Background: The relationship between bacterial function and inflammation in the female genital tract is not well understood. Here, we use a systems-biology approach to assess the relationship between vaginal microbiome function, structure, and host immunology in a cohort of Manitoban women.

Methods: Study participants included 50 women enrolled in the Vaginal Mucosal Systems (VMS) study in Winnipeg, Manitoba. Metaproteomics and 16S rRNA sequencing of cervicovaginal lavage (CVL) samples were used to characterize vaginal microbiome function, composition, and host inflammation pathways. Endocervical cytobrush samples were analyzed by flow cytometry to quantify levels of T cells (CD4+,CD8+), including expression of activation markers (CD38+,HLADR+), co-receptors (CCR5+,CXCR4+), homing markers (α4β7+), and Th17-like CD4's (CCR6+). Neutrophils (CD15+CD16+CD49d-), natural killer cells, and myeloid cells were also measured. Differences in microbiota, biological pathways, and immune cells were assessed by Mann-Whitney U test, permutation test, Fisher's Exact Test and Spearman's correlation when appropriate.

Results: Seventeen women (35%) had non-Lactobacillus dominated (non-LD) microbiome while 32 women (65%) had a Lactobacillus dominated microbiome (LD). Nugent score and Amsel's criteria were not different between non-LD and LD women ($P>0.05$), except non-LD women had elevated vaginal pH (5.1) in comparison to LD women (4.5) ($P=1.84E-04$). Non-LD women had reduced expression of epithelial barrier pathways, and increased expression of host carbohydrate metabolism and cellular immune response pathways ($P<0.01$). Non-LD microbiome profiles did not associate with increased CD4+ T cell subsets. Metaproteomic analysis revealed 7 bacterial metabolic pathways were different between non-LD and LD women ($P<0.05$) including enzymes involved in carbohydrate metabolism and homolactic fermentation, including: L-lactate dehydrogenase, pyruvate kinase, triosephosphate isomerase, and glyceraldehyde 3-phosphate dehydrogenase ($P<0.05$). Reduced carbohydrate metabolism correlated ($P<0.05$) with increased CD4+ T cell subsets including CD38+HLADR+CCR5+ ($r=-0.3133$), CD38+HLADR+ ($r= -0.3883$) and CXCR4+ ($r =-0.3083$). The absence of the enzyme lactate dehydrogenase was also a strong predictor of levels of CD4+ T cells, including CCR6+ (139% increased), CD38+HLADR+ (168% increased), CXCR4+ (129% increased), and CD38+HLADR+CXCR4+ (400% increased) ($P<0.05$).

Conclusion: These data show that HIV target cells in the female genital tract associate with a reduction in bacterial carbohydrate metabolism enzymes, but not overall bacterial community composition. This suggests that alterations to bacterial metabolism may be an important driver of genital inflammation processes important for HIV acquisition risk.

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Development of a High-Throughput Screening Assay to Identify Metabolites that Modulate HIV-1 Replication.

Background: The vaginal microbiome plays crucial roles in preventing infections of the reproductive tract such as sexually transmitted diseases, urinary tract infections, bacterial vaginosis, and HIV infections. Vaginal dysbiosis results in the reduction of protective Lactobacillus species and a corresponding increase in bacterial species diversity, a condition referred to as bacterial vaginosis (BV). Several labs have previously demonstrated that the metabolite profile of BV specimens is radically different, with a dramatic reduction in lactic acid and an increase in short-chain fatty acids such as butyrate, succinate, propionate, acetate and trace amounts of amines. These fluctuations were subsequently correlated with the increased production of pro-inflammatory cytokines, such as TNF-alpha and IL-8, that are thought to compromise the integrity of the epithelial barrier, potentially affecting the ease of HIV transmission and replication. In addition to these immunomodulatory effects, physiological concentrations of short-chain fatty acids produced during BV failed to inactivate HIV, which is in contrast to the in vitro irreversible inactivation by physiological levels of lactic acid. Whereas several studies have primarily focused on the effects of butyrate and other short-chain fatty acids on HIV replication, we have conducted a meta-analysis of metabolomics studies in the literature that compare BV positive and negative cohorts to identify other putative metabolites of interest in the context of HIV replication and transmission.

Material and methods: Several compounds of interest were selected for further analysis based upon either 1) a relatively large percent change in abundance between BV positive and negative cohorts (as determined by non-parametric Wilcoxon testing when possible) and/or 2) a correlation between relative metabolite abundance and increasing BV Nugent scores. We aim to determine whether these metabolites are capable of modulating HIV-1 replication.

Results: We have developed a high throughput screening (HTS) capable reporter assay. Indicator TZM-bl cells are seeded into 384 well plates and incubated with metabolites of interest for 24 hours prior to infection with the dual-tropic HIV-1 89.6 strain. Forty-eight hours post viral infection, the Promega Bright-Glo Assay System is used to quantify HIV-1 replication in the presence of our metabolites of interest. Under its current design, our optimized assay is well suited to identify inhibitors of HIV-1 replication in HTS applications with an average Z' factor of .60 ± 03. We are currently testing BV metabolites of interest manually in our assay system, resulting in identification of several that may enhance or inhibit HIV infection. Efforts are underway to conduct HTS of a ~1500 metabolite library at the University of Pennsylvania to identify metabolites that modulate HIV-1 replication.

Conclusions: Metabolic changes associated with BV can influence HIV infection efficiency, and may be important in HIV transmission.

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Anti-HIV-1 Activity of Lactic acid in Human Cervicovaginal Fluid

Background. Lactobacillus-dominated vaginal microbiota is associated with a reduced risk of acquiring and transmitting HIV and other sexually transmitted infections (STIs). Lactic acid, present as the D- and the L-isomer, is a major organic acid metabolite produced by lactobacilli that acidifies the vagina to a pH of ~ 4. Lactic acid at physiological levels has in vitro inhibitory activity against bacterial vaginosis-associated bacteria and viral STIs, including HIV. However, the anti-HIV properties of lactic acid in native vaginal lumen fluids of women colonized with Lactobacillus spp. have not yet been established.

Material & Methods. Cervicovaginal fluid (CVF) was collected from women of reproductive age (n=20, mean age ± SD, 23 ± 2.9) using a menstrual SoftCup. High titre HIVBa-L and HIVRHPA were incubated in neat and diluted CVF at 37°C followed by neutralisation and infectivity determined in TZM-bl cells. D- and L-lactic acid concentrations in samples were determined using an enzyme assay. Protonated levels of lactic acid were calculated using the Henderson-Hasselbach equation. CVF samples were fractionated by centrifugation through a 3-kDa molecular weight membrane. Protease digestion of CVF was performed using pepsin immobilised on agarose beads. Microbiota present in CVF were characterised by 16S rRNA gene sequencing. Generalised linear mixed modelling was used to estimate the association between HIV-1 infectivity and CVF sample lactic acid concentration, pH and neutralisation. Statistical significance was determined at the 1% level.

Results. The majority of native, minimally diluted CVF samples fully inactivated HIVBa-L and the transmitted founder HIVRHPA; however inactivation was lost upon neutralization of CVF. Inhibition of HIVBa-L in CVF was significantly associated with the % protonated form of D+L-lactic acid ($p<0.001$), which predominates at pH≤3.8, but not the % D+L-lactate anion ($p=0.280$) or with pH ($p=0.139$). Anti-HIVBa-L activity in CVF was also negatively associated with increasing levels of the % D-protonated lactic acid ($p<0.001$) and the % L-protonated lactic acid ($p<0.001$). The % D+L protonated lactic acid remained significantly associated with anti-HIVBa-L activity in a multivariable analyses accounting for pH and/or lactate anion. The pH-dependent anti-HIV activity in CVF was predominately observed in the acidic 3-kDa filtrate and was retained following pepsin digestion suggesting that the protonated lactic acid was responsible for most of the anti-HIV activity in CVF. CVF samples with high relative abundance of Lactobacillus spp., particularly, L crispatus, also had high levels of protonated lactic acid and anti-HIV-1 activity.

Conclusion. These ex vivo studies indicate that protonated lactic acid is a major anti-HIV-1 metabolite present in acidic native cervicovaginal fluid, suggesting a potential role in reducing HIV transmission by inactivating virus introduced or shed into the cervicovaginal lumen.

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Lessons learnt from the first South African health products regulatory authority (SAHPRA)-approved probiotics trial to improve cure of bacteria vaginosis (BV) in a region with high BV and HIV rates

Bacterial vaginosis (BV) is associated with genital inflammation, increased HIV risk and adverse reproductive outcomes. The standard of care for BV is antibiotics, although recurrence rates are high. In combination with antibiotics, biotherapeutics may improve efficacy and durability of BV treatment. No randomized trial comparing antibiotic treatment of BV to adjunctive vaginal probiotics has been conducted in South Africa, and no probiotic trial has yet been approved by SAHPRA.

A single-blind, randomized controlled trial in STI- BV+ women, with abnormal vaginal discharge compared metronidazole (n=20) to metronidazole with a commercially-available oral/vaginal probiotic (containing L. rhamnosus, L. acidophilus, B. longum and B. bifidum), available over-the-counter (OTC) in South Africa (n=30). BV was assessed by Nugent criteria. The primary endpoint was BV cure at one-month.

Among women screened with abnormal vaginal discharge, the prevalence of BV (Nugent ≥7) was 56.5 %, while 25.7% of BV+ women also had an STI, with a high screen failure rate. Vaginal discharge was a poor predictor for BV (positive predictive value [PPV] BV+/STI- 46.9%, BV-/STI+ 14.8%). To date, 24/50 women have completed the one-month visit and 14 have completed the study. Despite excluding STI+ women at enrolment, preliminary results showed a high rate of STI acquisition during the study: 20% incident STIs. The probiotic was well-accepted and no study-product related adverse events were reported. An interim analysis suggests no beneficial effects of this probiotic on BV cure and genital inflammation compared to metronidazole alone, although BV recurrence rates were lower. Similar concentrations of Lactobacillus spp. (L. crispatus, L. gasseri, L. vaginalis, L. jensenii, L. mucosae) was observed in both arms, and a similar decrease in BV-associated spp. (G. vaginalis, P. bivia, A. vaginae and BVAB2) was found in both treatment groups.

Although explicitly marketed for vaginal health, the probiotic tested did not contain Lactobacillus spp. typically found in the vagina. This OTC product provided little benefit to improving BV cure compared to metronidazole alone, although recurrence rates were lower. Importantly, this first to be registered by SAHPRA trial has laid the path for future probiotic product testing in South Africa.

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Strain-level variation in the microbiome of the female genital tract

Elevated inflammation in the female genital tract (FGT) is associated with an increased risk of HIV infection, and cervicovaginal bacteria have been shown to impact genital inflammation (Gosmann et al., 2017). These associations have been identified through bacterial 16S rRNA gene sequencing which has limited resolution and rarely achieves taxonomic assignment to the species-level. These shortcomings are relevant because within-species genetic differences can be vast, with some species-level pangenomes (all the unique genes observed within a species) exceeding the size of any single strain's genome by orders of magnitude. Furthermore, 16S sequencing provides no functional information, limiting our mechanistic understanding of disease associations.

To better characterize strain-level variation in the FGT microbiota we generated species-specific pangenomes from single cultured isolate genome sequences (1000 primary bacterial isolates and 2000 publicly available genomes). We produced sample-specific gene profiles by mapping metagenomic sequences from FGT samples from 300 North America and South Africa women to the species-specific gene catalogs. Profiles were partitioned into groups containing similar gene complements (strains) using centroid-based clustering. Host local inflammation was measured using Luminex cytokine assays performed on cervicovaginal lavages.

We show that most species possess a small core genome (~1000 genes) with an extensive pangenome (6000 to 30000 genes). We find that Gardnerella vaginalis comprises 4 distinct strains and that some women possess enough genes to account for 4 complete genomes, suggesting that these women are colonized by multiple strains. Furthermore, we show that these strain complexes are associated increased levels of cytokines in the FGT. Our findings signify the importance of using fine resolution methods to distinguish microbial strains in the FGT when linking the endogenous microbiome to local inflammation and adverse reproductive outcomes.

Gosmann, C. et al. (2017) 'Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women', Immunity. Elsevier Inc., 46(1), pp. 29–37. doi: 10.1016/j.immuni.2016.12.013.

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Female Reproductive Tract Microbiome Alteration following HIV Infection in a South African Cohort

Background:
Greater than 90% of global HIV transmission follows heterosexual intercourse, where women are twice as likely to contract HIV as men. The female reproductive tract (FRT) microbiome plays an important role in maintaining vaginal health, and is crucial in preventing urogenital diseases, including bacterial vaginosis (BV), sexually transmitted diseases (STDs), and HIV. Lactobacillus-dominant communities suppress inflammation and protect from infectious organisms, while diverse FRT bacterial microbiomes have been linked to increased inflammation and STD risk, including HIV transmission. Race and ethinicity appear to influence bacterial community independent of STD and sexual behavior. Commensal and pathogenic viruses in the FRT, the virome, have also been linked to microbiome alteration, inflammation, and HIV risk.

We hypothesized that there are significant alterations in FRT bacterial and viral microbiomes associated with HIV infection.

Methods and Materials:
We conducted a retrospective, longitudinal analysis of 310 vaginal swabs collected between October 2012 and October 2014 from 50 HIV-positive and 50 HIV-negative, sexually active women in Cape Town, South Africa. We performed 16S rRNA amplification of the V4 region from extracted total nucleic acid, and samples were sequenced on the Illumina MiSeq Platform. Generated sequences were quality-controlled and screened for homology to bacterial taxa using QIIME2. For virome analysis, samples were enriched for viral nucleic acid, libraries built, and sequencing performed on the Illumina Hiseq. Sequences were quality controlled and processed through a custom bioinformatics pipeline to stringently identify viral sequences.

Results:
Two participants tested positive at enrollment and two participants seroconverted during the study. Fifty-six percent of seropositive participants were on HAART. There was a significant difference in HPV and high-risk HPV strains in HIV positives compared to negatives ($p<0.0001$ and $p=0.0017$, respectively). There was no significant difference in abnormal PAP smear and BV diagnosis between groups. Baseline alpha diversity was higher in HIV-infected patients not on ART than healthy controls ($p=0.022$). Overall alpha diversity of bacteriomes significantly changed over time from baseline ($p=0.038$), but there was no significant difference in alpha diversity change between HIV negative and HIV positive groups. Weighted unifrac analysis between consecutive time points showed a significant decrease in beta diversity in HIV positive subjects on ART compared to HIV negative controls ($p = 0.003$). Preliminary results of virome analysis will be presented.

Conclusions:
In this South African cohort, HIV infection was associated with increased risk of HPV infection. Further, alpha diversity changed over time in all groups, while beta diversity decreased in HIV-infected subjects on ART compared to healthy controls.

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Persistence of a Transplanted Human Bacterial Gut Microbiome in Double Humanized BLT-mice

Background: Humanized mice are a fundamental animal model for the study of HIV-1. However, many important questions about how the gut microbiota impacts HIV-1 transmission, pathogenesis, and treatment remain unanswered. Within human populations, it is often difficult to control for factors that can influence the gut microbiome such as diet and life style choices. While informative, SIV infected non-human primate models do not fully recapitulate HIV induced gut microbial dysbiosis due to significant differences with the immune system, diet, and virus. In order to address some of these outstanding issues, we created a double humanized mouse model that features a functional human immune system and a stable human-like gut microbiome.

Methods: Humanized-BLT mice were generated by surgical engraftment of human liver and thymus tissues, followed by injection of CD34+ hematopoietic stem cells into irradiated NSG mice. Flow cytometry was used to confirm human immune reconstitution in peripheral blood. To reduce the level of the pre-existing murine gut bacteria, humanized mice were treated with broad-spectrum antibiotics. A combination of Vancomycin, Neomycin, Metronidazole, and Ampicillin was prepared fresh daily and administered in the drinking water. Following antibiotic treatment, the mice were given two fecal transplants via oral gavage using healthy human donor fecal preparations. The mice received one of three unique human donor samples or a mixture of all three. Mouse fecal samples were collected fresh before treatment and after fecal transplants for up to 14.5 weeks. 173 fecal samples were then sequenced for the V3-V4 region of the 16S rRNA gene. Amplicon sequence variants (ASV) were resolved using the DADA2 pipeline. We used the R package mctoolsr to calculate relative abundances, alpha diversity measurements, and to create non-metric multidimensional scaling (NMDS), principal coordinate analysis (PCoA), and dendrogram plots. We tested for statistically significant differences in bacterial relative abundance using FDR adjusted Kruskal-Wallis p values below .05 and linear discriminant analysis effect size (LEfSe). We calculated the percentage of shared taxa between the human donor samples and post-transplant double humanized mice samples. Further, we used the Bayesian SourceTracker algorithm to determine if the human donor samples were the source of gut bacteria found in the double humanized mice samples. Finally, we used PICRUSt to predict the metagenome functional content from our 16S rRNA data.

Results: We characterized the gut bacterial microbiome of our humanized-BLT mice, double humanized mice, and human donor samples. We found that our double humanized mice have unique human-like gut microbiome compositions compared to humanized mice. The gut bacterial microbiota in double humanized mice is human donor specific, which is an important aspect for personalized medicine applications and future experimental design. Double humanized mice have significantly higher species richness compared to humanized mice. We looked at taxonomic relative abundances and found 195 significant differences between the humanized mice and human donor samples. However, there were only 108 significant differences between the double humanized mice and human donor samples. We hypothesized that the pre-existing murine microbiome would slowly return after antibiotic treatment. However, we found that double humanized mice have stable human-like gut microbiomes for the duration of the study, up to 14.5 weeks post fecal transplant. We also characterized the predicted metagenome functional content from our 16S rRNA data and found that double humanized mice share many features of the human donor samples not found in humanized mice. We found that there were 1,604 (35.54%) significantly different predicted KO features between human donor samples and humanized mice samples. However, when we compared the

human donor samples and double humanized mice samples, there were only 61 (1.35%) significantly different predicted KO features.

Conclusions: Our data clearly show that we have created a mouse model with both a functional human immune system and stable human-like microbiome. This model has the potential to help answer difficult to study questions within human populations such as associating specific members of the microbiota with increased inflammation and immune activation during ART. Double humanized mice also provide an important pre-clinical model to study therapeutic strategies for altering the microbiome to impact HIV transmission or disease progression.

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Microbiome alterations in young and old non-human primates and impact of the SIV infection

Background: Advances in antiretroviral therapy (ART) have resulted in near normal life span such that >50% of people living with HIV are now over 50 years of age pointing to the importance of understanding how virally controlled HIV impacts biologic aging. This study is aimed to investigate the role of gut dysbiosis and the gut microbiome on health and immunity in a non-human primate (NHP) model of aging with and without infection with a simian immunodeficiency virus (SIV).
Methods: SIVmac239, 200 TCID50 was injected intravenously in Young (age 3-6 yr; n=4) and old (age >18 yr; N=8) Indian rhesus macaques (RM). Four old uninfected RM were included as controls. ART consisting of PMPA/FTC/L-000870812 was started on day 90 post-infection. Fecal and plasma samples were collected at baseline (pre-SIV infection), post infection (pi) at time of peak viremia (d14 p.i.), chronic infection (d43 p.i.), at ART initiation (d84 p.i.), and after viral suppression (d140 p.i.). Microbiome analysis was performed by shotgun sequencing of DNA isolated from fecal samples.
Results: In SIV uninfected stage age was associated with distinct microbiome profile when the two age groups were compared. Younger animals manifested an increased relative abundance of Streptococcus and Prevotella, while older animals had an increased abundance of Methanobrevibacter. Plasma inflammatory biomarkers were determined by ELISA/multiplex methods also revealed age associated differences. Older animals had higher plasma levels of CRP, neopterin, IFNγ, IL-2, IL-1β, IL-6, IL-8, TNFα, and LPS compared to young animals; while IL-17 was higher in young animals compared to older animals. Correlation analyses showed that microbes most upregulated in young animals had very few correlations with inflammatory cytokines while microbes upregulated in old animals had multiple positive correlations with inflammatory cytokines IFNγ, and TNFα as well as with CRP and neopterin.
Following SIV infection of young and old animals, distinct changes were noted in the microbial composition at the acute and chronic phase of SIV infection. Post ART microbiome became normal in the young animals. In old animals SIV infection and ART treatment led to fewer changes in microbiome. D-dimer transiently increased only in old animals during the acute phase of SIV Infection.
Summary and Conclusions. Our results in the NHP model captured many of the hallmarks of the aging process, including the age associated changes in the microbiome that correlated with inflammation, suggesting a link between microbiome and persistent inflammation in aging. Effect of SIV infection at young age on the microbiome was profound and differed from that of SIV infection in old age where the effect was minimal possibly because the microbiome was already altered in old age. ART treatment had minimal impact on microbial dysbiosis in older SIV animals. Future studies will use the microbiome to create a predictive metabolic map to identify the microbiome regulation of metabolites in aging and SIV. This project bears relevance for investigations of the microbiome in humans with HIV infection occurring at a young versus old age.

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Characterization of Rhesus Macaque Gut Microbiome during RhCMV/SIV vaccination and SIV challenges

BACKGROUND
Rhesus cytomegalovirus (RhCMV) based vaccines against simian immunodeficiency virus (SIV) have been shown to provide impressive protection against repeated low-dose challenges. Unlike conventional vaccines, these vaccines can superinfect previously CMV-exposed hosts and persist indefinitely, constantly stimulating the immune system to respond against SIV (2). We hypothesized that this modified virus interacts with the host in part via modulation of the host's interaction with other microorganisms, particularly the microbiome, possibly contributing to the protective mechanisms. We therefore characterized the gut microbiomes of wild-type RhCMV-seropositive (wtRhCMV+) and -seronegative (wtRhCMV-) animals before vaccination with RhCMV/SIV, after vaccination, and after SIV challenge.

MATERIAL & METHODS
We collected stool samples from 12 wtRhCMV+ and 12 wtRhCMV- animals before and after vaccination with RhCMV/SIV. Five animals each from wtRhCMV+ and wtRhCMV- groups were further challenged with SIV, and stool samples were collected after challenge. DNA from stool samples was extracted using the MoBio PowerSoil kit. Sequence libraries were generated by amplifying the V4 domain of 16S rRNA genes, and further sequenced on an Illumina MiSeq instrument. Sequence reads were analyzed using QIIME 1.9, using 97% sequence identity to Greengenes version 13_8 database.

RESULTS
We first examined the changes in microbiota before and after vaccination with RhCMV/SIV. Of the 165 total bacterial OTUs observed, 41 bacterial OTUs from phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were significantly changed by vaccination: in 33 cases abundance decreased after vaccination and in 8 cases abundance increased after vaccination. In contrast, only 9 bacterial OTUs were significantly changed by SIV challenge, possibly due to our small sample size. Next, we tested the effect of prior RhCMV serostatus on the microbial changes induced by vaccination. Animals that were previously wtRhCMV+ were found to have more significantly changed OTUs compared to seronegative animals (31 vs. 17 changed OTUs, respectively) after vaccination. Similarly, wtRhCMV+ animals experienced more drastic changes in microbiota after SIV infection compared to wtRhCMV- animals (28 vs. 3 significantly different OTUs, respectively).

CONCLUSION
Our results showed that vaccination with genetically engineered RhCMV/SIV vectors changes the recipient microbiota and that, surprisingly, previous wtRhCMV infection was associated with more impressive changes. Notably, both Blautia and Butyrivibrio were shown to decrease in relative abundance after RhCMV/SIV vaccination. Similar trends were observed even before vaccination, as both Blautia and Butyrivibrio were less abundant in wtRhCMV+ animals compared to seronegative animals, suggesting that the change in microbiome was partly because of RhCMV infection. Thus, the history of CMV infection prior to vaccination plays a role in the degree of change in microbiota during vaccination and SIV infection.