1

Fecal microvesicles differentially influence translocating bacterial taxa after SIV infection

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Background: Microbial translocation contributes to persistent inflammation in both treated and untreated HIV infection. Although translocation is due in part to a disintegration of the intestinal epithelial barrier, there is a bias towards the translocation of Proteobacteria, suggesting that translocation is not stochastic. In murine models, epithelial-derived microvesicles have been shown to influence bacterial gene expression and growth in a cargo-dependent manner. We hypothesize that intestinal epithelial microvesicles biologically differ after HIV infection and that altered microvesicular miRNA and/or antimicrobial peptide (AMP) content may contribute to biased translocation.

Methods: We isolated fecal microvesicles from 12 healthy and 12 chronically SIV-infected rhesus macaques (RM, Macaca mulatta) and co-cultured these microvesicles with isolates of translocated bacterial species in order to quantify any influence of microvesicles on bacterial growth. Fecal microvesicles were isolated utilizing the exoEasy protocol and quantified by NanoSight. Viable bacteria that had translocated were isolated from mesenteric lymph nodes, livers, and spleens obtained from end-stage, SIV-infected RM, cultured and passaged in 4 unique media under aerobic and anaerobic conditions, and identified by MALDI-TOF or 16S rDNA sequencing. Bacterial growth was kinetically assayed by spectrophotometer. Microvesicular miRNA profiles were assessed by human miRNA Array cards (n=768 miRNAs) and qRT-PCR. AMPs alpha defensin 1, beta defensin (bDEF) 1, bDEF2, bDEF4, Lysozyme C, PLA2G2a, and Reg3g were assayed by ELISA.

Results: Utilizing the SIV non-human primate model of AIDS, we observed that microvesicular miRNA profiles derived from uninfected and SIV-infected RMs differed significantly by principle coordinate analysis. Ninety-three of 100 differentially expressed miRNAs displayed upregulated expression, with miR-425 and -484 showing significant upregulation in microvesicles derived from SIV-infected macaques. Among AMPs, constitutively expressed bDEF1 showed a significant downregulation among microvesicles from SIV-infected RMs. Several bacterial species showed dose-dependent growth sensitivity upon microvesicle co-culture. Notably, Lactobacillus salivarius showed significantly accelerated growth when co-cultured with microvesicles derived from SIV-infected animals while in contrast Klebsiella pneumoniae displayed stunted growth.

Conclusions: Fecal microvesicles can differentially influence the growth of bacterial isolates known to translocate in SIV infection. This effect may be attributable to a quantifiable shift in microvesicular miRNA content and/or to a shift in AMP content. The identification of the precise mechanisms by which fecal microvesicles differentially regulate the behavior of translocating bacteria will inform the development of therapeutics aimed at impeding microbial translocation.

2

Glycomic determinants of gut microbial dysbiosis and translocation During suppressed HIV infection

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Introduction: An emerging paradigm suggests that gut glycosylation is a key force in maintaining a homeostatic relationship between the gut and its microbiota. In the general population, changes in the gut glycome can alter the gut microbial composition, leading to microbial dysbiosis and gut inflammation. In HIV-infected individuals, microbial dysbiosis and translocation contribute to the vicious cycle between HIV and immune activation/ inflammation. This cycle likely contributes to the development of non-AIDS inflammatory-related illnesses and HIV persistence. However, how gut glycosylation machinery contributes to this cycle is yet to be characterized.

Methods: We used ileum, right colon, and sigmoid colon biopsies, as well as plasma, from 20 HIV-infected individuals on suppressive antiretroviral therapy (ART) to examine: 1) gut glycomes using lectin microarray; 2)

mucosal-associated microbiome using 16S rRNA marker gene sequencing; 3) plasma markers of inflammation/microbial translocation using ELISA, and 4) gut-associated HIV DNA levels using qPCR. Analysis was performed using Spearman's rank correlation coefficient and linear mixed effects models. Nominal p-values and Spearman's Rho are reported.

Results: Increased levels of mucosal-associated, hypo-sialylated O glycans (glycans with low sialic acid) correlated with lower gut microbiome diversity (p=0.001, rho=-0.68), higher Bacteroidetes/Firmicutes ratio (a marker of microbial dysbiosis; p=0.003, rho=0.64), higher plasma levels of sCD14 (a marker of LPS-mediated inflammation; p=0.007, rho=0.58), and higher levels of ileum-associated HIV DNA (p=0.028, rho=-0.56). Levels of mucosal-associated α1-2 branched fucose correlated with higher microbiome diversity (p=0.032, rho=0.48), lower Bacteroidetes/Firmicutes ratio (p=0.009, rho=-0.57), and lower plasma levels of sCD14 (p=0.03, rho=-0.48). Last, levels of ileum-associated galactosylation correlated with lower levels of sCD14 (p<0.0001, rho=-0.8).

Conclusion: Our pilot study provides the first proof-of-concept evidence that differential gut glycomic patterns (mainly sialylated and fucosylated glycans), during ART-suppressed HIV infection, support distinct microbiome compositions that predispose to microbial translocation, inflammation, and HIV persistence. Our data are consistent with previous general population reports which demonstrated that sialic acid catabolism drives microbial dysbiosis and intestinal inflammation and that gut fucosylation sustains host-commensal symbiosis as well as prevents gut inflammation. Exploiting gut glycosylation machinery may allow the design of strategies to manipulate it to treat HIV and/or prevent/delay the development of HIV-associated co-morbidities.

3

Host genetic determinants of the vaginal microbiome and bacterial vaginosis in Kenyan women

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Background: The prevalence of HIV among adult women in western Kenya is 16%. Dysbiosis of the vaginal microbiome - reflected by high diversity and a paucity of Lactobacillus crispatus - is consistently associated with Bacterial vaginosis (BV), a commonly occurring infection that is associated with increased risk of HIV acquisition and transmission. Identifying genetic factors associated with the vaginal microbial composition and BV may aid in elucidating the biological mechanisms of these complex traits. Although a limited number of studies have explored this via a candidate gene approach, no study has performed a genome-wide association study (GWAS) on vaginal microbiome traits. Therefore, we conducted GWAS to identify novel host genetic loci associated with vaginal microbiome traits and BV.

Methods: We conducted GWAS on BV and vaginal microbiome traits on 176 Kenyan women who were assessed for BV four times over one year. Cervicovaginal lavage samples were obtained and 16S rRNA gene amplicon sequencing of the V3-V4 regions was performed to characterize the vaginal microbiome. Study participants were genotyped using the Illumina Global Screening Array using DNA collected from oral swabs. Linear and logistic regression were performed, adjusting for age and principal components, to evaluate the association between the proportion of BV-positive visits across follow-up, the relative abundance of L. crispatus and L. iners, and microbial Shannon diversity index with host genetic single nucleotide polymorphisms (SNPs). To identify biological processes putatively associated with the vaginal microbiome traits and BV, pathway analyses were performed using SKAT-O and WebGestalt. The most significant pathway for each trait is reported after Benjamini-Hochberg correction.

Results: At baseline, the median age of study participants was 22 years (IQR: 22 – 25) with 47% having BV (Nugent score 7-10) for at least one visit. L. iners was present in 83% of samples with a mean relative abundance of 45%. L. crispatus was present in 24% of samples with mean relative abundance of 31%. The mean Shannon diversity index was 1.06. The most significant SNPs associated with the outcomes were: rs72786240 in the intergenic region of LOC105371296-LOC107984867 (P=4.99x10-6) for L. crisp; rs988712 in BDNF-AS (P=7.38x10-7) for BV; rs527430 in the intergenic region of FOXD2-TRABD2B (P=5.20x10-7) for L. iners; and rs972741 in the intergenic region of ZKSCAN2-HS3ST4 (P=9.89x10-7) for Shannon diversity index. In pathway analysis, 'abnormality of the urinary system,' (P=0.005), NF-κB signaling (P=0.001), and 'gene expression (transcription)' (P=0.005) were significantly associated with L. crispatus, BV, and L. iners, respectively. Gene by environment analyses are planned.

Conclusions: To our knowledge, this is the first GWAS documenting host genetic contribution to vaginal microbiome traits and BV. We identified genetic loci and biologically relevant pathways associated with these

traits, providing evidence of host genetic influences on vaginal microbiome composition and BV. This new information should be replicated in larger samples, with comparison across microbiome sites within individuals, and across populations. Building this line of research has potential to guide predictive tools to identify women with unhealthy vaginal microbiomes, those susceptible to treatment failure/recurrence, and potentially aid in preventing further pathological conditions.

4

Elevated fecal inflammatory biomarkers in HIV-/+ MSM associated with gut microbiota

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Background: The gut microbiome of men who have sex with men (MSM) is dramatically altered in comparison to men who have sex with women (MSW), regardless of HIV infection status. In 2015, 82% of men newly diagnosed with HIV in the US were MSM, where the primary mode of transmission is anal intercourse. We recently demonstrated that MSM, relative to MSW microbiota, induced higher levels of immune activation when gavaged into gnotobiotic mice and increased HIV infection in vitro, suggesting that the MSM-associated microbiome may further increase HIV transmission risk in this vulnerable population. To further examine the impact of the MSM-associated microbiome on the gastrointestinal (GI) system we assessed GI symptoms and enumerated inflammatory biomarkers (cytokines/chemokines/adhesion molecules/growth factors) associated with GI inflammation from fecal samples.

Methods: Sixty individuals were placed in one of four cohorts: HIV- MSW, HIV- MSM, HIV+ ART treated (HIV+ART+) and HIV+ ART naïve (HIV+ART-) MSM. All participants completed a GI symptom questionnaire, Bristol stool scale assessment, and twenty-eight biomarkers were measured by ELISA either from the feces itself or water extracted from the feces. The fecal microbiome was assessed by 16s rRNA amplicon sequencing and the distances in fecal microbiome composition (calculated using UniFrac) between samples and distances in levels of fecal inflammatory biomarkers (calculated using Canberra) between samples were determined. Using these two distance matrices a Procrustes analysis was performed to assess the relationship between the microbiome and inflammatory biomarkers.

Results: HIV- MSM had a significantly higher GI symptom score than HIV- MSW but no significant difference in Bristol stool scale score or calprotectin levels were noted. However, thirteen fecal inflammatory immune biomarkers, including IL-12/23p40 (p=0.0006), GM-CSF (p=0.0163), TNF-β (p=0.0197), sCD14 (p=0.0234) and IL-1β (p=0.0304) were significantly elevated in HIV- MSM compared to HIV- MSW. When controlling for MSM behavior only C-reactive protein (CRP) was significantly elevated in HIV+ MSM, with the highest levels seen in the HIV+ ART+ cohort (p=0.0064). The Procrustes analysis significantly linked the microbiome to the fecal immune biomarkers (Mantel test; p=0.029) in our cohort and the concentrations of several biomarkers significantly correlated with the relative abundance of various bacteria.

Conclusion: These data demonstrate HIV seronegative MSM have higher levels of GI related symptoms and elevated fecal inflammatory biomarkers in comparison with MSW. Furthermore, elevated immune biomarker levels were associated with the MSM gut microbiome and only by a few measurements exacerbated with HIV infection, suggesting gut inflammation is more impacted by sexual behavior than by HIV infection itself. These data provide further evidence that the unique gut microbiome in MSM is inflammatory and potentially enhances HIV transmission via anal intercourse. Fecal biomarker analysis also provides a sensitive and noninvasive method for assessing GI health in this high-risk population.

5

Intestinal dysbiosis in simian immunodeficiency virus (SIV) infected rhesus macaques is partially reversed by antiretroviral therapy

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Background: Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infection associated dysfunction of the gastrointestinal (GI) tract has been extensively documented. This dysfunction includes dysbiosis of the GI microbiota and increased microbial translocation across the GI barrier. Prior studies have demonstrated that the GI dysbiosis of SIV-infected rhesus macaques (Macaca mullata, RM) is subtle; the overall diversity of the bacterial communities is largely unchanged. Instead, individual dysbiotic species are increased and certain beneficial species are lost during SIV infection. Whether antiretroviral therapy (ART) has the ability to effectively restore the GI microbiota to the healthy state is still not clear.

Materials and Methods: Fecal samples from healthy (n = 7), SIV-infected (n = 6), and SIV-infected RMs undergoing combination-ART (n = 10) were collected and used for microbial profiling; the V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq and the resulting data were analyzed using the mothur analysis pipeline.

Results: As with prior studies, we did not observe dramatic differences between the various groups based on the relative abundance of bacteria characterized to either the phylum or genus level. Furthermore, the overall diversity of the microbiota as measured by the inverse Simpson index was not different between healthy RMs and those with active SIV infection or undergoing ART. However, microbial dysbiosis between the groups was evident via multiple beta diversity calculators: Jaccard similarity coefficient, Bray-Curtis similarity coefficient, and Yue & Clayton theta similarity coefficient. Additionally, principle coordinate analysis clustered SIV-infected RMs away from healthy and treated RMs, indicating that the bacterial communities of SIV-infected RMs differed from the other two groups. Of note, RMs receiving ART clustered closely with healthy animals, indicating that ART was able to reverse the SIV-associated GI dysbiosis. Metastats analysis identified specific OTUs that were differentially represented across the various groups; these included members of the Streptococcus, Prevotella, Acinetobacter, Treponema, and Lactobacillus genera.

Conclusions: SIV infection of RMs appears to promote microbial dysbiosis of specific microbes and this dysbiosis appears to be partially reversed by cART.

6

A Streptococcus dominant dysbiotic gut microbiome in ART experienced HIV-1 infected adults from Ghana

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Background: A major driver of systemic inflammation and immune activation observed in HIV infected individuals is translocation resulting from gut microbial dysbiosis. This has been shown to persist even after initiation of effective combination ART therapy. Thus, modulating the gut microbiome has been proposed as potential therapeutic target for people leaving with HIV/AIDS. We describe the composition of the gut microbiome in ART experienced HIV-1 infected individuals with diverse treatment outcomes from Ghana.

Methods: In a cross-sectional case-control study, we enrolled 84 ART experienced (NNRTI based) HIV-1 infected individuals and 84 seronegative controls. HIV-1 infected individuals were recruited from the ART clinic at the Regional hospital, Koforidua, Ghana. While seronegative controls were recruited from the same community of residence as cases and matched by age and gender. Using the Illumina Miseq system, 16S rRNA gene (V3-V4 region) amplicon sequencing was performed on nucleic acids extracted from stool samples of study participants. Amplicon sequence data were analyzed by Quantitative Insights Into Microbial Ecology 2 (QIIME2 v2019.4). Assigning taxonomy by SILVA database (release 132), differentially abundant taxa were identified using the ANCOM and DEseq2 packages in R.

Results: When compared to controls, HIV infected individuals exhibited reduced alpha diversity (Kruskal-Wallis, $p<0.001$), measured by Shannon and Faith's-phylogenetic indices, irrespective of treatment outcome or duration of treatment (<1 year and over 1 year). Also, they exhibited significant separation of enteric bacterial community (PERMANOVA $p<0.05$, on Bray Curtis and weighted UniFrac distances) compared to controls. Among HIV infected individuals, there was no significant difference in both alpha and beta diversity analysis when compared by either duration of ART or treatment

outcome. Differential abundance analysis identified several genera including; Streptococcus, Blautia, Dorea, Gemella, Achromobacter to be significantly abundant in HIV infected individuals. In contrast, significant abundance of genera Faecalibacterium, Ruminococcaceae-UCG-014, Ruminococcaceae-UCG-002, Dialister, and [Eubacterium]-eligens-group were observed in controls.

Conclusions: Using a fairly appropriate control cohort, we show that the gut microbiome was significantly altered in HIV infected adults in our Ghanaian cohort. These individuals presented diverse treatment outcomes which is typical of low-income countries such as found in Sub-Saharan Africa. Our study importantly provides information from West Africa, a region where research on HIV-enteric microbiome is scarce.

7

HIV replication, transmission, and the metabolome of the female reproductive tract

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Background: HIV disproportionately affects young women, yet we have a poor understanding of the factors contributing to a successful sexual transmission event in the female genital tract. Colonization of the female reproductive tract with highly diverse, Lactobacillus-deficient microbial communities (HDCs) increase a woman's risk of acquiring HIV-1 compared to colonization with Lactobacillus-dominant communities (LDCs). The inherent polymicrobial nature of these communities has made it exceedingly challenging to elucidate the microbial mechanisms responsible for modulating HIV transmission in the genital tract. Despite this heterogeneity in microbial community structure, several studies have identified conserved changes in the small molecule metabolome of cervicovaginal lavage fluid collected from women colonized with HDCs or LDCs. Since bacterial production of metabolites can profoundly reprogram cell physiology, we interrogated whether any of these metabolites can modulate susceptibility to HIV-1 infection using a novel high-throughput screening method.

Materials and Methods: We developed a high-throughput screening (HTS) capable, TZM-bl based reporter assay to screen a library containing over 500 microbial and host derived metabolites. Indicator TZM-bl cells were seeded into 384 well plates and incubated with metabolites 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 was used to quantify HIV replication in the presence of our metabolites. The screen was conducted in duplicate and the results were interpreted in light of four published metabolomics studies comparing the small molecule metabolome of cervicovaginal lavage fluid of women colonized with HDCs (i.e. bacterial vaginosis) or LDCs. Conserved changes in the metabolome that putatively affect HIV-1 replication were validated using a combination of p24 ELISA assays in antiCD3 activated donor CD4+ T cells and/or flow cytometry staining for HIV-1 core antigen in unstimulated donor CD4+ T cells.

Results: Our HTS assay identified metabolites with previously demonstrated abilities to modulate HIV replication in primary T cells, validating the potential of our screen to identify biologically meaningful metabolites and/or pathways. Comparison of these results to the cervicovaginal metabolome of HDC and LDC colonized women identified several metabolites of interest that might play important roles in the context of the vaginal microbiome and HIV-1 infection. Preliminary results suggest that HIV replication in CD4+ T cells may be particularly sensitive to alterations in the concentrations of amino acids and amino acid derivatives produced by vaginal microbiota. We are currently exploring how these metabolites could be responsible for controlling HIV replication in the genital tract and aim to build a mechanistic understanding of how metabolites associated with these microbial communities work in concert to modulate T cell susceptibility to HIV infection.

Conclusions: HDC-associated metabolites can influence HIV replication efficiency and may play an important role in viral transmission. Ultimately, we hope this study will guide future efforts to modulate the environment of the female genital tract to interfere with HIV transmission.

8

The rectal microbiome in Kenyan men who have sex with men: associations with sexual behaviour and microbiome diversity

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Background: The rectal microbiome plays an important role in regulating mucosal immunity, which may have implications for rectal HIV acquisition in men. Both HIV infection and MSM status have been linked to altered gut microflora, but to date very few studies have been carried out in Africa.

Material and Methods: A cross-sectional study was conducted to characterize the microbial and immunological environment of the rectum and markers of systemic inflammation in the blood in a cohort of MSM men from Nairobi, Kenya. The microbiome was characterized pre and post enema using 16S rRNA sequencing. Concentrations of 37 inflammatory cytokines were analyzed using multiplex immunoassays from rectal mucosal fluid collected post-enema and plasma. Flow cytometry was done on PBMCs to quantify immune cell activation. Epidemiological data were also collected to account for different sexual behaviour and demographic information.

Results: Samples were obtained from three study groups of MSM: HIV negative (n=39), HIV treated (n=21), and HIV untreated (n=10). Alpha diversity was consistently lower in HIV positive men and treatment had minimal impact on reversing this trend. We did not observe any difference in the Prevotella or Bacteroides clusters based on HIV status, but ART trended towards an increase in Bacteroides and a decrease in Prevotella. At the genus level, we found HIV positive to have increased abundance of Streptococcus while those on ART were enriched in Roseburia. In the HIV negative men, receptive anal sex with paying partners was associated with a lower alpha diversity (p-value=0.00262), suggesting that specific exposure in the rectal mucosa contributes to microbiome changes. We also found douching to be associated with the Prevotella cluster and also higher IFABP and sCD14 levels. We did not find an association between alpha diversity and microbial translocation markers.

Conclusions: Changes in the rectal microbiome due to HIV was subtle as has been shown by others when MSM status is taken into consideration. The effect of ART was uncertain although some trends were evident in changing the beta diversity. We also found receptive anal sex to be associated with lower alpha diversity while douching was associated with the Prevotella cluster, thus showing how specific behaviours affect our rectal microbiome.

9

Gut fecal microbiome variation according to immune status of HIV infected children in Yaounde Cameroon

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Background: HIV has been observed that CD4+ T-lymphocyte depletion in the gastrointestinal tract leads to microbial disruption and intestinal permeability (translocation) which gives rise to opportunistic infections. The objective of this study was to describe gut fecal microbiome according to immune status of HIV infected children in Yaounde, Cameroon.

Methodology: A cross-sectional and case control study was conducted from January to October 2018. HIV infected and non-infected children, aged 1-19years were recruited at the Pediatric unit of Yaounde University Teaching Hospital and at the Centre for Mother and Child, Chantal Biya's Foundation. HIV infected children were on ART and prophylactic co-trimoxazole antibiotic. Fecal samples were collected from participants. DNA was extracted from fecal samples and the 16S rRNA was sequenced on the MiSeq platform. Statistic methods were Tukys's multiple comparison test, and correlations between taxa and with HIV status, CD4+ T cells, were performed using genus level using SILVA (v132) annotation and QIIME II.

Results: Among the 169 participants, 87 were HIV infected and 82 were HIV non-infected, healthy control children. Approximately equal number of female 84 (50.6%) and male 85 (49.4%) representation. Mean age of our study was 10.13 and median age was 10. Five (5.7%) infected children had CD4+ < 200 cells/mm3, 18 (20.7%) had CD4+ between 200-499 cells/mm3, and 64 (73.6%) had CD4+ >500 cells/mm3. We found a reduced diversity in the microbial community for participants with CD4 <200 cells/mm3, compared to those with CD4 >200

cells/mm3. Genera like Lachnospira (p=0.006), enterobacteriaceae like Escherichia-Shigella (p=0.043) and Citrobacter (p=0.044), and Oxalobacter (p=0.044) were significantly abundant among the HIV infected with CD4 T cell counts <200 cells/mm3 compared with those with CD4 T cell counts 200 – 499 cells/mm3. No significant bacteria were observed while looking for those with CD4 T cell counts <200 cells/mm3 and those >500 cells/mm3. When we went further to compare the infected group with CD4+ T cell counts 200 – 499 cells/mm3 and those with > 500 cells/mm3, we observed interestingly additional genera like Escherichia-Shigella (p=0.00007), Ruminococcus (p=0.0001), Lachnoclostridium (p=0.001), Rothia (p=0.004), and Klebsiella (p=0.022) that were significantly abundant.

Conclusion: CD4 counts appear to be reflected in the microbiota, enriched in proinflammatory pathobionts despite the fewer number of children with pronounced immune depression.

10

Impact of simian immunodeficiency virus (SIV) and antiretroviral therapy (ART) on plasma lipidomic profile of nonhuman primates (NHPs)

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Background: SIV/HIV related cardiovascular disease (CVD) is not understood. In general population, CVD is related to lipid abnormalities. Lipid profile changes occur in HIV infection. We hypothesized that disruptions of lipids occur in SIV-infected NHPs, induced by either ART or the virus. NHPs may be ideal to study the impact of ART on lipidomics, as well as the role of lipid classes in SIV pathogenesis due to their controlled, healthy diet, and the lack of other confounding lifestyle factors.

Method: We analyzed >1000 plasma lipid species in naïve and SIV-infected NHPs on ART. 25 pigtailed-macaques (PTMs) were infected with SIVsab and 6 received coformulated ART (emtricitabine, tenofovir, and dolutegravir) starting from 48 days postinfection. Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy was performed on samples collected throughout infection and treatment. After lipid profiling, the mean fold increase from the baseline was calculated for each timepoint, and statistical analyses were performed by ANOVA contrasts. Key parameters of SIV infection were monitored throughout the follow-up: proinflammatory cytokines (IL-6 and TNF-a), CD4+ T cells, D-dimer, and CRP, and correlated with the lipid concentrations by Spearman's correlation.

Results: SIV infection induced lipid profiles similar to those associated with CVD, including decreases in total lysophosphatidylcholine (LPC), numerous LPC species, cholesteryl ester (CE) (18:3), phosphatidylcoline (PC) (18:0/20:3) and increases in total lactosylceramides (LCER) and triacylglycerides (TAG) (54:2). Lipids altered in HIV-infected subjects on ART (LPC, and LPC species) were similarly decreased in SIV-infected NHPs on ART. 12/22 LPC species significantly decreased in chronic SIV infection and during ART. Total sphingomyelin (SM) levels transiently increased in acute infection. Total hexosylceramides (HCER) and total LCER were elevated throughout SIV infection. LCER returned to preinfection levels shortly after ART initiation, while HCER only normalized after prolonged ART. 6/12 HCER species were significantly elevated in chronically SIV-infected PTMs, with 4 remaining significantly elevated on ART. One LCER species was elevated in chronic infection, while 1 increased with ART. Total phosphatidylinositol (PI) levels decreased, and did not rebound to normal with ART. 10/28 PI species were significantly decreased in chronic infection, while ART decreased 18/28. Finally, total phosphatidylethanolamine (PE), total lysophosphatidylethanolamine (LPE), and total TAG were specifically decreased on ART.

Multiple lipid species decreased by SIV or ART negatively correlated with D-dimer, sCD14, IL-6 and TNF-a. Total PE and specific PC species which were also significantly decreased by SIV or ART positively correlated with CD4 counts. Total ceramides positively correlated with D-Dimer while PC (15:0/22:6) strongly positively correlated with CRP. Positive correlations were also found between TNF-a and TAG and LCER species that were increased by SIV or ART.

Conclusions: Multiple lipidome alterations occurred in SIV-infected PTMs. ART normalized some, yet significant abnormalities persisted, some specifically related to ART. Lipid changes significantly correlated with markers of disease progression, inflammation and coagulation, suggesting that a "proinflammatory lipidome" contribute to the pathogenesis of CVD in HIV/SIV. Our results may help identifying novel biomarkers predictive for SIV/HIV comorbidities. Further exploration into the benefits of interventions targeting dyslipidemia is warranted for the prevention of CVD and beyond.

11

Baseline characterization of the vaginal microbiome among secondary school girls enrolled in the cups and community health (CaCHe) vaginal microbiome study

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Background: We are evaluating the effect of menstrual cups on the vaginal microbiome and Bacterial vaginosis (BV) and sexually transmitted infections (STIs) among a sub-set of secondary schoolgirls enrolled in the Cups or Cash for Girls cluster randomized controlled trial in Siaya county, western Kenya. We characterized how the baseline vaginal microbiome (VMB) differed by BV and STI, and factors associated with VMB composition.

Methods: May through June 2018, 436 girls were enrolled in the CaCHe VMB sub-study. Girls completed self-administered survey (via electronic tablet) for socio-demographic and behavioral data, and self-collected four vaginal swabs. Vaginal swabs were tested for BV, T. vaginalis (TV), N. gonorrhoeae (NG), and C. trachomatis (CT). VMB was characterized via 16s rRNA gene amplicon sequencing. ElasticNet (EN) identified genus-level taxa that differed by BV and STI status. Hierarchical complete linkage clustering of species-level taxa identified community state types (CST), and multinomial logistic regression identified factors associated with CST.

Results: Girls were median age 16.9 years and 30% reported ever having sex and/or being coerced into having sex. The prevalence of STIs was 9.9%: 3.0% TV, 6.2% CT, 1.4% NG. The prevalence of BV was 11.2%. The prevalence of STI and/or BV was 17.4%, with 35% of girls with STI also having BV. Significant taxa associated with BV were: Lactobacillus (inverse association), Megasphaera, Atopobium, Gardnerella, Prevotella, Sneathia, Fastidiosipila, Eggerthella. Taxa associated with STI (composite of NG, CT, and/or TV) were Parvimonas, Mycoplasma, Gemella, Eggerthella, and Peptostreptococcus. Five CSTs were identified: CST-1 L. crispatus dominant (N=174, BV = 0%, STI = 2.9%, sexually active = 22%); CST-2 L. iners dominant (N=79, BV = 1.3%, STI = 7.6%, sexually active = 39%); CST-3 mixed, with moderate L. iners/L. crispatus (N=86, BV = 7.0%, STI = 7.0%, sexually active = 21%); CST-4 mixed, with moderate L. jensenii/L. crispatus (N=29, BV = 10.3%, STI = 13.8%, sexually active = 14%); CST-5 mixed, with moderate G. vaginalis (N=60, BV = 63.3%, STI = 36.7%, sexually active = 59%). Diversity was increased in CST-3, CST-4, CST-5. In multivariable adjusted analyses, more diverse CST was predicted by greater age, ever being sexually active, BV status, and STI status. CST was not associated with period characteristics (e.g., duration, pain/cramping, heavy/normal/light flow) or cloth vs. pad use.

Conclusions: The prevalence of STIs and BV were high. Data indicate sexual activity is underreported. Despite substantial overlap in girls with BV and STIs, the taxa discriminating these infections differed. Longitudinal analyses will identify change in VMB composition with incident BV and STI and changing sexual exposure.

12

Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and SCFAs associated with optimal and non-optimal vaginal microbiota

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Introduction: Non-optimal vaginal microbiota (VMB), such as bacterial vaginosis (BV), is characterised by a depletion of lactobacilli, cervicovaginal inflammation and increased risk of HIV infection compared to women with an optimal VMB dominated by Lactobacillus spp. Lactic acid (LA) is a major metabolite produced by vaginal Lactobacilli spp. that elicits anti-inflammatory effects from cervicovaginal epithelial cells and is depleted during BV. However, it is unclear if LA retains its immunomodulatory effects in the presence of physiological levels of short chain fatty acids (SCFAs) and succinic acid which are found in the FRT. In addition, the immunomodulatory effect of SCFAs and succinic acid on cervicovaginal epithelial cells at concentrations present during BV is unknown.

Methods: Cervicovaginal epithelial cells were treated with VMB metabolites in combinations and individually to mimic either eubiotic or BV conditions in the absence or presence of TLR agonists poly I:C and Pam3CSK4 to simulate pathogen challenge. Cytokines and chemokines were quantified using Luminex assays.

Results: The immunomodulatory effects of LA were maintained in the presence of SCFAs and succinic acid at pH<4.5 and physiological concentrations present in women with lactobacillus-dominated microbiota. In contrast, VMB metabolite mixtures present during BV had a dysregulated immunomodulatory effect on cervicovaginal epithelial cells, only evident during prolonged and sustained treatments. These BV VMB effects were cell-type specific, and pro-inflammatory effects were evidenced by increased levels of TNFα and the enhancement of TLR-stimulated production of TNFα and IL-8, attributed mainly to acetic acid. Certain SCFAs also dampened basal RANTES and IP-10 production, as well as TLR induced IL-6, RANTES and IP-10 production in a cell-type specific manner.

Conclusion: These findings indicate that elevated SCFAs are a potential source of cervicovaginal inflammation in women experiencing BV, and support the unique anti-inflammatory properties of the VMB LA on cervicovaginal epithelial cells. There may be a role for LA or LA-producing lactobacilli to reverse genital inflammation associated with increased HIV risk.

13

Effects of bacterial vaginosis on endogenous anti-HIV activity of female genital tract secretions and PrEP Efficacy

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Background: Phase 3 HIV PrEP clinicals trials in women have yielded suboptimal results in preventing HIV reflecting difficulties with consistent adherence and biological factors that may modulate HIV risk and drug pharmacokinetics (PK). Recent studies highlight the complex mechanisms by which individual bacteria and their metabolic products adversely affect PrEP PK by competing with human cells for drug uptake, inhibiting drug transport into human cells, or metabolizing drugs. Moreover, bacterial vaginosis (BV) is associated with increased HIV risk, possibly reflecting mucosal inflammation. However, the precise mechanisms linking BV, inflammation PrEP PK and HIV risk are not defined. We hypothesize that BV will be associated with decrease in PrEP efficacy reflecting either a decrease in drug efficacy and/or increase in HIV risk.

Methods: To test this hypothesis, we conducted a longitudinal study in 10 women with symptomatic BV. Vaginal swabs and cervicovaginal lavage (CVL) were collected at time of diagnosis and 1 week and 4 weeks after completion of treatment with oral metronidazole. Samples were also collected from 10 asymptomatic controls (no BV) frequency matched for age, race, and ethnicity at a single time point. The endogenous antiviral activity of secretions and their effects on PrEP efficacy were assayed by adding (filtered) secretions to cells in the absence or presence of tenofovir (TFV), tenofovir alafenamide (TAF) or dapivirine (DPV) and infected with HIV. The microbiome is being assessed by 16sRNA sequencing and quantitative PCR for select organisms.

Results: There was a trend for secretions from women with BV to increase HIV infection of cells. This enhancement was inversely correlated with the L. crispatus concentration (rho = -0.339, p= 0.006) and positively with several anaerobes including Sneathia, G. vaginalis, A. vagainae and BVAB3 (all $p < 0.05$). There was also a significant fold increase in the concentration of TFV needed to inhibit HIV infections (IC50) when assays were conducted in the presence of swabs from women with BV, which resolved after treatment and was not observed with swabs obtained from asymptomatic controls (fold increase in IC50 1.472 [0.83 μM, 4.24 μM] $p< 0.05$ compared to controls). This was not associated with any loss in radiolabeled drug levels, suggesting that the interference may be linked to ability of secretions to block TFV uptake by human cells [Taneva, et al. JCI Insight, 2018]. The increase correlated strongest with BVAB3. There was no significant effect of swabs on DPV or TAF antiviral activity.

Conclusions: Genital secretions from women with BV enhanced HIV infection and interfered with TFV anti-HIV activity, which resolved with treatment. These negative effects were associated with increased levels of select anaerobes; 16sRNA microbiome studies and levels of cytokines in genital secretions are in progress. The precise mechanisms and direct effects of bacteria and their soluble products are being investigated.

14

Colonic microbiota is altered in treated HIV infection independently of sexual practice and correlates with HIV disease progression

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Loss of gut mucosal integrity, and aberrant gut microbiota, and translocation of microbial antigens are posited mechanisms contributing to chronic inflammation and subsequent increased burden of morbidity and mortality during antiretroviral-treated and untreated HIV disease compared to the HIV-uninfected population. Although several studies report an HIV-associated gut microbiota signature, a dramatic shift in the gut microbiota dependent on sexual practice has recently been uncovered, potentially confounding the role of HIV in prior studies.

To overcome this confounding factor and others known to influence microbiota analyses, we recruited a well-powered cohort of HIV-infected subjects and seronegative controls that was both diverse in behavioral and physiological factors and matched for age, sex, sexual practice, body-mass index, and birth country between cases and controls.

Gut microbiota analyses revealed that treated chronic HIV infection was associated with significant alterations in the gut microbiota regardless of sex and sexual practice, which included enrichment of Gammaproteobacteria members, depletion of Lachnospiraceae and Ruminococcaceae, and decreased alpha diversity.

Men who have sex with men (MSM) exhibited a distinct microbiota signature largely characterized by Prevotellaceae enrichment and increased alpha diversity, with certain taxa exhibiting opposite abundance trends to those associated with HIV infection and likely contributing to spurious associations in prior studies. Receptive anal intercourse in both males and females was linked to the MSM-associated microbiota signature regardless of sex. Finally, we found that the HIV-associated microbiota signature correlates with inflammatory markers, clinical predictors of disease severity, and prevalence of age-associated noncommunicable comorbidities.

15

Metformin treatment and gut microbiota in non-diabetic persons living with HIV

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Background: Persons living with HIV (PLWH) on antiretroviral therapy (ART) present with increased risk of inflammatory co-morbidities linked with elevated microbial translocation of bacterial and fungal products. Metformin, an anti-diabetic drug was shown to improve glucose levels and also reduce inflammation by inhibiting mTOR signaling. Such effects were recently associated with changes in the gut microbiota and increased colonization of Akkermansia muciniphila in mice. Moreover, in diabetic individuals, metformin use was associated with modification of the microbiota composition and increased frequency of A. muciniphila. Herein, we evaluated the effect of 12 weeks of metformin therapy on inflammation in non-diabetic PLWH under ART, in association with the modification of bacterial and fungal stool microbiota.

Methods: As part of the LILAC study CTN PT027, 22 PLWH receiving ART were recruited. Participants were non-diabetic (HbA1c<6%), received ART and had <40 copies/ml of HIV RNA for more than 3 years, and a CD4/CD8 ratio below 0.7 to select participants with a higher risk of inflammation.
Participants received metformin (850 mg bid) orally for 12 weeks. Blood and stool samples were collected at baseline (visit 1), after 12 weeks of metformin (visit 2), and 12 weeks after metformin discontinuation (visit 3) to assess a carryover effect. Plasma markers of microbial translocation were assessed by ELISA for bacterial Lipopolysaccharide (LPS) or the Fungitel assay for the fungal 1->3-β-D-Glucan.

To assess composition of the microbiota, DNA was extracted from frozen stools. We analyzed the bacterial microbiota by sequencing the 16S rDNA region and the fungal microbiota by sequencing the internal transcribed spacer (ITS). Variations in microbiota composition were analyzed using the Lefse algorithm.

Results: Metformin was well tolerated in all participants. CD4, CD8 counts, fasting glucose, and HbA1c percentage remained stable following 12 weeks of metformin therapy in these non-diabetic participants. Plasma soluble CD14 levels decreased only after metformin discontinuation (visit 3) compared to baseline (1760 vs 1818 pg/ml, p=0.02). However, no differences were noted in LPS nor β-D-Glucan plasma levels between visits.

Stool bacteria diversity (Shannon and Simpsons indexes) tended to increase at visit 2 and 3 compared to baseline, without reaching significance. No differences were observed at each visit for the Prevotella/Bacteroides frequency ratio. We observed a significant increase of Escherichia/Shigella, Lachnoclostridium and A. muciniphila and a decrease of Collinsella frequencies at visit 2 compared to baseline. An increase of butyrate producing Lachnospiraceae was noted following after metformin discontinuation (visit 3) compared to baseline.

All participants had either Candida or Saccharomyces detectable in their stools. No variations in the diversity of the fungal microbiota were noticed between each visit. A slight increase in Saccharomycetales was observed only after metformin discontinuation (visit 3) compared to baseline.

Conclusion: 12 weeks of metformin therapy in non-diabetic PLWH under ART slightly decreased inflammation. This decrease was concomitant with a higher abundance of Lachnospiraceae and Lachnoclostridium, bacteria associated with lower inflammation in PLWH. A mild increase of Saccharomycetales was noticed after metformin intake. A longer metformin treatment may further reduce inflammation and prevent non-AIDS co-morbidities in PLWH.

16

Rational donor fecal microbiota transplantation in HIV (REFRESH Study)

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Background: An altered interplay between mucosal immunology and the microbiota might contribute to chronic immune dysfunction during treated HIV infection. It is unknown whether oral fecal microbiota transplants (FMT) can affect the gut microbiota and systemic immunity of HIV-infected individuals.

Methods: Thirty ART-treated HIV-infected subjects with a CD4/CD8 ratio <1 were allocated to receive either weekly oral fecal microbiota capsules or placebo for 8 weeks (10 capsules at week 0; 5 capsules/week from weeks 1-7). Three stool donors were selected from a universal donor stool bank based on bacterial abundance of Fecalibacterium and Bacteroides (high) and Prevotella (low) together with high fecal butyrate concentrations. We assessed 48-week safety (primary outcome) and efficacy (secondary outcome), including changes in CD4+/CD8+ T cells, microbiota engraftment using Illumina 16S rRNA sequencing, T cell activation/senescence, inflammation (sCD14, sCD163, sTNFr-2), bacterial translocation (LTA, LBP) and intestinal damage (FABP2) markers. Between-group differences in linear trajectories of continuous variables were assessed using linear mixed models.

Results: Twenty-nine participants, with a mean CD4 count of 641±286 cells/μL and CD4/CD8 ratio of 0.63±0.26 completed the 48-week follow-up. FMT was well tolerated, with no grade 3-4 related adverse events, although mild gastrointestinal symptoms were more frequent in the active arm. No significant changes were observed in CD4/CD8 T-cells, in T-cell activation/senescence or plasma levels of the inflammation/bacterial translocation markers. Significant between-group differences were observed in FABP2 changes (p=0.016), with a greatest decrease in fold changes from baseline at week 4 in the FMT arm (0.52 vs. 0.95, p=0.045). Alfa diversity significantly and incrementally increased until week 6 in the FMT arm (FMT vs. placebo arm, p=0.013) and returned to baseline levels at week 48. Beta-diversity analysis using Unifrac distance trajectories indicated mild engraftment of donor's microbiota that persisted until week 36 and greater engraftment among the 4 subjects who had received antibiotics in the 12-week period before FMT. Several taxa were acquired by the recipients and retained until the end of follow-up. LEfSe analyses indicated an incremental engraftment of different taxa in the active arm, being Lachnospiraceae family and Faecalibaculum, Faecalicoccus, Fusicatenibacter, Anaerostipes and Ruminococcus genus the taxa more robustly engrafted across time-points.

Conclusions: Repeated oral capsular FMT from rationally selected donors was safe in HIV-infected subjects on ART and introduced incremental compositional changes in the fecal microbiota. While it is unclear whether this strategy will help to attenuate systemic inflammation and immunoactivation, our results indicate that manipulation of the gut microbiota using a non-invasive and safe strategy of FMT delivery is feasible.

17

Oral fecal microbiota transplantation increases gut microbiome diversity and alters the microbiome distribution in people with HIV

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Background: Reduced microbiota diversity (dysbiosis) in people with HIV (PWH) can damage the intestinal barrier and increase microbial translocation, resulting in inflammation, a driver of morbidity and mortality. We hypothesized that multiple oral fecal microbiota transplants (FMT) would reverse dysbiosis in PWH.

Methods: We administered 6 weekly oral doses of a novel oral lyophilized fecal microbiota product from 2 healthy donors to 6 men who have sex with men with HIV on suppressive ART. Shotgun sequencing on stool from before, after 6 weekly FMT, and 20 weeks after completing FMT (Weeks 0, 6 and 26), and the donors, was performed to determine bacterial community profiles. Biomarkers were measured by Luminex assays and ELISAs. All comparisons used Wilcoxon matched-pairs signed rank test.

Results: Median age at Week 0 was 39 years, CD4+ T cell count 496 cells/mm3, BMI 27.7 kg/m2. All participants had HIV RNA<20 copies/mL. Median time since diagnosis was 5.5 (range 2-35) years, and median time on ART was 5.5 years (range 2-7 years). Mean α diversity by observed species index increased from Week 0 to 6 (61.2 to 70.2, P=0.29) and decreased by Week 26 (70.2 to 52.2, P=0.33) to be similar to the donors' (63.5, P=0.86). Microbiome distribution by principal component analysis (PCA) shifted towards the donors' distribution in most participants at Week 6 but shifted away by Week 26. Lachnospiraceae (unspecified genus) increased in 5 of the 6 participants between Weeks 0 and 6 from 0.92 to 4.39% (P=0.06).

BMI and CD4+ T cell counts did not change significantly between weeks 0 and 6 or 26. Two participants maintained HIV-1 RNA <20 copies/ml at weeks 0, 6, and 26. Three participants had confirmed HIV-1 RNA blips at Week 6 (30, 60, and 170 copies/ml respectively), all with HIV-1 RNA ≤20 copies/ml at Week 26. One participant paused ART due to unrelated factors and had HIV-1 RNA of 2,140 copies/ml at Week 6 and 130 copies/ml at Week 26.

One participant, with HIV >35 years, had persistent constipation that resolved with FMT, with a dramatic shift in microbiome communities. The constipation recurred at Week 26. Fusobacterium gonidiaformans, Porphyromonas somerae, and Haemophilus parainfluenzae comprised 27% of his microbiome at Week 0 but 0.73% at Week 6; untyped Bacteroides comprised 35% at Week 6 with a surge in Enterobacteria phages. I-FABP (6899 to 2736 pg/ml), sCD14 (1.67 to 1.31 ug/ml), IL-6 (1.51 to 1.13 pg/ml) and sTNFRII (11659 to 8300 pg/ml) levels decreased in this participant; Week 0 levels were higher than in other recipients. No related serious adverse events occurred.

Conclusions: Weekly FMT resulted in transiently increased intestinal microbiome α diversity and a shift in microbiome distribution in most participants. PWH with long-term HIV and/or greater inflammation or gut damage may be most likely to benefit from FMT. The effects of recurrent FMT were transient, suggesting longer duration of weekly treatment or intermittent FMT boosting may be required to maintain its benefits.

18

Cryptosporidiosis and other intestinal parasitic infections and concomitant threats among HIV infected children in southern Ethiopia receiving first-line antiretroviral therapy

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Background: Children infected with human immunodeficiency virus (HIV) are at high risk of acquiring intestinal parasitic infections. This study aimed to determine the magnitude of cryptosporidium and other intestinal parasitic infections and concomitant threats among HIV-infected children.

Methods: A hospital-based cross-sectional study was carried out at three antiretroviral therapy clinics in southern Ethiopia from February 2016 to June 2017 in a total of 384 HIV positive children. Socio-demographic and laboratory data were collected using structured questionnaires. Direct stool microscopic examination and modified Zeihl- Neelsen staining technique were performed by trained laboratory technologists. Chi-square test was conducted to determine the real predictors of the infection. A significant association was considered when p-value ≤0.05 at 95% CI.

Results: The overall magnitude of intestinal parasitic infections among the study population was 16.9% (95%CI: 13.0-20.8%). The most predominant parasitic infections were Cryptosporidium spp. (9.6%) and the least was Taenia spp. (0.78%). The presence of diarrhoea (χ 2 =7.653, df= 2, p=.022) was detected to be the only significant associated variable.

Conclusions: Cryptosporidium infection was found to be the most common intestinal parasitosis among HIV-infected children. Routine screening service for cryptosporidium and other intestinal parasites is important in the clinical management of HIV-infected children.

19

Transkingdom interactions between the microbiome and virome in the female reproductive tract

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Background: Human Immunodeficiency Virus (HIV) remains a major global health concern. More than 90% of HIV infections worldwide occur following heterosexual intercourse, where women are twice as likely to contract HIV as men. The female reproductive tract (FRT), the initial site of HIV infection in women, hosts a microbiome composed of bacteria, archaea, viruses, and fungi that plays an important role in maintaining vaginal health. Low diversity, Lactobacillus-dominant bacterial communities (bacteriomes) help prevent urogenital diseases including bacterial vaginosis (BV) and sexually transmitted infections (STIs) such as HIV, while diverse FRT bacteriomes have been linked to increased inflammation and HIV transmission. However, less is known about how alterations in the FRT viruses (virome), including transkingdom interactions with the bacteriome, affect FRT health and HIV transmission risk. We hypothesize that HIV will be associated with alterations in the FRT bacteriophage and eukaryotic DNA virus populations, and these alterations will correlate with shifts in the FRT bacteriome.

Materials and Methods: We conducted a retrospective, longitudinal analysis of vaginal swabs collected twice yearly between October 2012 and October 2014 from 50 HIV-positive and 50 HIV-negative, sexually active young women ages 16-21 in Cape Town, South Africa. 16S rRNA amplification of the V4 region was performed and samples were sequenced on the Illumina MiSeq Platform. Generated sequences were quality-controlled and screened for homology to bacterial taxa using QIIME2. A subset of 50 samples taken for virome analysis were enriched for viral nucleic acid, libraries built, and sequencing performed on the Illumina Novaseq. Sequences were quality controlled and processed through a custom bioinformatics pipeline to stringently identify viral sequences.

Results: The cohort consisted of HIV positive and negative women that were on average 20 and 18 years old, respectively. HIV positive women had an average CD4+ T cell count of 477.5 cells/µL. The majority of HIV positive and negative women, 96% and 94 % respectively, had at least 1 sexual partner within 6 months of initial study collection. Samples clustered into five distinct community state types (CSTs) where CST 2 was correlated with BV diagnosis. Associations were also found between Atopobium vaginae, a dominant member of CST 4, and HIV positive, not on HAART status. Bacterial alpha diversity was significantly associated with BV while phylogenetic diversity was associated with both BV and HIV positive, not on HAART status. The eukaryotic virome

consisted almost entirely of HPV. HIV infection was associated with the presence of HPV infection in this South African cohort during 2 of the 5 visits that were tested (p=0.0005 and p= 0.0078). We did not observe differences in bacterial diversity with HPV infection; however, increased alpha diversity was observed with HPV Type 6 infection (p=0.029). Bacteriophage data will be presented.

Conclusions: In this South African cohort, HIV infection was associated with HPV infection at certain time points. Changes in bacterial diversity were associated with HIV positive, not on HAART status, HPV type 6, and BV. Bacterial community clusters were consistent with previous studies of this population.

20

Topical penile microbicide for heterosexual men – evidence of protection from previous microbicide studies

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Purpose: Research studies on penile microbicide use by heterosexual men to prevent transmission of HIV are rare. We analysed results from previous microbicide research studies for evidence of protection from HIV by topical microbicide use over penis.

Method: A literature search was conducted using Pubmed and Google scholar tools using words: microbicides, HIV, penis, prepuce for studies conducted with topical microbicide use on penis. RCT and invitro studies with data on microbicide effect on penis were included in our analyses.

Results: A total of 6 RCT and 1 in-vitro study were included. All 6 RCT studies Tabet et.al (2003), Jaspers et.al (2007), Kilmarx et.al (2008), Schwartz et.al (2009), Chen et.al (2010), Cranston et.al (2014) established safety of topical microbicides on penis with no serious adverse events.

One in vitro study on penile explant tissue (Fischetti & Barry et.al, 2009) established good tissue compatibility and efficient prevention of HIV 1 by topical PMPA, PRO 2000 and Cyanovirin-N gel.

Only one RCT study MTN 012/IPM 010 studied systemic absorption of topical microbicide through penis. Dapivirine (0.05%) gel used as a topical penile microbicide was systemically absorbed in 100% of male participants. Uncircumcised men had 54% higher drug levels than circumcised men.74% men were more likely to use it on every sexual encounter.

Conclusion: Topical penile microbicide can be absorbed through prepuce and penile tissue providing protection from HIV. Uncircumcised men have preputial mucosal compartment providing increased exposure to the drug. It is safe to use topical microbicide on penis. Circumcision is not universally acceptable across globe due to cultural, religious and reasons due to resource limitations and invasive procedures. Topical microbicide for heterosexual men should be further investigated as a prevention tool for HIV.

21

Monitoring the effects of Vedolizumab treatment on the gut microbiome

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Background: Vedolizumab (VDZ) is a monoclonal antibody that targets the α4β7 integrin, which controls immune cell homing to the gastrointestinal tract. This drug is licensed for treatment of inflammatory bowel disease (IBD) and is also under investigation for its therapeutic effects in HIV infection. Given its mode of action, we've hypothesized that VDZ might change the regulation of the gut microbiome. This has only been investigated in one study to date, and the mechanisms that underlie any effects are unclear.

Materials and Methods: Stool samples from IBD participants on/off VDZ are being compared to each other and to healthy controls. We are currently comparing 16S amplicon sequencing and metatranscriptomics to identify microorganisms down to the species level, and to detect significantly enriched catabolic or biosynthetic pathways within each participant's microbiome. These microbiome parameters will be compared to immune cell populations in the blood, small and large intestine, and to measures of systemic and mucosal inflammation.

Results: We compared multiple kits and lysing strategies to identify the best approach for characterizing the stool microbiome in IBD patients. As indicated by mock microbial communities and spike-ins, our pipeline can identify a wide range of microorganisms with little observed bias due to GC content or gram status. In a small sample size of IBD patients, predominant microbes are Bacteroides, Eubacterium, Lachnoclostridium, Roseburia and Bacteroides spp., with an overall agreement at the family level between amplicon- and metatranscriptomics-based approaches. Due to the conserved nature of the 16S gene V4 region, taxonomic differentiation at the genus and species levels was often not possible with the amplicon-based approach; however, metatranscriptomics data correctly identified mock communities down to the species. Using metatranscriptomics we could monitor gene expression of multiple pathways within the gut microbiome, particularly those related to amino acid and carbohydrate metabolism. This study will be expanded to include additional samples and test for statistically significant changes to microbiome composition and metabolism in individuals with IDB both on/off VDZ.

Conclusion: The information from this study will be useful to understand how VDZ impacts the gut mucosa and microbial environment. These data are being expanded to a larger sample size and could be applied in future studies that investigate VDZ's effect on the gut microbiome in the context of HIV infection.

22

Dynamic changes of the gut microbiota in SIV-infected rhesus macaques on longitudinal antiretroviral therapy

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Background: Recent studies suggest that gut microbial dysbiosis may be associated with chronic immune activation and inflammation in HIV-1 infection. However, the extent of changes of gut microbiota in HIV-1 patients with antiretroviral therapy (ART) is unclear. Limited studies in the SIV-infected rhesus macaque model have shown that the gut microbiome is changed similarly to HIV-1 patients. To understand the impact of ART on extent of dynamic changes and restoration to normal gut microbiota, we characterized and compared the gut microbial community in groups of healthy controls, SIV-infected animals with ART, and without ART. Further, we analyzed longitudinal changes in a subset of treated animals at different time points of therapy.

Methods: Eleven Indian rhesus macaques (iRM) were infected with SIVmac239 intravenously, 4 of which received ART, and 14 healthy SIV-naïve animals were used as control for comparison. Blood and fecal samples were collected from all the groups. Longitudinal fecal samples from ART animals were used for analysis of dynamic changes of gut microbiota. Genomic DNA was extracted from fecal samples and used for sequencing of V3 and V4 regions of 16S rRNA gene. Data analyses using mothur and microbiome R packages were performed to identify and compare the composition and abundance of microbiome in the gut. Monocyte chemoattractant protein-1 (MCP-1 or CCL2), blood lipopolysaccharide (LPS), and soluble CD14 (sCD14) were also measured to monitor microbial translocation.

Results: SIV infection was associated with very distinctive changes in gut community. The macaque gut microbiota communities had altered taxonomic composition among different time points and between infected and ART groups, paralleling to human studies. The predominant phyla Firmicutes, Bacteroidetes, and Proteobacteria made up for > 90% of the total community in all the animals. A total of 9,380 distinct operational taxonomic units (OTUs) were identified, based on which global diversity indices were calculated. As compared with controls, SIV-infected animals showed decreased diversity (e.g., Shannon Diversity Index), while ART group appeared to recover to the levels (or towards the direction) of normal healthy animals. From 137 core OTUs, 9 OTUs were identified at FDR<0.10 for differential relative abundance among SIV, ART and control groups. These OTUs belong to Bacteroidetes, Firmicutes and Proteobacteria. Longitudinal analysis of 4 animals under ART revealed several OTUs with differential abundance across different time points, e.g. OTU00139 (Firmicutes/Clostridia/Clostridiales/Clostridiales) and OTU00003 (Bacteroidetes/Bacteroidia/Bacteroidales/Prevotellaceae/Prevotella). Meanwhile, plasma LPS, CCL2 and sCD14 levels in the ART animals also returned to levels comparable with healthy animals.

Conclusion: Intestinal microbiota communities appeared to change after SIV infection. ART may benefit infected animals by enhancing the microbiome diversity that was compromised by SIV infection. Long-term ART may improve the gut microbiota towards the compositions

found in uninfected animals and alleviate immune activation.

23

A complete solution for microbiome data analysis and publication of files using Nephele 2.0 and METAGENOTE that increases reproducibility

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The interest in investigating the role of the microbiome in health and disease continues to grow, and with it, the number of datasets generated from a large variety of studies of diverse populations. The availability of data at the NCBI Sequence Read Archive alone provides the hope that greater insights could be obtained through reproducibility of published studies and even by pooling datasets and performing multi-omics studies. The field has greatly benefited from improvements in tools and databases, yet challenges still exist in the ability to access reproducible analysis pipelines. In addition, microbiome data integration depends strongly on consistent use of controlled vocabularies to describe the environment and library generation method for example, but datasets currently available in public repositories lack metadata or show many inconsistencies in the use of metadata standards and ontologies, which limits our ability to perform cross-study comparisons.

At NIAID, we have released an improved version of NEPHELE (2.0), an online platform for microbiome research, that provides a consistent and centralized environment for high-throughput metagenomics data analysis. It focuses on providing access to published open-source tools and databases for amplicon and WGS data analysis. The revised pipelines are based on the popular algorithms in DADA2, QIIME2, BioBakery, and others. Finally, Nephele 2.0 includes a general sequencing data pipeline with preprocessing trimming and QC steps as well as a QIIME2 based downstream analysis pipeline with added visualizations.

Once the analysis is complete, a user can reuse the analysis mapping file, to start the metadata annotation and publishing process using METAGENOTE. This newly released tool is a web-based notebook for easily and consistently applying MIxS-compliant metadata to genomics samples. It also automates the submission of metadata and associated sequence files to the SRA repository, creating the needed bioProject, BioSamples, and Experiment records in one step and thereby contributing to a much richer standardized annotation.