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Abstract Book

1st International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment

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1st International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment

Abstracts

Oral Presentations

Abstract: 1

Pathogenesis

Gut microbiota diversity predicts immune status in HIV-1 infection

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Introduction: Progressive HIV-1 infection is characterized by dysregulation of the intestinal barrier and systemic inflammation, in part driven by translocation of immunostimulatory microbial products. However, little is known on the role of gut microbiota alterations in the pathogenesis, and whether the microbiota is restored by antiretroviral treatment (ART).

Methods: We conducted a prospective, observational study including 28 viremic patients, three elite controllers, and nine uninfected controls. Fecal microbiota composition was determined by 16S rRNA sequencing at baseline and for 19 patients at follow up during ART (median 10 months [4-15]). Soluble markers of microbial translocation and monocyte activation were analyzed by LAL assay (for LPS) or ELISA (for sCD14, LBP, sCD163).

Results: Several alpha diversity measures, including number of observed bacterial species and Shannon alpha diversity index, were significantly lower in viremic patients compared to uninfected controls, whereas the microbiota of elite controllers resembled that of the controls. In viremic patients, alpha diversity correlated significantly with CD4+ T cell counts, markers of microbial translocation, and monocyte activation. In multivariate linear regression, for every age- and gender-adjusted increase in number of bacterial species, the CD4+ T cell count increased with 0·88 (95% CI 0·35-1·41) cells /µl (p=0·002). After introduction of ART, microbiota alterations persisted with further reduction in alpha diversity (p<0.001). The changes of the bacterial taxa within the individuals were reflected by the overall significant (p<0.001) increase in beta diversity. Additionally, the microbiota composition at follow up changed for several taxa on phylum and genus level, with a significant reduction in *Prevotella* genus after adjustment for multiple testing (p=0.007).We could not observe any influence of different ART regimes (NNRTI vs. PI) on richness or intra/inter diversity at follow up.

Conclusions: The gut microbiota is altered in HIV-1 infected patients. Our data implicate that re-shaping the microbiota may be an adjuvant therapy even in patients commencing successful ART.

No conflict of interest

Abstract: 2

Pathogenesis

Feeding Practices and HIV Infection Modulate Infant Gut Microbiota Maturity: A Cross-Sectional Study Across Four Geographic Sites

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Background: Establishment of the infant gut microbiome is influenced by myriad factors including mode of delivery, diet, antibiotic exposure and environmental contacts. The relationship between some of these exposures and maturation of the microbiota is poorly understood. We performed a meta-analysis of 1,910 infant stool samples collected across four disparate geographical locations to characterize the impact of feeding practices and maternal HIV infection on the infant gut microbiome maturity.

Materials and Methods: Demographic data, clinical data, and infant stool samples were collected prospectively from study sites in Port au Prince, Haiti (50 infants at a single time point), and the USA (119 infants followed with between 1 and 11 number of samples from Tampa, Florida and Los Angeles, California),. Metagenomic sequencing was performed using standard protocols and data were analyzed using the QIIME 1.8.0 pipeline. We compared these data to those from 176 infants downloaded from a previously published study (Subramanian et al., Nature 2014). A random forest regression model was trained on the healthy cohort using the OTU table as input and chronological age as response. A sparse model comprising 33 predictors was selected as optimal based on cross-validation error, and this was subsequently applied to predict microbiota maturity in the remaining datasets.

Results: Initial examination of the 1,910 samples revealed clear geographic separation between all cohorts. Bacterial compositions tended to be more diverse in the US/Haiti groups, particularly when compared to children with Severe Acute Malnutrition ($p = 0.015$, PD_whole_tree metric). Levels of *Bifidobacterium* were lower and levels of *Enterobacteriaceae* and *Bacteroidaceae* were higher in the US/Haiti samples (all p-values < 2.2e-16) when compared with the healthy Bangladeshi samples.
We next sought to characterize the developmental trajectory of the infant gut microbiome across the four study sites. Using a random forest regression model, we observed significant microbiota immaturity ($p=0.025$, Kolmogorov-Smirnov test) in babies of Haitian HIV+ mothers, relative to the babies of HIV- Haitian and US cohorts. The infants of US/Haiti HIV negative women possessed a more mature microbiome ($p=0.023$, Kolmogorov-Smirnov test) compared to healthy Bangladeshi subjects. We also found differences in microbiota maturity as a function of feeding practices with percentage of breastfeeding and introduction of solid foods corresponding with an increase in microbiota maturity ($p=0.04$ and $p=0.02$, respectively, Kruskal-Wallis test).

Conclusions: Our cross-sectional study highlights key differences in the infant gut microbiome across the developing and developed world. In particular, subjects from the US/Haiti cohorts showed increased microbiota maturity and a corresponding shift from *Bifidobacterium* to *Enterobacteriaceae* and *Bacteroides*. Within these subjects, we also observed differences in microbiota maturity when comparing across feeding practices and mother's HIV status. Taken together, our data demonstrate the significant impact of these factors on the long-term development of the infant gut microbiome.

No conflict of interest

Abstract: 3 – *invited lecture*

Pathogenesis

Potential cross reactions between HIV-1 specific T cells and the microbiome

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Introduction: T cells normally focus on a small number of discrete epitopes. Antigen processing explains some but not all of this immunodominance. The pre-immune T cell repertoire may also contribute but has been difficult to examine.

Methods: We measured the pre-immune repertoires of CD4+ and CD8+ T cells in HIV-1

negative and unexposed blood donors adapting the Sallusto library method (JEM 7,1525 2009). Sorted naïve and memory T cells were plated at low numbers and non-specifically expanded for ≥12 divisions. Then each well was challenged with autologous monocytes pulsed with HIV-1 or control peptides. Proliferating T cells were expanded further and peptide specificity confirmed.

Results: Both naïve and memory CD4+ and CD8+ T cells responded to HIV-1 peptides. The peptides matched known immunodominant epitopes in infected patients, implying that pre-immune T cell frequency contributes to immunodominance. To determine what primed the memory cells, matches in HMP database microbial sequences were sought. For 35 CD4+ epitopes there were > 2000 matches of 8 or more contiguous amino acids. Because only 4-5 amino acids are needed for a cross reaction the potential for cross-reactive priming is enormous.

Conclusions: Commensal microbes provide a rich source for priming memory T cells.

No conflict of interest

Abstract: 4

Pathogenesis

The short chain fatty acid butyrate reduces HIV-1 and pathobiont-associated increases in intestinal myeloid dendritic cell and T cell activation

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Background: We previously reported that untreated chronic HIV-1 infection is associated with an altered intestinal microbiome that was linked to mucosal myeloid dendritic cell (mDC) activation and to mucosal and systemic T cell activation. HIV-1-infected subjects had increased abundances of commensal bacteria with potentially pathogenic properties (pathobionts) including *Prevotella* and *Acinetobacter* species and decreased abundances of bacterial families that contain known butyrate-producing bacterial species. Butyrate is a short chain fatty acid and has immune modulating effects on both intestinal and peripheral immune cells. We hypothesized that butyrate would decrease pathobiont-driven mucosal mDC and T cell activation and investigated this using an *ex vivo* intestinal cell model.

Methods: Species identification was performed on bacterial 16S ribosomal DNA sequences previously obtained from colon biopsies of 17 untreated chronic HIV-infected and 14 uninfected subjects. For analysis, the percent abundances of known colonic butyrate-producing bacterial species (n=15) were pooled for each subject. Lamina propria mononuclear cells (LPMC) from normal human intestinal tissue (n=4-9) were infected with HIV-1_{BAL} (80ng p24/10^6) and cultured with *Prevotella stercorea* (2.5 bacteria:1 LPMC) in the presence or absence of butyrate. Non-toxic concentrations of butyrate were chosen to reflect a 'low dose' (0.2mM) and potentially 'physiologic dose' (2mM) of butyrate. Multi-color flow cytometry was used to measure mDC activation (CD40 expression levels) after 24 hours and frequencies of activated (CD38+HLA-DR+) and infected (intracellular Gag-p24; ip24) CD4 T cells after 4 days. Levels of T cell-associated cytokines IFNγ and IL-17 in culture supernatants were measured by ELISA. t-tests were used for statistical analysis.

Results: Abundances of known butyrate-producing bacterial species were lower in HIV-infected subjects compared to uninfected controls (p=0.009). In HIV-1_{BAL}-infected LPMC, without butyrate, *P. stercorea* increased CD40 expression on mDC (p=0.003) and increased frequencies of CD38+HLA-DR+ (p=0.003) and ip24+ (p<0.0001) CD4 T cells above levels detected in cultures without *P. stercorea*. Significant increases in levels of secreted IFNγ (p=0.005) and IL-17 (p=0.001) were also observed. In the presence of 2mM butyrate, *P. stercorea*-induced CD40 expression on mDC

was decreased by 47%, frequencies of CD38+HLA-DR+ CD4 T cells decreased by 85% ($p<0.05$) and frequencies of ip24+ CD4+ T cells decreased by 57% ($p<0.01$). Production of IFNγ ($p<0.01$) and IL-17 ($p<0.01$) were completely abrogated. Lower butyrate concentrations (0.2mM) failed to inhibit *P. stercorea*-induced mDC activation as well as *P. stercorea*-associated T cell activation and enhancement of infection. Lower levels of butyrate decreased production of IFNγ and IL-17 by 2 fold ($p<0.05$).

Conclusions: Communities of butyrate-producing bacterial species are reduced in the colonic mucosa of untreated HIV-1-infected individuals compared to control subjects. In an *ex vivo* intestinal cell model, the HIV-1-associated pathobiont, *P. stercorea*, promoted mucosal mDC and T cell activation, CD4 T cell infection levels and increased T cell cytokine production. Levels of mDC and T cell activation, infection and cytokine production were reduced by butyrate in a dose-dependent manner. These findings suggest that a lower abundance of intestinal butyrate-producing bacteria may exacerbate inflammatory mucosal immune responses to pathobionts and contribute to HIV-1-associated mucosal pathogenesis.

Some of the data will be presented at the Conference on Retroviruses and Opportunistic Infections (CROI), Seattle, Washington, February 23-25, 2015.

No conflict of interest

Abstract: 5

Dynamic Vaginal Microbiota in Macaques Associated with Menstrual Cycle and Inflammation

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Introduction: A challenge in developing interventions to prevent mucosal HIV transmission is incomplete understanding of correlates of vaginal transmission, including mucosal inflammation. Vaginal SIV/SHIV transmission in pigtail macaques is an excellent model for HIV transmission, however little is known about how genital bacterial species may influence inflammation and SIV transmission.

Materials and Methods: Broad-range 16S rRNA gene PCR and pyrosequencing was performed on vaginal swabs for assessment of vaginal bacterial communities in a cohort of pigtail macaques for a cross-sectional analysis (N=22), and a limited longitudinal analysis (N=3). Bacteria were identified to the species or genus level using a custom designed reference set of sequences, providing comprehensive characterization compared to previous studies focused on genera or phyla. Luminex was used to measure cytokine and chemokine levels in vaginal swabs.

Results: Pigtail macaques had diverse bacterial communities at all phases of the menstrual cycle, with a range of dominant bacteria in different animals, including *Prevotella, Porphyromonas, Dialister, Streptococcus* and *Lactobacillus* species. In contrast to previous studies, we found three animals had *Lactobacillus*-dominant vaginal microbiota, all at peak sex skin swelling (indicating ovulation). Several species were identified that are commonly found in bacterial vaginosis (BV) in humans, including *Atopobium vaginae, Prevotella buccalis,* BV-associated bacterium-2 *(*BVAB2*), Sneathia, Peptoniphilus lacrimalis, Prevotella timonensis and Gardnerella vaginalis.* Longitudinal sampling demonstrated that vaginal bacterial communities were dynamic and possibly influenced by alterations in environment throughout the menstrual cycle. Animals which had *Lactobacillus*-dominant vaginal microbiota had decreased IL-8 (p=0.009) and decreased IL-1 receptor antagonist (p=0.044), indicating decreased innate inflammation in a *Lactobacillus* rich environment.

Conclusions: The macaque vaginal microbiota is diverse, dynamic, and can resemble the human vaginal microbiota, including BV-associated bacteria as well as *Lactobacillus* spp. Also like humans, the presence of *Lactobacillus*

was associated with decreased inflammatory proteins, which may explain why BV is associated with increased HIV transmission. These data provide a foundation for understanding how vaginal microbial communities may impact risk of HIV/SIV transmission using these models.

No conflict of interest

Abstract: 6

Vaginal Microbiomes Alter the Expression of Antiretroviral Drug Transporters Impacting Drug Efficacy

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Introduction: The vaginal microbiome (VMB) impacts the physiology and health of the female reproductive tract including changes in susceptibility to infection, inflammation and gene expression in the mucosa that phenotypically alter this site of first contact with vaginally-applied interventions and sexually-transmitted pathogens. These studies are complicated not only by the diversity, dynamics and complexity of the VMB, but also by host genetic and environmental differences and the limited number of bacterial taxa from the VMB (<40%) that can be cultivated. We have established the first system that allows for the reproducible propagation of intact human VMBs in the context of a cultured human vaginal mucosa providing for controlled studies of the molecular dialog between the epithelia and the VMBs. Using this system, we previously reported that specific dysbiotic VMBs reduced the efficacy of vaginally-applied antiretrovirals including tenofovir (TFV). Topical application of TFV and other antiretrovirals in pre-exposure chemoprophylaxis (PrECP) is a promising strategy for HIV-1 prevention. Molecular transporters in the vagina play a pivotal role in determining drug disposition and, consequently, pharmacodynamic outcomes. Using vaginal tissue and swab samples and our human vaginal mucosa culture system we studied the impact of representative VMB upon host protein expression patterns involved in the movement and processing of topically-applied antiretrovirals.

Materials &Methods: We completed genome-wide transcriptome analysis in vaginal tissue samples (n=44) from reproductive-age women undergoing gynecologic surgeries. Vaginal swab and lavage samples were collected during routine gynecological visits and molecularly characterized for bacterial communities using both deep sequencing and a custom qPCR array. Representative VMBs for each of the common community state types were used to colonize immortalized human vaginal epithelial cell (VEC) multilayers that contained physiologic numbers of macrophages. Established cultures colonized with selected VMBs were exposed to varying concentrations of antiretrovirals, apically, simulating topical administration and were challenged with HIV-1 strains. The cultures were subjected to microarray, qRT-PCR array and immunolocalization evaluations.

Results: The expression patterns of ARV transporters and metabolizing enzymes showed excellent correlation between the tissues, swabs and VEC culture samples. Expression patterns were most impacted by the VMB community present. Collectively the data indicated that expression of more than 35 ABC and SLC transporter gene family members was significantly altered by specific VMBs. Six distinct efflux and 3 influx transporters associated with antiretroviral movement were selected for focused study and were found to be frequently suppressed by dysbiotic VMBs helping to explain our published observations on reduced TFV efficacy. Colonizing VEC cultures with *Lactobacillus*-dominated VMBs produced distinct expressions profiles and should help to explain the molecular impacts that should be considered during development and testing of PrECP approaches.

Conclusions: The genetically diverse clinical materials validated our findings in the controlled culture system and together provided a potential explanation for clinical differences in vaginally-applied compounds designed to increase resistance to HIV-1. Our data indicate that the VMB composition influences transporter expression in the vagina and, consequently, the disposition of ARV administered systemically or topically supporting a role in HIV PrECP efficacy.

No conflict of interest

Abstract: 7

Diversion of HIV-1 Vaccine-Induced Immunity By Dominant Gp41-reactive Non-Neutralizing Antibodies that Cross-react with Intestinal Microbiota

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Introduction. A DNA prime, recombinant Adenovirus Type 5 (rAd5) boost HIV vaccine showed no efficacy for prevention of HIV-1 transmission. Vaccination induced higher levels of plasma gp41-specific compared to gp120-specific antibodies (Abs). Gp41 Abs induced in HIV-1 infection have been reported to be cross-reactive with intestinal microbiota (IM). Thus, we studied plasma Abs in 40 HIV Vaccine Trials Network (HVTN) phase IIb (HVTN 505) trial participants who were HIV-1 negative at the final month 24 visit, as well as plasma and blood memory B cell Abs in 8 phase Ib (HVTN 082) and phase II (HVTN 204) vaccinees with high titers of plasma binding Abs to vaccine strain rgp140 Envs in order to understand the nature of the failed vaccine-induced Abs, including their reactivity with IM.

Materials and Methods. HIV-1 reactive plasma and memory B cell Abs from blood at 4 weeks post rAd5 boost were screened for binding specificities via binding Ab multiplex assay. Blood HIV-1-reactive Abs were isolated via Env-specific memory B cell sorts. IM whole cell lysates were extracted and pooled from stool samples of 6 heterologous HIV-1 uninfected adults. Next generation sequencing (NGS) was used to study pre-vaccination immunoglobulin (IG) heavy variable (VH) gene repertoire in 8 vaccinees (HVTN 082, 204).

Results. We found that plasma-binding Ab titers were higher to Env gp41 than gp120. Repertoire analysis demonstrated that the dominant vaccine-induced memory B cell response was to Env gp41; 205/221 (93%) gp41, 16/221 (7%) gp120. The dominant vaccine-induced gp41-reactive Ab response from blood memory B cells preferably used the IGHV gene segment 1-69, compared to in non-vaccinated HIV-1-uninfected individuals where 6% of the Ab repertoire utilized IGHV1-69 *(Boyd et al, J Immunol, 2010)* (p<0.0001, Chi square test). Remarkably, 92% (125/137) of HIV-1 reactive IGHV1-using Abs used IGHV1-69, while only 12% (18/145) of non-HIV-1-reactive Abs used IGHV1-69 (p<0.0001, Fisher's exact test). Thus, VH1-69 predominance was not an artifact of primer bias. In contrast to vaccine-induced gp120 Abs, gp41 Abs were non-neutralizing, and were more polyreactive with environmental antigens including IM (p=0.003, Cochran-Mantel-Haenszel Test controlling for Antigen). NGS of pre-vaccination IGHV genes in 8 vaccinees (HVTN 082, 204) revealed a gp41-reactive, IM-reactive IGHV gene in one subject that belonged to the same clonal lineage as a vaccine-driven gp41-reactive Ab. To investigate the binding specificities of the pre-

and post-vaccine clonally-related Abs, pre- and post-vaccine IGHV genes were paired with the natural light chain of the post-vaccine Ab. Both the pre-(DH477) and post-(DH476) vaccine Abs reacted with IM, but DH477 was more reactive with IM when compared to DH476. Additionally, DH477 was more reactive with cardiolipin than DH476, and both Abs weakly bound one auto-antigen.

Conclusion. HIV-1 Env DNA, rAd5 vaccine induced a diversionary, IGHV-restricted, dominant, polyreactive non-neutralizing gp41 Ab response incapable of protecting against HIV-1 transmission. These data suggested that HIV-1 vaccine-induced responses may be shaped by IM or other HIV-Env cross-reactive environmental antigens.

No conflict of interest

Abstract: 8

Gut microbiome, HIV-exposure, and vaccine responses in South African infants

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Background: The gut microbiome is crucial for mucosal and systemic immune development. In mice, certain bacteria are required for induction of Treg and Th17 cell development in the gut. Likewise, gut microbiota enhance immune responses to influenza vaccination. HIV-infected individuals have altered gut microbiome, and HIV-exposed infants (HEU) and their mothers receive antibiotics for PCP prophylaxis, therefore HEU may have altered gut microbiota. HEU have increased morbidity and mortality than HIV-unexposed (HU) infants, and respond poorly to certain infant vaccinations. We hypothesized that HEU have impaired immune responses due to dysbiosis of the gut microbiome.

Methods: HEU and HU infants were recruited from informal settlements of Cape Town. Stool DNA was extracted using MoBio PowerFecal DNA kit and 454 or Illumina sequencing of the V4 or V6 region of the 16srRNA gene was performed. Data was preprocessed using QIIME and UPARSE and imported into R for further analyses using the R package phyloseq. Differential abundance testing was performed at Operational Taxanomic Unit (OTU) level using the R metagenomeSeq package. Whole blood was incubated with BCG and proliferation and cytokine expression measured using multiparameter flow cytometry.

Results and Discussions: We found substantial differences in the relative abundances of certain microbial taxa between the HEU and the HU. HEU had higher bacterial diversity than HU infants by Shannon index. In HU infants, several key species were significantly correlated with both proliferative and cytokine responses to BCG. For example, at 6 weeks of age, significantly decreased abundance of Bacteroides spp. and in particular B. fragilis was observed in infants with high CD4+IL-2, CD8+ki67+, and CD8+IL-17+ responses at week 6.

Conclusions: Gut microbial makeup could explain the immunological differences between HU and HEU infants. These differences should be considered in development of HIV vaccines for HIV-exposed neonates

No conflict of interest

1st International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment

Abstracts

Poster Presentations

Abstract: 9

Comorbidities

HIV Symptoms Reported in HIV Disease May Indicate Gut Microbiome Dysfunction and Inflammation from Microbial Translocation

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Background: Microbial translocation leads to immune activation and chronic systemic inflammation. Dysfunctional alterations in the gastrointestinal epithelial barrier and gut microbiome in HIV infection have been associated with the translocation of microbial products, systemic inflammation, and poor clinical outcomes. Microbial translocation and inflammatory processes may play a role in symptom development in HIV disease as it does in other disease models such as Inflammatory Bowel Syndrome. Our aim was to examine whether symptom clusters reported in HIV-infected patients seeking outpatient care at the University of Alabama at Birmingham HIV outpatient clinic were inflammatory related.

Materials & Methods: We conducted a retrospective data analysis of symptoms reported by men (*n* = 1,511, 78%) and women (*n* = 434, 22%) ages 19-79 years at routine clinical visits in 2011 by using the HIV Symptom Index, a 20-item survey capturing prevalence and symptom distress using the scale 0 = I do not have this symptoms, 1 = I have the symptom, does not bother me, 2 = bothers me a little, 3 = bothers me some, 4 = bothers me a lot. Symptom prevalence and distress were determined using the first routine clinic visit of that calendar year for each patient. Principal components analysis was used to identify clusters of symptoms using within-subject mean item scores for the year of 2011.

Results: The mean age of study participants was 44 years, 96% of patients were on HIV antiretroviral therapy, 76% of patients had a viral load of <500 copies/ml, and 71% had a CD4 count of >500. The mean number of visits for the calendar year was 3. Two main clusters were identified encompassing both physical and psychological symptoms. The symptoms with the highest prevalence were poor sleep (51%), muscle aches/joint pain (48%), and fatigue (47%). Among those reporting these symptoms 82% of patients with poor sleep stated it was bothersome, 84% of those with fatigue reported it as bothersome, and 85% of muscle aches/joint pain reported it as bothersome. Bloating/abdominal pain was reported as being present by only 25% of patients, but 83% of those patients reporting bloating/abdominal pain stated that it was bothersome.

Conclusions: The study results revealed clusters of symptoms known to have an association with inflammation and are comparable to symptoms that are known to be associated with microbial translocation and alterations in the gut microbiome (i.e., Inflammatory Bowel Disease and Irritable Bowel Disease). Future research into changes in the microbiome due to HIV infection is warranted as well as diversity changes in the microbiome in relationship to symptom development and prevalence.

No conflict of interest

Abstract: 10

Pathogenesis

Intestinal pro-inflammatory disbyosis in HIV-1 infection

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Introduction. Recent studies suggest a role of the gut microbiome on HIV/AIDS pathogenesis, but it is unknown if the gut microbiota differs by HIV-1 control and immune status, or if antiretroviral treatment (ART) affects the intestinal microbial content to any extent.

Material & Methods. This cross-sectional study compared HIV-1-negative (HIVneg) subjects with HIV-1-infected individuals with the following phenotypes: late presenters (LP: no ART, CD4≤200 cells/mm3), elite controllers (EC: no ART, HIV-1 RNA (VL)<50 copies/mL for 1 year), viremic controllers (VC: no ART, VL 50-2000 copies/mL for 1 year), ART-naïve (AN: no ART, CD4≥500 cells/mm3, VL>2000 copies/mL), early treated (ET: on ART started ≤6 months from HIV-1 infection, VL<50 copies/mL), immune concordant (IC: on ART≥2 years, CD4≥500 cells/mm3, VL<50 copies/mL), and immune discordant (ID on ART≥2 years, CD4≤300 cells/mm3, VL<50 copies/mL). Participants were 18-60 years old, had body mass index 18.5-30 and, except for LP, had no antibiotic usage during the previous 3 months. The fecal microbiota was characterized by massive 16S rRNA sequencing (MiSeq^{TM}). Richness (Chao1 estimator), diversity (Shannon index) and microbial taxonomic analyses were performed using Mothur and R/Vegan software packages and the SILVA database. The LEfSe algorithm was used to identify which bacterial taxa contributed to differences between HIV-infected and HIVneg individuals.

Results. The study included 80 individuals: 16 HIVneg and 64 HIV-1-infected (5 LP, 3EC, 6 VC, 7 AN, 5 ET, 27 IC and 11 ID). The gut microbiome of HIV-infected subjects was significantly less rich and diverse than that of HIVneg individuals. HIV phenotypes corresponding to more advanced HIV disease stages, ie: LP, ID, but also IC subjects, had the lowest richness and diversity, whereas AN and ET subjects had similar richness and diversity to HIVneg individuals. HIV-1-infected subjects in the LP, ID, and IC groups also showed the lowest Firmicutes:Bacteroidetes phyla ratio. Using LEFse, the only unbalanced phylum between HIV positive and negative subjects was Lentisphaerae, which was enriched in HIVneg individuals. At the family level, HIV-1 positive subjects were enriched in Bacteroidaceae and Porphyromonadaceae, and depleted in Victivallaceae, Peptostreptococcaceae, Clostridiaceae and Prevotellaceae. At the genus level, HIV-infected individuals were enriched for Bacteroides, Bilophila, Parabacteroides, Alistipes and Odoribacter, and depleted for Dialister, Butyrivibrio, Coprococcus, Catenibacterium, Desulfovibrio, RC9, Clostridium, Oribacterium, Prevotella and 3 uncultured or unclassified genus (one Ruminococcaceae, one Victivallaceae and one Peptostreptococcaceae).

Conclusions. HIV-1 infection is associated with significant decreases in gut microbiome richness and diversity as well as to an intestinal pro-inflammatory dysbiotic profile. Our findings thus suggest a central role of the intestinal microbiome on HIV pathogenesis.

No conflict of interest

Abstract: 11

Pathogenesis

Functional profiling of the gut microbiome in HIV infection

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Introduction. The gut microbiome plays an essential role in human physiology. We investigated to what extent HIV infection could modify its functions.

Materials & methods. We used PICRUSt to infer the functional profile from 16S rRNA Miseq^{TM} data, which was obtained in a cross-sectional study comparing the intestinal microbiome of HIV-negative (HIVneg) and HIV-1-infected subjects with different phenotypes, i.e., late presenters (LP: no ART, CD4≤200 c/mm^3),

elite controllers (EC: no ART, HIV-1 RNA (VL)<50 c/mL for 1 year), viremic controllers (VC: no ART, VL 50-2000 c/mL for 1 year), ART-naïve (AN: no ART, CD4≥500 c/mm^3, VL>2000 c/mL), early treated (ET: ART started ≤6 months from HIV-1 infection, VL<50 c/mL), immune concordant (IC: on ART≥2 years, CD4≥500 c/mm^3, VL<50c/mL), and immune discordant (ID: on ART≥2 years, CD4≤300 c/mm^3, VL<50c/mL). Gene abundance data predicted by PICRUSt was analyzed with HUMann to reconstruct the metabolic pathways and modules. Then we ran the LefSe algorithm in order to identify which pathways significantly contributed to differences among different HIV phenotypes.

Results. The parent study included 80 subjects: 58 men, 21 women and 1 transgender woman. Men and women showed statistically significant differences (p-value log (LDA score) < 0.05) in 38 metabolic pathways. Within men, HIV+ subjects (5 LP, 1 EC, 3 VC, 6 AN, 5 ET, 17 IC, 8 ID) showed enrichment in genes implicated in biotin metabolism; alanine, aspartate and glutamate metabolism and sulfur metabolism relative to HIVneg (n=13), and were depleted in genes related to synthesis and degradation of ketone bodies and fatty acid biosynthesis. Most differences among HIV+ men involved the ID group, whose microbiome was enriched for genes related to biotin metabolism, RNA degradation and beta-lactame resistance. The microbiome of VC patients contained more genes involved in Alanine metabolism as well as in the citrate cycle (TCA cycle). ET patients showed an increase in ABC transporters relative to other phenotypes, whereas AN showed an enrichment in genes implicated in the atrazine degradation.

Conclusions. HIV infection is associated with bidirectional unbalances in intestinal metabolic activity affecting mucosal barrier integrity and function, local and systemic inflammatory processes and energy storage and consumption.

***Abbreviations**. ART (Antiretroviral treatment), CD4 (Cluster of differentiation 4), VL (Viral load).*

No conflict of interest

Abstract: 12

Pathogenesis

Oral microbiome dysbiosis in HIV-associated periodontal disease

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Background. HIV infection increases the risk of periodontal diseases (PD). The oral microbiome structure differs between HIV-infected (HIV+) and uninfected patients (HIV-), even when Antiretroviral Therapy (ART) successfully suppresses viral replication and can be related to an increased risk of developing PD. Here, we characterize the oral microbial signatures of HIV+ and HIV- at different levels of PD.

Methods. This cross-sectional study included both HIV+ and HIV- with varying degrees of PD, as assessed by oral exam using CDC/AAP criteria (severe (S-PD), moderate (M-PD), mild/none (N-PD)). A total of 5 oral samples (2 tooth, 2 cheek, 1 saliva) were used for sequencing with Illumina/MiSeq standard protocols. Mothur phylotype pipeline and SILVADB were used to classify sequences at the genus level. R/Bioconductor (Vegan, PhyloSeq and DESeq2) were used for statistical analysis. Redundancy analysis (RDA) and Bray-Curtis distances were used both for constrained and unconstrained ordination on genus proportions. Adonis was used to assess overall microbiome structure differences between groups. A negative binomial fit and Wald test were run on every genus to assess differential abundance. P-values were adjusted using Benjamini-Hochberg correction.

Results. 250 samples from 50 patients were included. Of these, 40 (80%) were HIV-infected patients. 18 (36%), 16 and 16 (32%) patients presented N, M and S-PD, respectively. Sequencing data were available for 242 (96.8%)

samples.Globally, unconstrained ordination analysis showed clustering by anatomic site, [Adonis test (p<0.001)]. Constrained ordination analysis showed that sampling site explained 25% of the total variance, while HIV infection and PD status explained only ~1% of such variance. Genera Abiotrophia (0.017), Neisseria (adj-p=0.003), Kingella (p=0.04) and unclassified Neisseriaceae (p=0.001) were enriched in HIV+ samples while Leptotrichia (p=0.04) and Selenomonas (p=0.04) were depleted. Neisseria genus enrichment was detected in cheek and teeth samples, but not in saliva, where no difference between HIV+ and HIV- was found. PD was also associated with enrichment of several genera. Of these, only Veillonella was depleted in S-PD samples both from cheek (p<0.001) and saliva (p=0.006).
Interestingly, samples from HIV+ patients with N-PD showed a strongly significant enrichment in genus Aggregatibacter (p<0.0001) along with an unclassified Neisseriaceae family genus when compared to HIV- N-PD patients. Kingella (p=0.006) and Neisseria (p<0.001) genera enrichment was also found for HIV+ samples with M-PD. Conversely, those HIV- samples within the M-PD showed an enrichment of Treponema genus (p=0.005). Differential abundance between HIV+/HIV- of these genera was not found for samples within the S-PD group.

Conclusions. HIV infection-driven changes on oral microbiome structure result in subtle but distinct microbial signatures along different stages of PD.

No conflict of interest

Abstract: 13

Pathogenesis

Gut microbiome alterations in ART-controlled HIV-infected patients with abdominal adiposity

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Introduction: Lipohypertrophy is a common manifestation associated to metabolic complications in HIV infected patients under ART for many years. The study ROcNRAL was designed to evaluate whether a change in antiretroviral therapy with a strategy excluding nucleosides analogues and protease inhibitors could have an impact in lipohypertrophic HIV+ patients. One hypothesis to explain this phenotype is a permanent modification of the gut microbiota and\or the intestinal permeability facilitating abdominal fat accumulation. We postulated that lipohypertrophic HIV+ patients could present a dysmetabolic profile as observed in non-infected subjects with a metabolic syndrome for whom a modification of the intestinal microbiota is described.

Material & Methods: The objective of this case control study was to assess the gut microbiome profile of 11 lipohypertrophic HIV+ patients from the ROcNRAL study in comparison with 11 non HIV-infected individuals from the French MicroObes cohort matched for BMI, age and sex (Cotillard et al. Nature, 2013). The 11 HIV+ patients where at baseline on NRT/or PI regimen, of whom 7 were re-evaluated under raltegravir/maraviroc. Whole metagenomic deep sequencing (>40M reads) was performed on

fecal samples from all individuals using SOLiD technology. Mapping and counting against the 3.9M gut gene catalog (Nielsen et al. Nat Biotech, 2014) was performed using the MeteorStudio and analyses with the MetaOMiner suite. The final abundance table was rarefied at a common level of 11M mapped reads and normalized using RPKM and TC methods. Differentially abundant genes were searched and projected onto the metagenomic species (MGS) catalog for further characterization.

Results: The HIV-infected patients were aged a median 54 years (IQR=[50,58]) and had a median BMI 26.2 kg/m^2 (IQR=[25.2,28.7]). We found 47805 differentially abundant metagenomic genes falling onto 85 different MGS. Only 40 of these MGS are known at the species level. We saw a lower prevalence of *Faecalibacterium prausnitzii* strains (n=10 MGS) in the HIV-infected patients than in control group. Four of these MGS were negatively correlated with insulin levels. Most of the species enriched in HIV+ were unknown, some of them belonging to the *Prevotella* (n=8) and others to the *Bacteroides* genera (n=4). Some differentially abundant MGS were small in size and may correspond to the small genomics units or bacteriophages. HIV+ patients had a trend of being enriched in the *Bacteroides* enterotype and controls in *Prevotella* ($p<0.083$). We did not find any effect of the switch from NRTI/PI to raltegravir/maraviroc treatment on the composition of the gut microbiome (paired tests). Findings were comparable using a different control cohort from another country (MetaHIT; Le Chatelier et al. Nature, 2013; data not shown).

Conclusions: This preliminary data on gut microbiome of HIV+ patients with a lipohypertrophy syndrome suggest different profile compared to non-HIV infected patients with a metabolic syndrome. ART-controlled HIV+ patients were depleted in *F. prausnitzii*, which was inversely associated with insulin sensitivity markers. Our results indicate that gut microbiome does not seem to be dependant upon the ART regimen, suggesting long-term alterations related to the HIV status.

No conflict of interest

Abstract: 14

Gut Microbiome and Breast Milk Oligosaccharides (GuMBO) in HIV Positive and Negative Mother Infant Pairs from Haiti

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Introduction: Over 1 million HIV-exposed, uninfected (HEU) infants are born annually to HIV positive (HIVpos) mothers. This growing population of infants experience higher morbidity and twice the mortality of unexposed infants in the same community. Furthermore, many of these infants experience immunologic derangements that may persist into adolescence. Breastfeeding conveys health benefits in HEU-infants, but the mechanism remains unclear. Human milk oligosaccharides (HMOs), which are the third largest constituent of human milk, are not digested by the infant and their main purpose appears to be nourishing the infant's microbiome. The infant's microbiome in turn conditions the developing immune system. We hypothesize that perturbations of both mother's milk composition and mother's microbiome by HIV infection alters the microbiome in HEU-infants and may account for some of the immunologic and survival differences seen in HEU-infants.

Materials and Methods: We conducted a cross-sectional study of 50 mother and infant pairs attending a GHESKIO nutrition clinic in Port au Prince, Haiti: 25 HIVpos mothers and 25 HIV negative (HIVneg) mothers. All infants were breastfed. We prospectively collected demographic and clinical data and six samples from each pair (mother: vagina, areola, and breast milk; infant: stool, oral mucosa, and skin).

For each sample we performed 16S bacterial metagenomic sequencing and analyzed the data using QIIME 1.8.0. High-performance liquid chromatography was used to characterize HMOs in breast milk.

Results: The microbiome in samples collected from individual mother-infant pairs was closely related. The microbiome in the mother's breast milk was most closely associated with that in the infant's stool. Maternal HIV-infection was associated with significant differences in bacterial diversity and taxonomic composition. The stool of infants born to HIVpos mothers had more *Proteobacteria* and *Actinobacteria* whereas non-exposed infants had more *Bacteroidetes* and *Fusobacteria*. Further, individual classes of the phyla *Acidobacteria*, *Bacteroidetes*, and *Proteobacteria* were significantly different in the stools of HEU infants. The *Bacteroidetes* to *Firmicutes* ratio, a link to overall diversity, was also lost in HEU infants.
HMO composition significantly differed between HIVpos and HIVneg mothers. 3'-sialyllactose (3'SL) and 2'-fucosyllactose (2'FL) were increased in breast milk from HIVpos mothers. In the milk from HIVpos mothers, the increase in 3'SL levels was associated with an increase in *Proteobacteria* and a decrease in *Firmicutes*. This increase in mother's 3'SL was then also associated with a decrease in *Actinobacteria* in the stool of HEU infants. In contrast, milk lacto-N-neotetraose (LNnT) was increased in HIVneg mothers. An increase in HIVneg mother's lacto-N-fucopentaose 1 (LNFP1) levels was associated with an increase in *Actinobacteria* and a decrease in *Bacteroidetes* in the stool of non-exposed infants.

Conclusions: The microbiomes of mother infant pairs as well as the HMO composition are altered with maternal HIV-infection. Furthermore, distinct differences in select HMOs based on the mother's HIV status directly correlate with the infant's microbiome composition. These data suggest that maternal HIV-infection disrupts the normal development of the infant microbiome and may help explain why HEU infants experience higher morbidity and mortality than unexposed infants.

No conflict of interest

Abstract: 15

Microbicides

Vaginal Microbial Biofilms and Topical HIV-1 Prophylaxis

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Introduction: Vaginal microbial biofilms play a critical role in determining the vaginal status in women, healthy or dysbiotic, can affect susceptibility to HIV-1 infection via vaginal intercourse, and can affect the safety and efficacy of topical microbicides for HIV-1 prevention. However, the mechanisms that underpin the development of complex, polymicrobial biofilms in vivo and the consequences to HIV-1 prevention remain poorly understood. Here, in vivo vaginal microbial biofilms are compared to corresponding ex vivo sessile communities developing on the apical surface of human vaginal epithelial cells (VECs) in a novel, multilayer tissue culture model. The impact on susceptibility to HIV-1 infection is investigated.

Material & Methods: Vaginal smears and swabs were collected from six reproductive-age women. The swabs were cryopreserved and used to colonize the apical surface with an air interface of a novel vaginal mucosal culture model based on VECs co-cultured with HIV-1-susceptible cells. The ex vivo development of microbial biofilms on the VECs was studied using molecular, microscopic, and metabolomic techniques and compared to the biofilms collected in vivo. The impact of these sessile vaginal microbiomes on HIV-1 infection susceptibility in the presence and absence of antiretroviral agents was investigated.

Results: In vivo microbial biofilms were complex and could be classified into a series of phenotypes based on molecular and microscopic data. Culture-independent methods showed that

the cryopreserved vaginal microbiomes developing on the VECs ex vivo maintained similar composition as communities from the corresponding in vivo specimens. The kinetics of biofilm development were investigated. HIV-1 challenge studies found that different biofilm phenotypes could either increase or decrease susceptibility to productive infection relative to controls.

Conclusions: Vaginal microbial biofilms were studied for the first time in an ex vivo model that faithfully recreated the complex community composition of the in vivo specimens. The structure and dynamics of these sessile microbial architectures can have a significant effect on the success of HIV-1 prophylaxis. The novel, genetically controlled, high throughput mucosal culture system allows unprecedented mechanistic insights into vaginal HIV-1 transmission and should be applied in the development and evaluation of prevention products.

No conflict of interest

Abstract: 16

Comorbidities

The effect of the penile microbiome on BV, GUD, and genital epithelial trauma

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Introduction: The goal of this study is to determine the effect of the penile microbiome on women's risk of Bacterial vaginosis (BV) and other outcomes in a cohort of 204 heterosexual couples in Kisumu, Kenya.

Materials & Methods: Men are aged 18-35 and female partners are aged 16 years and older. Stratified sampling for 50% circumcised and 50% uncircumcised men. Penile and vaginal microbiome are measured via 16s rRNA amplicon sequencing at baseline, 1 month, 6 months, and 12 months. BV, GUD, and genital epithelial trauma are primary endpoints, measured at each study visit. Cytokine, antimicrobial peptides, pregnancy testing, complete medical history and examination, and extensive behavioral survey are assessed at each study visit. HIV, syphilis, and HSV-2 serostatus are assessed at baseline, 6 months, 12 months.

Results: April 1, 2015 to January 16, 2015, 107 couples have been enrolled. The couples-level prevalence of HIV is 11%, with most couples (9/11) being discordant. Over half (51%) of women and 40% of men are HSV-2 seropositive. At baseline, 22% of women with uncircumcised male partner (UP) were diagnosed with BV by Nugent score 7-10, vs. 14% of women with circumcised male partner (CP). This discrepancy persisted and strengthened at 1 month and 6 month visits, producing an age-adjusted relative risk of 0.42 (p=0.10) for BV among women with CP vs. UP. Syphilis was 9% among women with UP vs. 2% for women with CP (p=0.06), and HSV-2 was 60% among women with UP vs. 47% for women with CP (p=0.10). Incidence of self-reported genital coital injuries at 6 months were higher for women with UP vs. CP. DNA extraction is underway, with microbiome results expected in March.

Conclusions: Baseline and follow-up measures and associations are in keeping with original estimates and hypotheses. Subsequent analysis will identify how the penile microbiome leads to increased risk of these outcomes; factors causing changes in key genital microbiome community types and bacteria; inflammation associated with specific genital microbiome dysbiosis.

No conflict of interest

Abstract: 17

Glycogen levels in undiluted genital fluid and their relationship to vaginal pH, estrogen and progesterone

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Background: Heterosexual transmission of HIV is responsible for 70-80% of new infections worldwide. Colonization of the female lower genital tract with *Lactobacillus* species provides protection against HIV infection and growth of this bacterium is thought to be dependent on glycogen produced by genital epithelial cells. However, the amount of cell-free glycogen in genital fluid available for utilization by *Lactobacillus* is not known. Previous studies suggested that glycogen expression in the vaginal epithelium decreases during times of low estrogen, thereby reducing *Lactobacillus* levels. The goal of the present study was to determine cell-free glycogen levels in undiluted genital fluid, and to also assess its relationship to estrogen and progesterone.

Materials and Methods: Genital fluid samples were collected from 7 pre-menopausal women over a 6-week period using the Instead Softcup. Cell-free glycogen and glucose were quantified in undiluted genital fluids. Estrogen and progesterone levels in blood were determined by ELISA. Twenty nine genital fluid samples, for which all variables were available, were analyzed in a multivariable analysis. Mixed effects linear regression models were used to examine univariable and multivariable associations of predictor variables with log glycogen concentration.

Results: We found glycogen levels varied substantially between women and over time in the same woman, ranging from 0-32.3 µg/µl. Glucose levels ranged from 0-13.3 µg/µl. In multivariable analysis, the association between estrogen and glycogen concentration was not statistically significant, but glycogen levels were significantly negatively associated with vaginal pH ($p<0.001$), and progesterone levels ($p = 0.004$). Glucose levels, age, sexual intercourse 24 hours prior to visit, and days after the initial visit were not significantly associated with glycogen.

Conclusion: Our results show that cell-free glycogen concentrations can be very high, up to 3% of genital fluid, but point to the complexity of the relationship between sex hormones, glycogen and vaginal microbiota. Further studies of factors that affect glycogen levels are needed.

No conflict of interest

Abstract: 18

Pathogenesis

Impaired pulmonary macrophage migration in HIV-1 transgenic mouse model of LPS-associated lung disease

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Introduction: Circulating microbial products, such as lipopolysaccharide (LPS), derived from the gastrointestinal tract microbal translocation, are significantly increased in individuals with chronic HIV-infection. Circulating LPS stimulates HIV-related chronic innate immune activation and may lead to development of multiple organ

pathologies. Chronic obstructive pulmonary disease (COPD) is associated with an abnormal inflammatory response and HIV-1 is an independent risk factor for it development. Pulmonary non-infectious lung diseases remain the leading causes of morbidity and mortality in HIV-1 positive persons despite the antiretroviral therapy (ART). The mechanism leading to COPD and its progression in HIV-1 positive individuals is poorly understood.

Material and Methods: We established a mouse model of noninfectious lung disease using intra-peritoneal LPS administration in HIV-Tg_{26} transgenic mice. Organs were collected at 24-72 h after LPS administration, and immunostaining of activated macrophages (MΦ) and neutrophils (Ne) was performed. Lung and intraperitoneal MΦ were isolated, and expression levels of Nef and env proteins were analyzed by quantitative RT-PCR. In inhibition experiments, HIV-Tg mice received intraperitoneal injection of either 1E7-03 inhibitor or 80% DMSO (control) 15 min after LPS challenge, and organs were collected at 24 h. Plasma cytokines level was determined by Bio-Plex Pro Mouse group1 Th1 panel L60-00004C6 kit (BioRad). One way ANOVA was used for comparison of multiple groups. Statistical significance was defined as $P<0.05$.

Results: Our studies demonstrated that LPS administration significantly increased mortality and lung inflammation in HIV-Tg_{26} mice comparing to WT littermates. Moreover, Ne lung infiltration was significantly higher in HIV-Tg_{26} mice than in WT mice. In contrast, MΦ alveolar infiltration was significantly lower in HIV-Tg_{26} mice than in WT controls with significant accumulation of activated MΦ in the lung capillaries. The levels of renal Ne and MΦ infiltration were similar in both HIV-Tg_{26} and WT mice. We found increased HIV-1 genes expression in MΦ infiltrated in the lung capillaries but not in the intraperitoneal MΦ. Previously, we demonstrated that high oxygen stimulated HIV-1 expression in MΦ. Administration of novel inhibitor of HIV-1 transcription (1E7-03) significantly reduced lung Ne infiltration, prevented MΦ accumulation in the capillaries, and reduced inflammation in HIV-Tg_{26} mice.

Conclusions: Lung-specific high oxygen environment stimulates HIV-1 expression in LPS-activated MΦ that reduces their ability to migrate and contain activated Ne. Supplementation of ART with HIV-1 transcription inhibitors may be beneficiary for treatment of non-infectious lung disease in HIV-1 positive individuals.

No conflict of interest

Abstract: 19

Effects of the cervicovaginal microbiome on the cervicovaginal proteome in African sex workers

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Introduction: Cervicovaginal microbiome dysbiosis has been associated with increased HIV acquisition and shedding in epidemiological studies but the biological mechanisms are unclear.

Material & Methods: Fifty African female sex workers had previously been clustered into four groups based on their cervicovaginal microbiome composition. The microbiome composition was determined by phylogenetic 16S rDNA microarray (TNO, Zeist, Netherlands) and neighborhood co-regularised multi-view spectral clustering. Microbiome group 1 had a *Lactobacillus crispatus*-dominated microbiome, group 2 a *L. iners*-dominated microbiome, and groups 3 and 4 had a microbiome containing multiple genera of anaerobic bacteria, with group 3 representing transition to or from dysbiosis and group 4 full dysbiosis. We subsequently conducted proteome analysis by mass-spectrometry on cervicovaginal lavages from the

same 50 women. We used on-line nanoflow liquid chromatography using the nanoACQUITY-nLC system coupled to an LTQ-Orbitrap Velos, default settings of Progenesis LC–MS, and Mascot, Uniprot 2013_06, AmiGO, Proteinatlas and EMBL-EBI Expression Atlas for peptide identification and classification. Only human proteins with >2 unique peptides were included. Data were analysed by one-way ANOVA on log transformed ion intensities with Bonferroni correction (p-value cut-off of 9.1*10-5) and 'nptrend' function in STATA (extension of the Wilcoxon rank-sum test) on predefined proteins based on the literature.

Results: We identified 549 human proteins, of which 82 were differentially abundant among the four microbiome groups. The total protein concentration in the cervicovaginal lavages was not associated with microbiome group. With increasing bacterial diversity (going from groups 1 to 4), we found: mucus alterations (increasing MUC5AC and 5B; unaltered MUC6 and 16), epithelial remodelling and cell death (increasing actin-organising proteins and LDH-A/B; decreasing cornified/keratinized cells), altered enzyme balance (increasing proteasome core complexes/proteases; decreasing antiproteases), altered antimicrobial peptide balance (increasing psoriasin, calprotectin, and histones; decreasing lysozyme and ubiquitin; unaltered levels of elafin, SLPI, LL-37, α-defensins, and lactoferrin; and no β-defensins), increasing proinflammatory cytokines, and decreasing IgG1/2 (other immunoglobulins unaltered).

Conclusion: We conclude that the cervicovaginal human proteome is associated with the cervicovaginal microbiome. A *L. crispatus*-dominated microbiome, and to a lesser extent a *L. iners*-dominated microbiome, are associated with a healthy vaginal microenvironment, whereas transition to or from dysbiosis and full dysbiosis are increasingly associated with the breakdown of mucus, epithelial, and antimicrobial peptide barriers and inflammation. These alterations may explain the increased vulnerability to HIV acquisition in women with vaginal dysbiosis.

No conflict of interest

Abstract: 20

Effect of low pH on glycogen breakdown by lower genital tract enzymes

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Background: While glycogen expressed by the lower genital tract epithelium has long been believed to support *Lactobacillus* growth and colonization in the vagina, there has been an enigma in understanding how this bacterium is able to maintain dominance in the genital tract since most genital isolates of *Lactobacillus* are not able to use glycogen as an energy source in vitro. We recently reported that α-amylase is present in the genital fluid of women and that this enzyme can break down glycogen. Genital α-amylase was shown to process glycogen into maltose, maltotriose and maltotetraose which are good sugar sources for growth of lactobacilli. Since the pH of the lower genital tract can be very low, we assessed the effect of low pH on glycogen processing by α-amylase.

Methods: Cervival vaginal lavage was obtained from healthy subjects. Glycogen degradation was measured in the samples chromogenically at 565 nm using iodine and a glycogen standard curve. High-performance anion-exchange chromatography (HPAEC) was used to detect the size of glycogen breakdown products. PCR was used to quantify bacteria.

Results: α-amylase in saliva degraded glycogen similarly at pH6 and 7, but activity was reduced by 40% at pH 5 and by 55% at pH 4. α-amylase in 9 genital samples showed a similar profile with an average reduction of more than 50% at pH 4. However two samples collected from one woman at different times had a strikingly different pH profile with increased glycogen degradation at pH 4, 5 and 6 when compared to pH 7. This

second pH profile did not correlate with genital levels of human α-acid glucosidase, human maltase, or genital levels of *L. iners*, *L. crispatus*, *L. jensenii* or *G. vaginalis*. HPAEC showed that mostly maltose was produced from glycogen by this second enzyme profile while genital α-amylase produced maltose, maltotriose and maltotetraose.

Conclusions: These studies show that even at low pH, some α-amylase activity remains, which we speculate may help maintain *Lactobacillus* growth at a slower but sustained rate. However, the results indicate some women have an enzyme that appears distinct from α-amylase with higher activity at lower pHs. Further studies are needed to determine if this second type of enzyme is human or bacterially derived, how widespread its distribution is, and whether its presence influences the makeup of genital microbiota.

No conflict of interest

Abstract: 21

Pathogenesis

Fast disease progression in SHIV infected female macaques is accompanied by a robust local inflammatory innate immune and microbial response

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Introduction: Gender differences in immune response and the rate of disease progression in HIV-infected individuals have been reported but the underlying mechanism remains unclear, in part due to the lack of relevant animal models. Here, we report a novel nonhuman primate (NHP) model for investigation of sex disparity in HIV disease progression.

Methods: Viral load and rate of disease progression were compared between male and female rhesus macaques infected intrarectally with lineage-related subtype C R5 SHIVs. A pilot longitudinal study was conducted to assess gender differences in rectal innate immune and microbial responses.

Results: SHIV infected female rhesus macaques progressed faster to AIDS than males, recapitulating the sex bias in HIV-1 disease in humans. The sex disparity in SHIV disease susceptibility cannot be readily explained by differences in the rectal mucosal immune environment prior to infection. However, a longitudinal study in six infected macaques indicates that the females mounted an earlier and more robust proinflammatory skewed rectal immune response to infection. Moreover, expansion of adherent bacteria that increase in other intestinal inflammatory disorders was significantly higher in the rectal mucosa of female than male macaques during acute infection.

Conclusion: These findings suggest that sex differences in local innate immune activation and compositional shifts in the gut microbiota could be the drivers of increased disease susceptibility in females. Further studies with this novel NHP model of HIV infection could lead to innovative research on gender differences, which may have important therapeutic implications for controlling disease in infected men as well as women.

No conflict of interest

Abstract: 22

Pathogenesis

Oral microbiome in HIV-infected women: pathogenic profile is increased by aging, disease progression, and opportunistic infections

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Introduction: Although human immunodeficiency virus (HIV) remains a leading cause of death worldwide, effective combinatorial antiretroviral therapy (cART) has dramatically increased life expectancy of individuals in developed countries. Data from the Centers for Disease Control and Prevention (CDC) indicate that more than half of HIV-infected individuals in the United States are now over the age of 50. HIV-Associated Non-AIDS (HANA) conditions, such as cardiovascular disease, diabetes, osteoporosis, and dementia are more prevalent in older HIV-infected populations than young adults. Although dysbiosis of the oral microbiome has been studied as a window into pathogenesis of various human conditions and diseases associated with aging, the role of age-related dysbiosis in the development of HANA conditions and opportunistic infections in HIV patients is not well understood.

Methods: We utilize 16S rRNA-based pyrosequencing to characterize and compare the salivary microbiome in 3 Groups: Chronically HIV-infected women enrolled in the Women's Interagency HIV Study (WIHS) who are 1) >50 years old [aging], or 2) < 35 years old [young adult], and 3) healthy age-matched uninfected women. We also examine correlations between dysbiotic profiles in the salivary microbiome, clinical parameters of disease progression, and manifestation of opportunistic oral infections.

Results: HIV infection results in dysbiosis of the salivary microbiome that is enhanced in aging individuals, and characterized by increased abundance of pathogenic bacteria and a decline in healthy probiotic microbes. Higher proportions of *Prevotella*, *Staphylococcus*, *Moryella*, *Peptostreptococcus*, *Ruminococcus*, and *Oribacterium* were detected in both aging and young adult HIV infected women than in uninfected controls. *Prevotella*, *Moryella*, and *Oribacterium* increases were higher in aging than in young HIV patients. HIV infection in older patients was associated with greater salivary shedding of Epstein Barr Virus (EBV). Increased EBV shedding, higher peripheral HIV burden, and reduced CD4+ T cell counts correlated with increases in *Prevotella* and decreases in probiotic *Lactobacillus*. Patients with opportunistic oral infections (e.g. *Candida albicans*, Epstein Barr virus, Kaposi's Sarcoma-associatred herpesvirus, and human papilloma virus) also showed distinctly elevated salivary levels of *Porphyromonas*, *Lachnospira*, *Capnocytophaga*, *Aggregatibacter*, and *Actinobacillus*, and reduced levels of *Streptococcus* and *Lactobacillus*.

Conclusions: Our results indicate that age, severity of disease progression, and emergence of opportunistic infections all contribute to various degrees in increasing the pathogenic footprint of the salivary microbiome during chronic HIV infection. The study provides new and intriguing insights into age-related dysbiosis of the oral microbiome and its role in HIV pathogenesis and disease progression. Importantly, our findings also underscore the need for expanded comprehensive investigations designed to dissect the mechanisms of oral dysbiosis and fully exploit the experimental potential of the wealth of archived saliva samples and associated metadata available through the WIHS.

No conflict of interest

Abstract: 23

Pathogenesis

Impact of two antiretroviral regimens on fecal microbiota composition and diversity, systemic inflammation and epithelial barrier damage

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Introduction. The degree to which antiretroviral therapy (ART) restores microbial composition remains unclear. We looked at the impact of Efavirenz-based (EFV) and protease inhibitors-based regimen (PIs) on fecal microbial composition and diversity, systemic inflammation, microbial translocation and epithelial barrier damage in a cohort of HIV-infected individuals on long-term ART.

Materials and Methods. We did a cross-sectional study on 20 HIV-infected individuals treated with EFV, 17 treated with PIs and 9 seronegative individuals (SI), who gave their informed consent. Fecal samples were obtained from all participants. DNA was extracted and sequenced for the 16S ribosomal DNA-V3 region using the Ion Torrent Personal Genome Machine (PGM). Taxonomy assignment at 97% ID was performed using QIIME 1.8.0. Plasma markers of immune activation (25-plex cytokines), monocyte activation (soluble CD14) and enterocyte death (Intestinal-Fatty Acid Binding Protein; I-FABP) were measured using commercial kits. Statistical analysis was performed using two-tailed Mann-Whitney U- test (GraphPad Prism 6).

Results. Median CD4 counts were 457 (235-1247) and 559 cells/mm3 (240-1177) for individuals on EFV- and PIs respectively (p=0.174). All individuals on ART had undetectable viral loads. Median duration of ART was 67 months ± SD 32.5 and 75 months ± SD 35.7 for EFV and PIs respectively. Four predominant phyla were found: *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Fusobacteria*. The phylum *Fusobacteria* was only observed in individuals under EFV (overall RA= 3.591% ± SD 9.05%). The relative abundance (RA) of *Bacteroidetes* was significantly reduced in individuals receiving EFV as compared to those receiving PIs (p=0.0054) and to SI (p=0.0175). At the genus level, the RA of *Prevotella* was significantly reduced (p=0.0415) and the RA of *Bacteroides* was significantly enriched (p=0.0328) in individuals receiving EFV compared to those on PIs. Increased plasma levels of I-FABP and inflammatory cytokines were observed in PIs compared to EFV and SI (p=0.0230 and 0.005 respectively). No difference in plasma levels of sCD14 was observed between PIs and EFV (p>0.05).

Conclusions. This study supports the notion that different ART regimens have distinctive impact on the microbial composition and diversity. Furthermore, PIs seems to have a more profound effect on epithelial barrier damage and systemic inflammation. The impact of ART in the gut microbiome of treated HIV infection warrants further investigation.

Results were also presented at the conference on retroviruses and opportunistic infections, Seattle, february 2015 and Keystone Microbiota, Keystone Resort, Colorado, march 2015

No conflict of interest

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