**HIV-associated alteration in gut microbiota are associated with increased inflammation and infection of enteric CD4+ T cells**

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**Conclusions**

- Both HIV infection and high-risk MSM behavior influence microbiome composition.
- Stimulation of PBMC and LPMC with bacteria isolated from stool indicated that bacterial communities in HIV positive individuals are more pro-inflammatory, and less anti-inflammatory than that of HIV negative, indicating a potential direct role for gut bacteria in driving inflammation in HIV positive individuals.
- Higher levels of HIV infection of LPMC are obtained in the presence *P. stercoralis* (enriched in high-risk MSM subjects) than *B. uniformis* (enriched in low-risk HIV negative subjects), indicating a potential role for the high-risk MSM associated gut microbiota in HIV disease transmission and progression, although similar experiments with stool bacterial community preps are needed.
- Further comparisons of the immune-modulatory properties of bacteria that differ between HIV-negative low and high risk populations and HIV-positive populations, will allow us to better define gut microbial drivers of gut and systemic inflammation and its role in disease progression, acquisition and co-morbidity.

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Abstract

• Background: HIV infection has been associated with dramatic alterations of gut microbiota composition that often persist despite long-term otherwise successful Antiretroviral Therapy (ART). Alterations in gut microbiota have been correlated with HIV disease progression, inflammatory markers in the gut and with inflammatory and bacterial translocation markers in blood. A better understanding of the immune-modulatory properties of gut microbes that correlate with disease will allow for the exploration of microbial drivers of immune activation, HIV disease pathogenesis and co-morbidity.

• Methods: We are collecting gut microbiota data from a large cohort of individuals living in the United States using 16S rRNA sequencing, and identifying bacteria whose prevalence correlates with disease status, ART, and with inflammatory and metabolic disease markers. To explore the immune-modulatory properties of these bacteria, we culture peripheral blood mononuclear cells (PBMC) and lamina propria mononuclear cells (LPMC) isolated from resected gut tissue with bacteria isolated from patient stool and cultured bacteria that are increased or decreased with HIV infection. We then measure the impact of the stimulation on pro and anti-inflammatory cytokines, T cell activation, T regulatory cells, HIV co-receptors and levels of HIV infection.

• Results: Some aspects of the previously reported HIV-associated gut microbiota appear to be enriched in high-risk men who have sex with men (MSM) independent of HIV status, particularly a gut microbiota characterized by high relative abundance of the bacterial genus Prevotella and low Bacteroides. However, when correcting for this confounding factor, such as by exclusively comparing HIV negative and HIV positive women, we are identifying bacterial species that specifically associate with HIV. Although stimulations of PBMC/LPMC with most bacteria induce both pro and anti-inflammatory cytokines, fecal bacteria from HIV-infected individuals induce lower levels of T regulatory cells and anti-inflammatory IL-10 and higher levels of pro-inflammatory protocols. Furthermore, incubation of LPMCs with numerically dominant bacteria of the MSM-associated (Prevotella stercorea) but not the non-MSM-associated (Bacteroides uniformis) gut microbiota resulted in increased infectivity of immune cells by HIV, indicating that the MSM-associated gut microbiota may promote disease transmission.

• Conclusions: These data suggest that a loss of anti-inflammatory (beneficial) bacteria and an increase in pro-inflammatory bacteria with HIV infection has the potential to drive chronic inflammation observed in HIV-infected individuals. Furthermore, our infectivity assays indicate a potential role of the MSM-associated gut microbiota in disease transmission and progression. We are currently further exploring microbiota associations with HIV disease, inflammation and metabolic disease in populations in both the US and Zimbabwe.
**Background**

- Bacteria that inhabit the gastrointestinal tract have the potential to play an important role in many aspects of HIV infection (Figure 1).
- Our group had previously reported that in contrast to uninfected subjects, the microbial community in HIV-infected subjects is enriched in the bacterial genus Prevotella and depleted in Bacteroides and that the use ART does not consistently restore the gut microbiota to that of a seronegative subject.\(^1,2\).

![Diagram](image_url)

**Figure 1. Gut health during HIV infection.** 1) A healthy gut is characterized by homeostasis between the immune system and the microbiome. 2) Though the impact of the baseline microbiota on HIV infection is unknown, it is possible that gut microbes could impact (A) transmission, potentially by activating CD4+ T cells. (B) HIV infection leads to CD4+ T cell death and immune depletion, and loss of immune regulation of gut bacteria, resulting in a dysbiotic microbiome. (C) Translocation of dysbiotic bacteria leads to (D) immune activation and further HIV infection of activated CD4+ T cells. 3) ART suppresses viral replication but does not fully restore gut immunity; dysbiosis is sustained during ART and microbial translocation continues to cause chronic immune activation. 4) A healthy gut may be restored by supplementing ART with prebiotics or diets that encourage growth of beneficial bacteria, and with immunotherapy that could help reconstitute gut immunity and restore immune regulation of the microbiome.
**Microbiome Characterization**

**Methods**

- We used 16S rRNA targeted sequencing to evaluate the gut microbiota of 145 individuals from the United States, including 41 untreated, 51 on successful ART and 53 uninfected controls. Our HIV negative control population included 13 individuals who were at a high risk of contracting HIV because of sexual behavior, and of these 10 were confirmed MSM. Although our cohort was 78% male, it included 17 HIV positive females and 15 HIV negative control females.

**Results**

- The fecal microbiomes of HIV-positive individuals in the United States generally clustered apart from HIV negative, with HIV positive individuals having significantly more bacteria in the genus Prevotella and HIV negative having more Bacteroides (Fig. 2 left panel).
- HIV negative individuals from our high-risk cohort (MSM) frequently clustered with HIV positive rather than non-MSM HIV negative, as has been previously reported.*
- HIV positive women clustered closer to HIV negative non-MSM, although they still were compositionally different from HIV negative women (Figure 2 right panel).
- Mann Whitney U comparisons showed that HIV positive women had several differentiating taxa, including significantly less of taxa in the genus Coprococcus ($p<0.001$; FDR $p=0.11$) and family Christensenellaceae ($p<0.01$; FDR $p=0.17$) and more of taxa in the genus Paraprevotella ($p<0.01$; FDR $p=0.17$).

![Figure 2. Principal Coordinates Analysis (PCoA) of 16S rRNA data from fecal samples. Sequences from the V4 region of rRNA were generated using the MiSeq platform. The composition of the bacterial communities were compared based on unweighted UniFrac and PCoA clustering using QIME.](image)

**Immune response characterization**

**Methods**

- Bacterial communities were isolated from fecal samples as described in Fig. 3.
- The level of induction of pro-inflammatory cytokines, T regulatory cells, and immune cell activation markers after 4 day stimulations of PBMC and LPMC were compared between bacteria isolated from the stool of HIV positive and HIV negative individuals (Figs. 4 and 5).
- The level of infection of T cells in LPMC was compared when done in the presence of *Prevotella stercorea* (high-risk MSM associated) versus *Bacteroides uniformis* (low-risk non-MSM associated).
Fig 3. Isolation of bacteria from fecal samples. A. Whole stool was collected from subjects with and without HIV infection. B. Homogenized stool was overlaid on Histodenz, subjected to density gradient separation with ultracentrifugation and then the infranephase layer containing bacteria was collected. C. Visualization of bacteria fraction. D. Enumeration and viability of bacteria was evaluated by staining with propidium iodide (PI) and thiazole orange (TO). E. The degree to which fecal isolated bacterial populations matched populations in whole stool were evaluated with 16S rRNA sequencing. Two representative example taxa bar charts of isolated bacteria and whole stool are shown.
Bacteria isolated from the stool of HIV infected subjects induce high levels of inflammatory cytokines in PBMC and LPMC.

**Figure 4.** Stool bacteria from HIV positive subjects induce higher levels of inflammatory cytokines than stool from HIV negative subjects in vitro. PBMC and LPMC from healthy subjects were stimulated with bacteria isolated from the stool of HIV-infected and uninfected subjects for 4 days. On day 4 the cultured cells were restimulated with PMA/Ionomycin for 6 hours and intracellular cytokine (IFN-γ, IL-17 and IL-22) staining of CD4+ T cells was performed. A. PBMC and B. LPMC. Supernatant were collected on day 4 before restimulation from C. PBMC and D. LPMC and IL-17 and IL-22 levels were measured by ELISA.
Figure 5. Stool bacteria isolated from HIV-infected subjects induces more inflammation and less regulatory T cells that stool bacteria from HIV-uninfected subjects. PBMC and LPMC from healthy subjects was stimulated with isolated stool bacteria from HIV-infected and uninfected subjects for 4 days. A. Proportion of CD4+ T cells that are T regs (CD38+FoxP3+HLA-DR+CD127-) in A. PBMC and B. LPMC after 4 days of culture with isolated stool bacteria. Percent of CD4+ T cells that are activated (CD38+HLA-DR+) in B. PBMC and E. LPMC. C and F. ratio of T regulatory cells per CD38+HLA-DR+ activated CD4+ T cells in PBMC and LPMC.

Figure 6. Bacteria prevalent in the stool of high risk MSM subjects enhances HIV infection of lamina propria CD4+ T cells to a greater extent than bacteria prevalent in the stool of non high risk MSM HIV negative subjects. LPMC from healthy subjects was infected with HIV in the presence of Proteus mirabilis or Enterococcus faecalis. After 7 days the percentage of CD34, CD8- T cells were examined for HIV p24 by flow cytometry.

References