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HIV-induced alteration in gut microbiota
Driving factors, consequences, and effects of antiretroviral therapy

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C onsistent with an important role for adaptive immunity in modulating interactions between intestinal bacteria and host, dramatic alteration in the composition of gut microbes during chronic HIV infection was recently reported by ourselves and independently by four other research groups. Here we evaluate our results in the context of these other studies and delve into the effects of antiretroviral therapy (ART). Although gut microbiota of HIV-positive individuals on ART usually does not resemble that of HIV-negative individuals, the degree to which ART restores health-associated prevalence varies across bacterial taxa. Finally, we discuss potential drivers and health consequences of gut microbiota alterations. We propose that understanding the mechanism of HIV-associated gut microbiota changes will elucidate the role of adaptive immunity in shaping gut microbiota composition, and lay the foundation for therapeutics targeting the microbiota to attenuate HIV disease progression and reduce the risk of gut-linked disease in people with HIV.

HIV infection causes rapid and substantial depletion of lamina propria CD4+ T cells that are important for modulating interaction with intestinal bacteria.1 In five recently published, independent research studies that used the 16S ribosomal RNA (rRNA) gene to compare gut microbiota composition between individuals with chronic HIV infection and seronegative controls,2-6 strong and characteristic compositional differences were observed in the relative abundance of bacteria in feces2,6 as well as in mucosal samples from the ileum through the rectosigmoid colon.2,4,5 Gut bacteria have been proposed to play a key role in HIV disease progression, since translocation of bacterial products such as lipopolysaccharide to blood may cause systemic activation of T cells, and HIV preferentially infects activated T cells.7-9 Furthermore, many diseases that increase in prevalence with chronic HIV infection have been linked with gut microbiota composition including metabolic and cardiovascular disease.10-12 Now that it has been well established that gut microbiota compositional changes occur with HIV infection, understanding why they occur and the health consequences of these alterations is of paramount importance.

There was some consistency in the types of bacteria reported to differ with chronic HIV infection across the five independent studies. For instance, the most profoundly enriched genus in feces of untreated HIV-infected individuals in our study (Prevotella) was significantly enriched with chronic HIV infection in two other studies2,4 and the most profoundly depleted genus in our study (Bacteroides) was significantly depleted in three other studies.2,4,5 These shared observations occurred despite substantial methodological differences, including (1) the types of samples evaluated (e.g., mucosal biopsies from different regions of the intestine vs. feces), (2) whether the HIV-infected individuals were receiving ART, and (3) the techniques used to characterize gut microbiota composition, including DNA extraction protocol, PCR
primers, use of hybridization arrays (the PhyloChip) vs. next generation sequencing, which sequencing platform was used, and how data were bioinformatically analyzed.

Given the substantial methodological differences between the studies, it is also not surprising that there were notable ways in which the results varied across studies. For instance, various types of Proteobacteria significantly increased in relative abundance with HIV infection in all three papers that evaluated biopsies,4-6 suggesting that gut microbiota alterations could contribute to the pathogenesis of diseases that occur and/or persist with ART. Here we expand our analyses by doubling the size of our ART cohort from 14 to 28 individuals (Table 1).

In this augmented cohort, our original result was supported by the finding that at the community level, individuals on ART usually resemble individuals with untreated HIV infection more than HIV-negative controls. Specifically, various taxa that significantly decrease with HIV infection, such as the genera Bacteroides and Odoribacter or an OTU classified as Parabacteroides distasonis, remain at low prevalence in the majority of individuals on ART (Fig. 1A). Similarly, some taxa that significantly increase in relative abundance with HIV infection, including the genus Prevotella, the family Paraprevotellaceae, and an OTU classified as Eubacterium biforme, which we previously have shown to induce a pro-inflammatory cytokine profile in in vitro stimulations,6 have increased variance in relative abundance in individuals undergoing ART, but overall do not decrease toward levels typical of HIV-negative individuals (Fig. 1B). In contrast, some taxa that were increased with untreated HIV infection, such as the genus Peptococcus, significantly decrease with ART (Fig. 1). Other taxa that had increased representation in people with untreated HIV infection, such as

**Table 1. Patient Clinical Characteristics**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>Age (years)*</th>
<th>Duration on ART (months)*</th>
<th>CD4+ T cells (cells/ul)*</th>
<th>HIV Viral Load (copies/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B1: Chronic HIV untreated</strong></td>
<td>12</td>
<td>32 (25–46)</td>
<td>0</td>
<td>523 (270–1095)</td>
<td>62745 (1350–301 000)</td>
</tr>
<tr>
<td><strong>B1 ART: Chronic HIV short-time ART &lt;12 mo</strong></td>
<td>11</td>
<td>35 (28–58)</td>
<td>8.5 (7–10)</td>
<td>658 (36–1118)</td>
<td>40 (&lt;20–297)</td>
</tr>
<tr>
<td><strong>B2: Chronic HIV long-term ART &gt;12 mo</strong></td>
<td>17</td>
<td>46 (32–57)</td>
<td>45 (13–101)</td>
<td>611 (204–1491)</td>
<td>&lt;20 (&lt;20–44)</td>
</tr>
<tr>
<td><strong>A2: HIV-seronegative</strong></td>
<td>15</td>
<td>40 (25–57)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Median and range

For instance, the most highly depleted Operational Taxonomic Unit (OTU; cluster in which sequences have ≥97% identity over their aligned 16S rRNA genes, which approximates assignment to the same species) in the fecal samples in our study had a representative 16S rRNA gene sequence that was 100% identical to *Bacteroides uniformis*, a species previously reported to be found in higher abundance in the lumen than in the mucosa.6 We did not detect a significant change in *Bacteroides fragilis*, a species that is much more prevalent in mucosal samples than feces. In contrast, by using BLAST to find the most closely affiliated species of the significantly depleted PhyloChip probes in mucosal samples from HIV-positive individuals in Vujkovic-Cvijin et al., we observed that a probe with 99% identity over aligned 16S rRNA genes to *B. fragilis* (probeset ID 69014) was among the most significantly depleted in HIV-infected individuals, showing a 5.6-fold depletion, whereas no significantly depleted probes were highly related to *B. uniformis*.5 Thus with regard to species in the Bacteroides group, those with niches that are relatively luminal or mucosal were both depleted with chronic HIV infection, but change was more readily detected in the appropriate sample type.

**Effect of ART on the Composition of Gut Microbiota**

One conclusion drawn independently by all research groups whose cohorts included individuals undergoing ART, is that individuals on long-term successful treatment rarely had a gut microbiota composition that resembled that of HIV-negative individuals, suggesting that gut microbiota alterations could contribute to the pathogenesis of diseases that occur and/or persist with ART. Here we expand our analyses by doubling the size of our ART cohort from 14 to 28 individuals (Table 1).
the Desulfovibrio and Catenibacterium genera, with ART trended back toward proportions seen in HIV seronegative individuals, although these decreases were not statistically significant after correcting for multiple comparisons (Fig. 1C). These taxa furthermore showed intermediate levels in individuals on ART for less than one year compared with those undergoing long-term treatment (1–9 years; Fig. 1C). Differential recovery of various taxa with ART may reflect differences in the recovery of various components of the host immune system in gut-associated lymphoid tissue (GALT) and different underlying factors that control prevalence.

Occasionally, the microbiota composition of individuals on long-term ART resembled HIV-negative individuals more than those with untreated HIV infection. For instance, a minority of individuals on ART had Bacteroides to Prevotella ratio typical of HIV-negative individuals. We showed previously that an overall “correction” of microbiota composition was not associated with the duration of ART\(^6\); however, other factors that may be involved have not been explored. These additional factors may include the duration of HIV infection and extent of CD4\(^+\) T cell depletion (i.e., CD4\(^+\) T cell nadir) before ART commenced, the specific class of antiretroviral drugs used, or the state of CD4\(^+\) T cell recovery in GALT.

Our ART-treated cohort also had lower stool microbiota diversity as measured with the Shannon diversity index compared with untreated individuals with untreated HIV infection ($P = 0.029$; T test with Welch’s correction). This result is supported by Mutlu et al. who observed a significant decrease in mucosal bacterial diversity in their HIV-positive cohort who were receiving ART compared with seronegative controls.\(^4\) Studies that compared diversity in mucosal samples
Why Does HIV-Infected Immunodeficiency Cause a Compositional Change?

Now that alteration in gut microbiota composition with chronic HIV infection has been demonstrated, important questions that remain are: (1) Why do these specific changes occur? and (2) What are the implications for health?

One possible driving factor is that the loss of effector CD4+ T cells results in the failure to mount an effective immune response to pathogens or, resulting in the outgrowth of certain bacteria. However, this view ignores the concept that adaptive immunity has the means to both promote and to temper innate immune responses for microbial clearance. Compositional change with HIV infection may also result from the loss of mutualistic bacteria that depend on interaction with the adaptive immune system for persistence (Fig. 2). Consistent with this notion, B. fragilis, a species that was significantly depleted in relative abundance in mucosa samples from HIV-infected subjects relies on a CD4+ T cell-interacting coat polysaccharide called polysaccharide A (PSA) to establish a niche in the gut; B. fragilis isolates in which the PSA operon has been knocked out have a decreased ability to colonize the mouse colon mucosa. Taken together, this suggests that the combined effects of ART and of HIV infection itself, whose effects are not entirely mitigated by ART, may induce a “double hit” to the microbiota. However, the effects of ART on microbiota composition appear to be subtle compared to the effects of HIV infection itself. Further study of the effect of different types of antiretroviral drugs on the microbiota is an important future direction.

Do Alterations in Gut Microbiota Mediate Chronic Inflammation in HIV-Infected Individuals?

Immune activation and chronic inflammation have been linked with HIV disease progression and co-morbidities that occur in both untreated and treated chronic infection, including metabolic and cardiovascular disease. Determining whether gut microbiota alterations that occur with HIV infection are direct drivers of inflammation will establish whether these alterations may be a part of the etiology of these diseases. Consistent with a role for gut microbiota alterations in HIV disease progression, the only long-term non-progressor (LTNP, i.e., a person in an “elite” group of suppressors in whom HIV disease does not progress despite not undergoing ART) evaluated in any of the existing studies had a gut microbiota
composition that was more similar to HIV seronegative subjects than to HIV-positive subjects.  

Interestingly, gut microbiota composition correlated with markers of immune activation in both blood and gut mucosal tissues, although in all reports no effort was made to correct for multiple comparisons or potentially confounding variables such as viral load, shedding some doubt on the strength of the associations. Both Dillon et al. and Vujkovic-Cvijin et al. detected a correlation between overall microbiota composition in rectosigmoid biopsies and HLA-DR/CD38 expression on blood and mucosal CD4+ and CD8+ T cells. Specifically, Dillon et al. found that the relative abundance of the genus Prevotella was positively correlated with the number of activated (CD38+HLA-DR+) CD4+ and CD8+ T cells per gram of mucosal tissue and the level of CD1c+ mDC activation based on CD40 expression. Associations were also seen between the relative abundance of specific bacterial taxa in mucosal tissues and plasma cytokines and/or chemokines levels including IL-6, TNF-α, IL-10, and IP-10. For example, Mutlu et al. showed that plasma IL-6 levels were inversely associated with Bacteroides relative abundance, which is consistent with anti-inflammatory properties of at least some of the species in this genus. Dillon et al. also found that systemic biomarkers of bacterial translocation in plasma, sCD14 and LPS, were correlated in an increase in Lachnospira and Roseburia, respectively, in the gut. Although the presence of these associations is intriguing, correlations do not define cause and effect and may just be a reflection of HIV disease progression. No associations were found between changes in the composition of gut microbiota and CD4+ T cell count or viral load in blood in any of the studies. 

Positive or negative correlations between specific bacteria and inflammatory markers are particularly compelling if the identified bacteria were independently shown to have pro- or anti-inflammatory properties. As already noted, several species that decrease in relative abundance with HIV infection are known to stimulate anti-inflammatory Tregs. Furthermore, a couple of these Treg inducers have been shown to protect against colitis in mouse models, at least in part by competitively inhibiting the colonization of pathogenic bacteria. B. fragilis protected against colonization of Helicobacter hepaticus and of 2,4,6-trinitrobenzenesulfonic acid (TNBS) -induced colitis in a PSA dependent manner. Furthermore, oral treatment of mice with P. distasonis lysate reduced the severity of dextran sodium sulfate (DSS)-induced intestinal inflammation. This protection was mediated at least in part by the adaptive immune system, since severe combined immunodeficient (SCID) mice were not protected.

As already noted, phylotypes related to Proteobacteria that can be pro-inflammatory, such as Escherichia and Campylobacter, were increased in mucosal but not fecal samples of HIV-infected individuals. Heat-killed commensal E. coli exposure has previously been shown to enhance HIV-1 replication, CD4+ T cell activation and infection in vivo. The high relative abundance of the genus Prevotella in HIV-infected individuals and a positive correlation with immune activation markers in mucosal tissues has led to speculation about whether bacteria in the Prevotella genus are themselves...
pro-inflammatory and Prevotella-rich communities have indeed been associated with inflammation in other studies. For instance, in a recent study that correlated Prevotella copri with disease in new-onset untreated Rheumatoid Arthritis (RA) patients, the colonization of antibiotic-treated C57BL-6 mice with P. copri by oral gavage resulted in increased inflammation and led to more severe symptoms in DSS-induced colitis. In another study, deficiency of the NLRP6 inflammasome in mouse colonic epithelial cells resulted in a shift in the microbiota to one with a significant increase in Prevotellaceae, and transmission of this microbiota to wildtype mice via co-housing also lead to more severe symptoms in DSS-induced colitis.

Although these studies both indicate an association of Prevotella with inflammation and that Prevotella-rich communities may fail to protect against bacteria-mediated inflammation upon chemical injury, neither directly show that Prevotella itself is driving the inflammation. Consistent with the Prevotella-dominated community type in humans, an increased representation of Prevotella was associated with other complex community changes, including in both cases a community shift away from the anti-inflammatory Bacteroides genus just discussed. Genomic analysis of P. copri revealed genes that allow for the tolerance of inflammation, such as superoxide dismutase, but not genes that promote inflammation. Furthermore, our stimulations of human peripheral blood mononuclear cells (PBMC) with P. copri lysate showed that the cytokine production was not any more pro-inflammatory (as assessed by TNF-α/IL-10 ratio) than two different Bacteroides species tested and stimulations of human dendritic cells with three different species in the Prevotella genus showed them to have a moderate pro-inflammatory profile that was weaker than the assayed opportunistic pathogens Hemophilus spp and Moraxella spp. Although multiple studies have suggested that sulfatases may allow Prevotella to degrade mucins and induce inflammation by that mechanism, many of the Bacteroides species that decrease with HIV infection are also known to degrade mucins using sulfatases. Taken together, these results suggest that it may be aspects of the Prevotella-rich community type, including a corresponding loss of anti-inflammatory Bacteroides or co-occurrence of directly pro-inflammatory bacteria, rather than Prevotella-richness itself that results in an inflammatory phenotype.

It is also important to note that Prevotella-richness is commonly observed in health and associated with diet. A Prevotella-rich and/or Bacteroides-poor community profile highly similar to that observed in HIV-infected patients is regularly observed in healthy individuals in agrarian cultures, including Malawi, the Amazonas states of Venezuela, and Burkina Faso. Among healthy Thai subjects, vegetarians had high abundance of Prevotella and non-vegetarians of Bacteroides, and within the United States population, Prevotella-richness has been described in healthy individuals who consume diets relatively rich in carbohydrates and poor in animal products.

Mouse studies have suggested that at least some of the differences between the microbiota that is typical of Western vs. agrarian cultures (and HIV-negative vs. -positive people in the USA) have the potential to protect against health consequences of a Western diet. As an example, B. uniformis is highly enriched in HIV-negative individuals in the USA compared with HIV-positive and compared with individuals in Malawi and Venezuela American Indians and oral administration of B. uniformis reduced high fat diet (HFD)-induced inflammation and metabolic disease in mice. This protection was mediated, at least in part, by interaction with the adaptive arm of the immune system. B. uniformis intake restored an otherwise compromised capacity of intestinal dendritic cells to induce a T cell proliferative response to LPS, and reduced the prominence of Enterobacteriaceae and the overall pro-inflammatory signal from the gut (as assessed by in vitro stimulations of macrophage and dendritic cells with feces). However, in the absence of the HFD, the mice did not have an overall pro-inflammatory signal in the gut, nor did they have as high populations of Enterobacteriaceae, indicating that the presence of B. uniformis only had a health benefit in a particular dietary context.
In our work, we did not collect dietary information on study participants, and so we do not know if dietary differences between HIV-negative and HIV-infected persons were influencing observed differences in the fecal microbiome. Dillon et al. also observed an increase in Prevotella and decrease in Bacteroides with chronic HIV infection and determined that this relationship was not associated with dietary differences between their cohorts.2 Interestingly, Bacteroides relative abundance positively correlated with red-meat intake in HIV-negative, but not HIV-positive, individuals.2 This observation, together with the finding that certain Bacteroides species protect against metabolic disease in HFD-fed mice, suggests that dysfunction of the adaptive immune system that occurs with HIV infection compromises the ability to select for bacteria that protect against metabolic disease in individuals who are eating a Western HFD, potentially resulting in a mismatch between diet and microbiota composition that is detrimental to health.6

Conclusions

HIV infection clearly is associated with alterations of the gut microbiota composition. New challenges are (1) to understand why the change occurs, (2) to determine whether this altered microbiota plays a role in HIV disease progression and in the high prevalence of co-morbidities such as cardiovascular and metabolic diseases, and (3) to determine the influence of ART. The pronounced loss of Bacteroides spp. such as B. uniformis, which is associated with the reduction of HFD-induced inflammation, suggests that HIV-infected individuals who consume diets high in fat and protein and low in carbohydrates and fiber may have an increase in inflammation because of the absence of beneficial bacteria that attenuate diet-driven inflammation. This notion is consistent with previous reports that diet modulation has the potential to attenuate both metabolic diseases in HIV-infected individuals31,32 and SIV disease progression in SIV-infected macaques.34 Tailoring diet modulations aimed at promoting health to the microbiota composition of HIV-positive individuals and developing probiotic therapies that are based on a mechanistic understanding of the drivers and consequences of the compositional changes that occur with HIV infection, are promising strategies for promoting health in HIV-infected individuals.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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