HIV-Associated Changes of the Gut Microbiome in ARV-Treated, Untreated, and Controllers

Poster #261

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Introduction

The human intestine harbors an enormously diverse community of microorganisms, the "microbiome", with a cumulative genome, or "metagenome", that has co-evolved with humans to aid in critical host metabolic and immune functions. To better understand the progression of HIV and its effect on the host immune system, we aim to study the differences between the intestinal microbiome of different communities of human patients who are infected or uninfected by HIV.

Prior work looking at patients with acute & and chronic HIV demonstrated altered gut microbiota when compared to a seronegative patient; the effect was more profound with chronic infection [1]. In HIV infected patients, there was an altered functional profile, with enrichment of genes for pathogenic processes such as LPS biosynthesis, bacterial translocation, and pro-inflammatory pathways; at the expense of metabolic expression [2].

Methods

Phenol-chloroform was used to extract DNA from each fecal sample, which was used for 16S rRNA V4 region PCR amplification with a unique barcoded primer. Amplicons were sequenced on the Illumina MiSeq platform. For community analysis, QIIME was used to organize viable sequences into OTUs with >97% similarity in sequence data. Alpha (intra-sample) and beta (inter-sample) diversity was calculated at a rarefaction depth of 10,000 seqs/sample.

From patient serum samples we used ELISA kits to measure levels of soluble CD14 (sCD14) and soluble CD163 (sCD163) as indirect markers of microbial induced inflammation, and which has been associated with increased gut epithelial barrier dysfunction.

Comprehensive diet surveys included food consumed 24-hours prior to sample submission, and monthly average consumption over the past year.

Bacterial strains of interest from community analyses were cultured and exposed to the Caco2 colonic adenocarcinoma cells to determine their effect on epithelial cell integrity via a trans-epithelial resistance (TER) assay; capsaicin was used as a positive control.

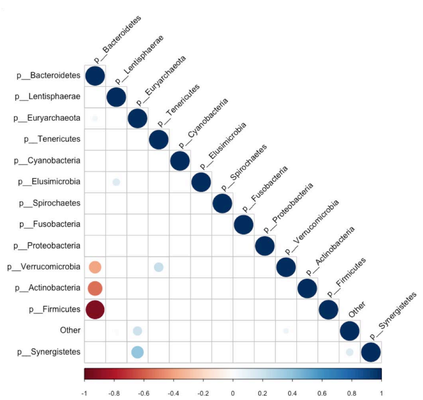


Figure 6: Correlation amongst Bacterial phyla: Spearman correlation by taxonomic phyla abundance. Significant values (p<0.05) colored.

Results and Figures

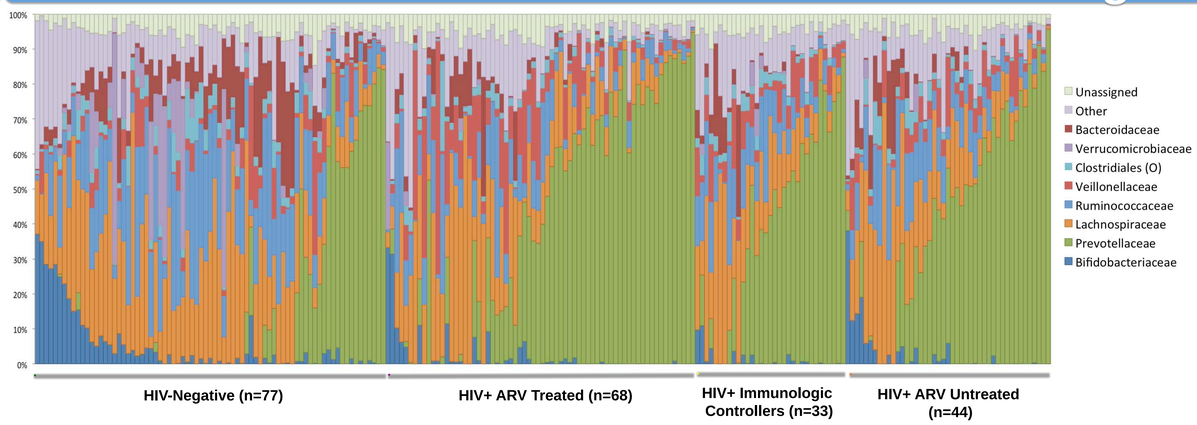


Figure 1: **HIV Infection significantly alters the human gut microbiota, irrespective of ARV or immunologic control:** The bacterial family composition of the human fecal microbiota for each member of the cohort, sorted by HIV clinical status. Each column represents one individual.

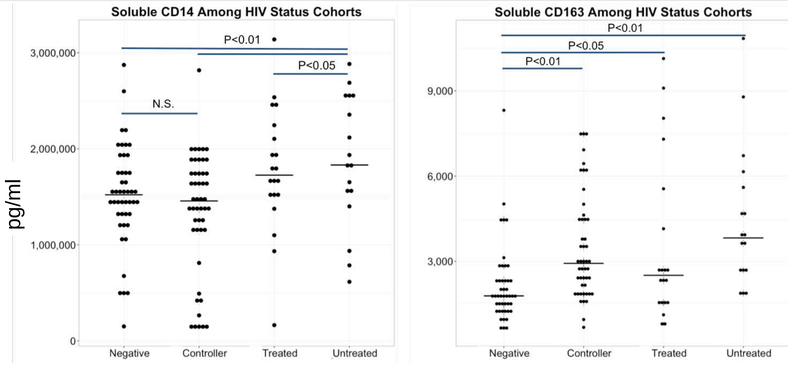


Figure 2: sCD14 Increases with Viremia, while sCD163 Decreases with ARV Treatment: ELISA Quantification of **(a)** sCD14 and **(b)** sCD163 serum levels from among cohort sub-groups that were HIV-negative, immunologic controllers, ARV-treated, or chronic untreated. KW test was followed with Dunn's Post-hoc.

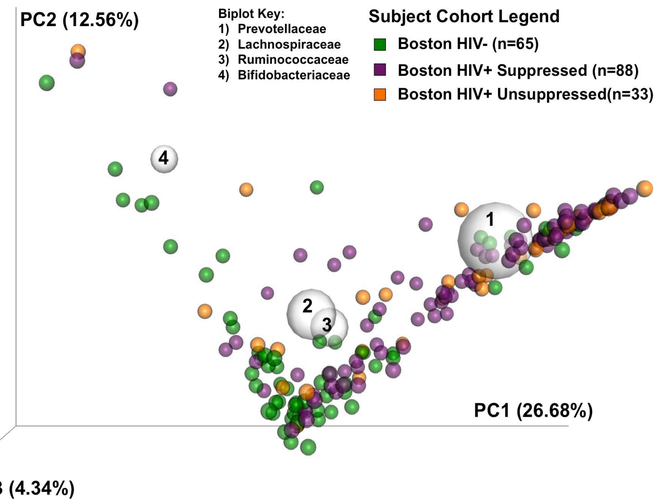


Figure 3: Human gut microbial community structure clusters based on HIV infection status: Weighted Unifrac PCoA of the cohort, organized by HIV infection sub-groups, with overlaid Family level taxonomy biplot of the four most influential taxa. Size of the sphere represents effect size, and location is in relation to the microbial community structure.

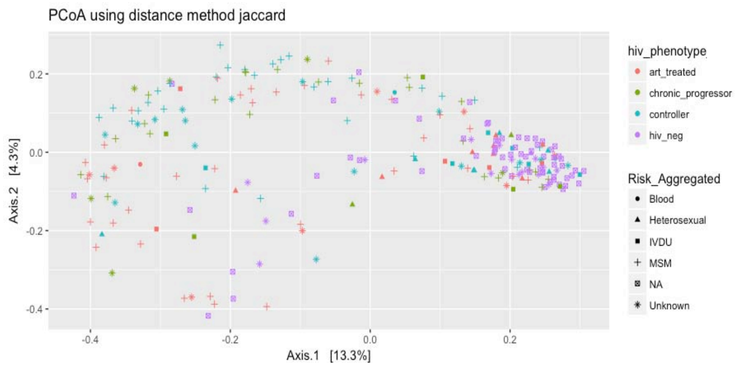


Figure 4: **Risk Factor PCoA:** Weighted Unifrac PCoA of the cohort. HIV acquisition risk factor (organized by shape) correlates but does not stratify based on HIV subgroup (organized by color).

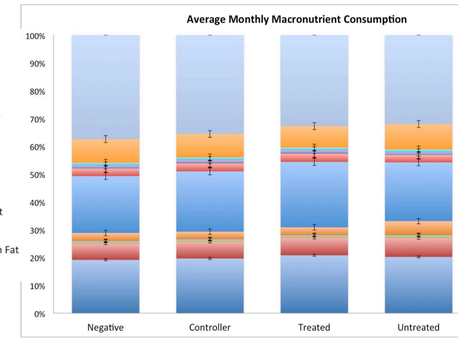


Figure 5: Diet consumption does not significantly differ amongst HIV subgroups: Dietary information for macronutrient consumption by monthly averages for the year prior were taken and compared amongst sub-groups with no statistical differences.

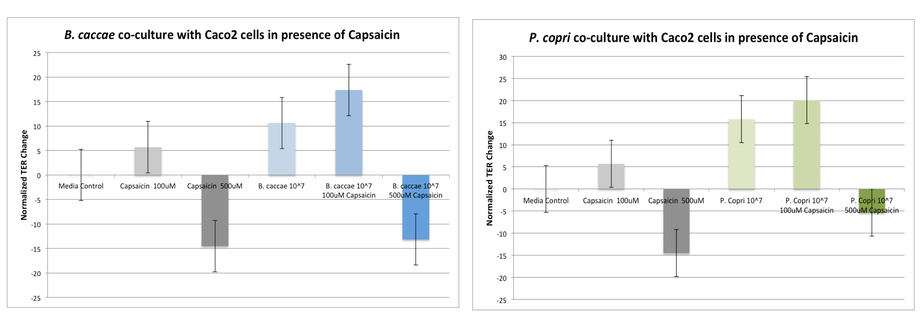


Figure 7: *In Vitro* co-culture of Caco2 cells with selected bacterial strains: Bacterial species that significantly differentiate amongst HIV sub-groups were selected for co-cultured *in vitro* with the colonic adenocarcinoma Caco2 cell line with a 'two-hit' model. A representative strain from each family was co-cultured and exposed to various levels of capsaicin to see the effects of **(a)** *Bacteroides caccae* (enriched in HIV uninfected) and **(b)** *Prevotella copri* (enriched in HIV-infected).

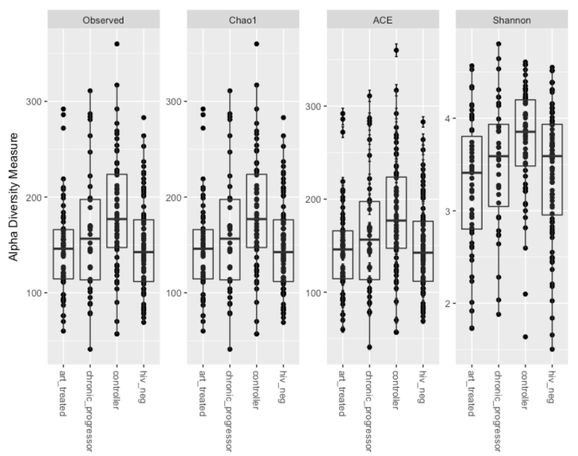


Figure 8: **Metrics of Alpha Diversity:** The enteric bacterial communities were analyzed in terms of intra-sample (alpha) diversity by various measures: Observed Species, Chao1, ACE, and Shannon. Organized by clinical subgroups, represented by 1st and 3rd IQR, and median.

Conclusions

Our findings demonstrate a complex model for how HIV may alter the gut microbiome and human host health. Our data suggests that bacterial communities, irrespective of means of HIV control, and inflammatory responses, with nuances, could be driven by HIV infection and baseline microbial community structure. However, this alone does not prove the bacteria enriched in HIV infection elicit a pathogenic effect on the host and colonic epithelium as was previously presumed. Further work is planned to isolate individual strain effects on intact and non-intact epithelium.

References

1.Lozupone, C.A., et al., *Alterations in the gut microbiota associated with HIV-1 infection.* Cell Host Microbe, 2013. **14**(3): p. 329-39.

2.Vazquez-Castellanos, J.F., et al., *Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals.* Mucosal Immunol, 2014.

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