Acute HIV-infection and Human Gut Microbiome before and after Anti-Retroviral Therapy

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Background and Study Rationale

- The etiology of persistent immune activation in chronic HIV infection is still incompletely understood and is probably multifactorial encompassing residual HIV replication, co-infections, mucosal alteration and deficient immune restoration.
- The intestinal gut microbiota is considered an additional possible driver of pathologic inflammation that may persist even after the administration of antiretroviral therapy (ART).
- Several studies have indicated both a decrease in the percentage of Bacteroidetes and Firmicutes and an increase in Proteobacteria and Fusobacteria in HIV+ compared to HIV- people.
- In addition, at genus level an expansion of Prevotella has been routinely observed in chronically HIV-infected individuals.
- The impact of acute HIV-infection and the immediate ART initiation (2NRTI + Efavirenz) on gut microbiome has not yet been evaluated.

Goals

- To address if acute infection affects the microbiome and the potential impact of subsequent ART administration.
- To assess the impact of ART on gut microbiota in a longitudinal, well characterized, cohort of acutely infected HIV subjects who started ART during acute infection.
- To study associations of microbiome composition with systemic inflammatory and gut epithelial integrity or mucosal function markers (sCD14, CRP, Th17).

Methods

- Anal swab samples were collected from 59 acutely HIV-infected Thai subjects (Fiebig stages I-IV) at baseline and 6 months after ART initiation (2NRTI + Efavirenz).
- 4 anal samples, derived from a cohort of HIV- subjects, with similar demographic characteristics, were used as controls.
- Total DNA was extracted from anal swabs.
- Bacterial profiles of study participants were generated by broad-range amplification and sequencing by Illumina of the V4 variable region of the 16S rRNA gene.
- Computational analysis was performed using QIIME 1.8 implemented in Nephele https://nephele.niaid.nih.gov.
- Association between soluble biomarkers and taxa was assessed using Spearman's test.

Results

Table 1. Characteristics of study participants

	Uninfected subjects	HIV-infected subjects
Number of subjects	26	59
Median Age (yrs)	29	28
Gender	Male	Male
Risk	MSM	MSM
Median CD4 count (cells/µl):		
At baseline	-	386
After 6 months of ART		600
Median viral load (HIV-1 RNA copies/ml):		
At baseline	-	334889
After 6 months of ART		<50
Race	Thai	Thai

Results

- Comparisons between HIV+ post-ART and HIV- showed a higher proportion of Fusobacteria (30% vs 10%) in HIV+.
- Paired comparisons between baseline and 6 months post-ART showed an increased percentage of Fusobacteria, eg. *Fusobacterium*, (9% vs 30%); Proteobacteria eg. *Campylobacter* (12%vs 16%) and Tenericutes eg. *Mycoplasma* (0.9%vs 4.5%). In contrast, a decrease in the percentage of Bacteroidetes, *eg. Prevotella* (58% vs 31.5%) and Firmicutes *eg. Lactobacillus* (20%vs 17%) (p<0.05 for both).

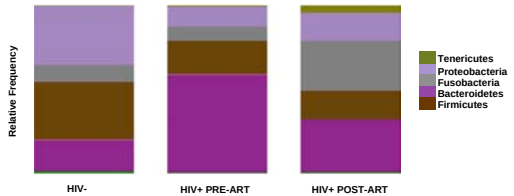

Figure 1. Abundance of bacterial phyla in cases pre and post-ART and controls.

- Subjects post-ART treated at Fiebig (F) stages (III-IV) had a higher proportion of Proteobacteria (18% vs 7%) (p<0.05), a higher percentage of Fusobacteria (9% vs 4.5%) and a lower proportion of Bacteroidetes (37% vs 49%) compared to those treated at FI-II.

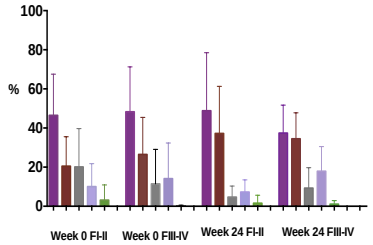

Figure 2. Bacterial phyla prevalence at week 0 Fiebig I-IV and week 24 Fiebig I-IV.

- At baseline (pre-ART) Bacteroidetes and Firmicutes proportions negatively correlated with levels of C-Reactive Protein (CRP) and sCD14 respectively.

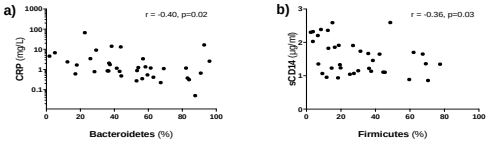

Figure 3. Association between inflammatory markers and GI tract taxa, at baseline.

- After 6 months of ART, Fusobacteria positively correlated with CRP and sCD14 levels, while the percentage of Firmicutes remained negatively associated with sCD14.
- The amount of Proteobacteria negatively correlated with IL17^{+}CD4^{+}T cells.

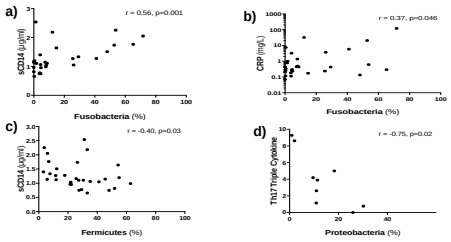

Figure 4. Associations between inflammatory markers and GI tract taxa post-ART.

Conclusions

- Our results suggest changes in the composition of the gut microbiome in persons treated with ART during acute HIV infection with decreases in Bacteroidetes and Firmicutes, consistent with previous findings in chronically infected subjects.
- At genus level there appear to be a decrease in the amount of Prevotella, in contrast to the expansion of this genus that has been previously reported in chronically HIV infected.
- The association between pathogenic taxa and markers of microbial translocation and systemic inflammation, warrants further investigation of the potential role of microbiome alterations in HIV-associated inflammation.

Acknowledgments

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