Fecal Microbiota of HIV Controllers is Similar to Non-HIV-Infected Individuals

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Background

Less than 5% of the HIV-infected population is able to control HIV infection in the absence of antiretroviral therapy (ART), maintaining viral loads below 2,000 copies of RNA/mL for at least 1 year. This population is commonly defined as HIV controllers[1]. Studying this population represents a unique opportunity to understand the mechanisms responsible for HIV control. The aim of this study was to address whether HIV controllers have the same alterations in the composition of their gut microbiota as described in chronic HIV-infected individuals naïve to ART[2-3].

Methodology

The protocol was approved by our local ethics committee (CCBI-INER) and all participants gave written informed consent.
Stool samples were collected from:

- 20 **CI** (chronic HIV-infected individuals naïve to ART).
- 6 **HIC** (HIV controllers).
- 9 **NI** (non-HIV-infected individuals).

1. Microbial DNA was extracted from stool samples using the QIAamp® DNA stool mini Kit (QIAGEN, Valencia, CA).
2. Extracted DNA was PCR-amplified with barcoded primers targeting the V3 region of 16S rRNA gene in triplicate.
3. V3 amplicons were sequenced using the Ion PGM (Life Technologies, Carlsbad, CA). Negative (water) and positive (*E. coli*) controls were included.
4. Raw sequences were filtered by both size (>129 bp) and quality (Q20) using an adapted version of cutadapt[4]. After filtering, an average of 281,867 ± 168,001 sequences per sample was obtained.
5. Filtered sequences were analyzed using QIIME 1.8.0[5].
6. Statistical comparisons between groups were calculated with GraphPad Prism 5.

Results

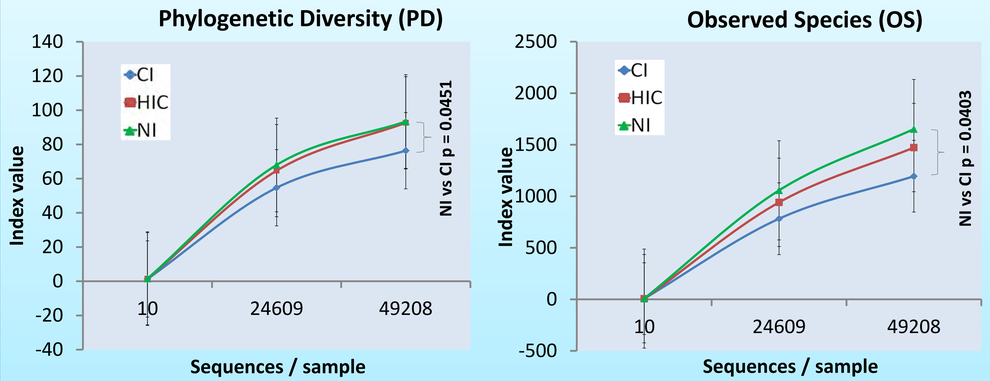
Table1. Cohort characteristics.

Group		CI	HIC	NI
N		20	6	9
Age (years)		28±6.8	34±15.21	38±12.43
Gender	Male	19	3	5
	Female	1	3	4
Body Mass Index (Kg/m^2)		22.3±5.0	25.9±7.4	24.5±2.8
CD4 (cells/mm^3) *		436±164.3	947±298.3	965±251.0
Viral Load (copies of RNA/mL) **		74,408±515,774.2	62±158.9	-

Values are expressed as Median±SD. *CI vs HIC p=0.002. **CI vs HIC p=0.0003 (Mann-Whitney U test).

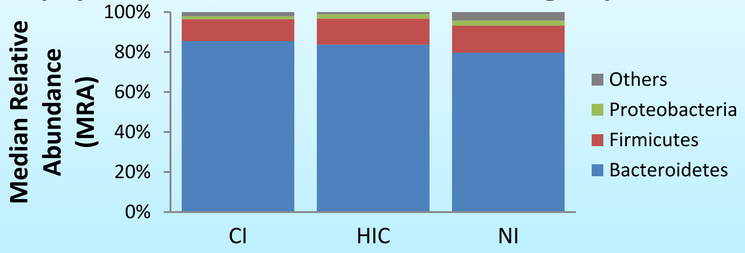
No statistical differences were found for both age and body mass index between the groups. CI had significantly lower levels of CD4 T cell count and higher viral load when compared to HIC and NI groups.

Figure 1. Decreased microbial diversity was only observed in CI individuals.



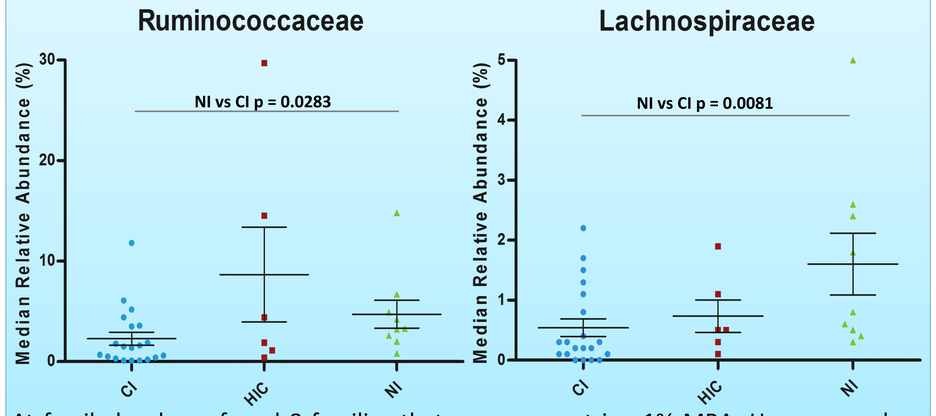
As previously reported[2-3], a decrease in microbial diversity was observed in CI when compared to NI (p=0.04). No difference was observed between HIC and NI. Four Alfa diversity indices were computed using QIIME 1.8.0: PD and OS (Mann-Whitney U test), and Chao1 and Shannon´s index (not statistically different between groups, data not shown). Alpha diversity was rarefied at 49,208 sequences/sample.

Figure 2. At phylum level, no differences between groups were observed.



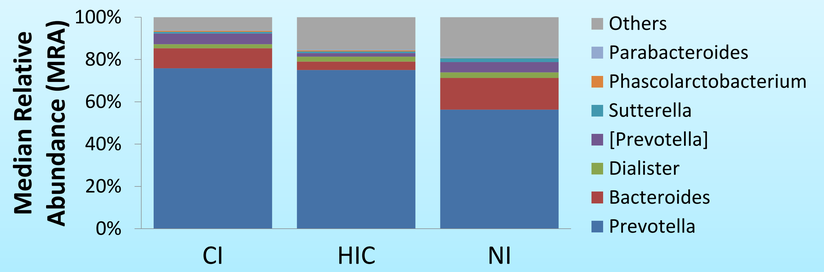
We found 3 different phyla that were predominant in the cohort (>1% MRA): Bacteroidetes, Firmicutes and Proteobacteria. Others represent the 8 minor phyla (with an MRA<1%) considered rare, and unclassified sequences. At phylum level, no significant overall differences were observed (p=0.964, Kruskal-Wallis test). Taxonomic classification was assigned using "pick_open_reference_otus.py" command/QIIME 1.8.0 with the default parameters (Uclust and Greengenes 13.5 reference database).

Figure 3. The MRA of both Ruminococcaceae and Lachnospiraceae are decreased in CI compared to NI.



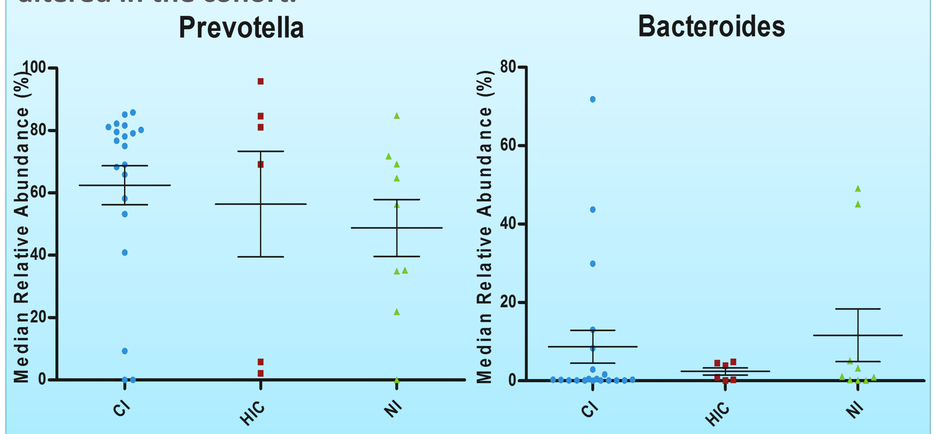
At family level, we found 8 families that were present in >1% MRA. However, only a significant alteration was observed in two families belonging to Firmicutes phylum . A significant decrease in the MRA of Lachnospiraceae and Ruminococcaceae was observed in CI individuals when compared to NI (Mann-Whitney U test).

Figure 4. Most abundant genera in the cohort did not show differences between groups.



Seven genera were observed in our cohort with a MRA >1%. None showed significant differences between groups (p=0.872, Kruskal-Wallis test). Others represent >200 minor genera (with an MRA<1%) considered rare, and unclassified sequences.

Figure 5. The MRA of both Prevotella and Bacteroides was not altered in the cohort.



Changes in Prevotella/Bacteroides ratio has been reported as the principal alteration in CI individuals[2]. However, in this study alterations in both Prevotella and Bacteroides did not achieve statistical differences (p=0.335 and 0.536, respectively. Kruskal-Wallis test).

Conclusions

HIV controllers do not present significant alterations in their gut microbiota composition as reported in chronic HIV infected individuals naïve to ART[2-3] when compared to non-HIV-infected individuals. The fecal microbiota of HIV controllers seems to resemble that of non-HIV-infected individuals. However, it is necessary to take in account the small number of individuals in both CI and NI groups.

References

1. Deeks, Steven G. et al. "Human Immunodeficiency Virus Controllers: Mechanisms of Durable Virus Control in the Absence of Antiretroviral Therapy". Immunity 27.3 (2007):406.
2. Lozupone, Catherine A. et al. "Alterations in the Gut Microbiota Associated with HIV-1 Infection." Cell host & microbe 14.3 (2013): 10.
3. McHardy, Ian H., et al. "HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota." Microbiome 1.1 (2013): 26.
4. Courtesy of Dr. George Watts, University of Tucson, Arizona, USA
5. Caporaso, J Gregory et al. "QIIME Allows Analysis of High-Throughput Community Sequencing Data." Nature methods 7.5 (2010): 335–336.

Acknowledgments

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