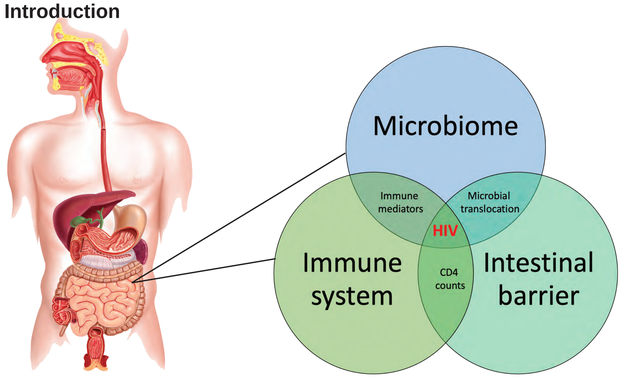
LOSS OF DIVERSITY & RICHNESS OF INTESTINAL MICROBIOME IN HIV+ NAÏVE INDIVIDUALS IS REVERSED BY ART

0225

Angélica Cruz-Lebrón[1], Danielle Labbato[2], Theresa Rodgers[2], Julia Kosco[2], Grace McComsey[2,4,*], Alan Levine[1,2,3,4*]

Departments of [1]Molecular Biology & Microbiology, [2]Pediatrics and Medicine, [3]Pathology, and [3]Pharmacology, Case Western Reserve University, and [4]University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA; *Equal contribution of authors

Introduction



Gut microbiome in HIV infection:

- Decreased bacterial diversity
- Increased *Prevotella* & decreased *Bacteroidetes*
- Increased inflammatory markers (sCD14, IL-6 & sCD163)
- Imbalance of bacterial components could lead to immune activation and promote viral replication
- HIV infection depletes CD4+ T cells within gut associated lymphoid tissue, affecting gut homeostasis
- A wide spectrum of microbial communities (genus & species) are associated with HIV infection

Study Aims

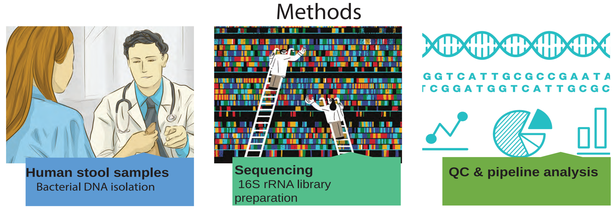
Characterize the gut microbiome of persons with HIV (Naive & ART-treated) and identify potential confounders associated with dysbiosis:

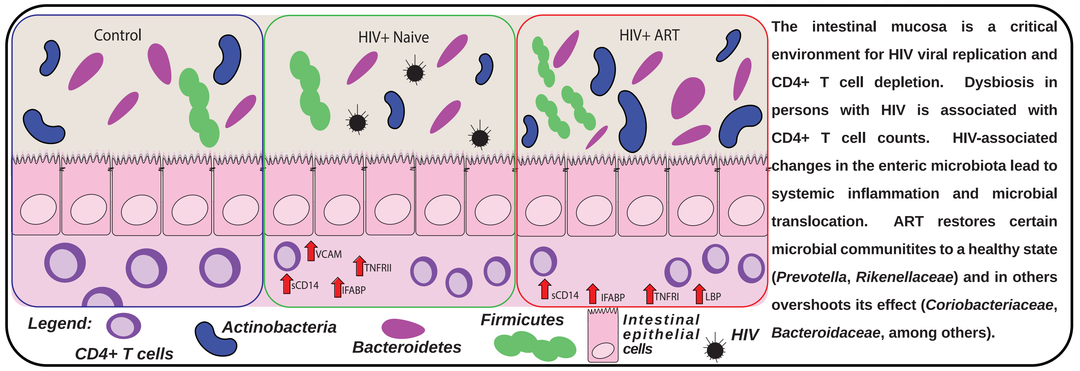
- -Body Mass Index (BMI)
- -CD4+ T cell

Table 1. Study population demographics

	Control (58)	Naive (14)	ART (60)	Statistics
Age range (median)	19-60 (44)	18-54 (44)	23-69 (45)	<0.0001
Sex (F/M)	15/43	2/12	14/46	ns
Race				
Caucasian	44	4	19	<0.0001
African American	11	9	39	
Hawaiian/ Pacific Islander	1	-	-	
Biracial	1	-	2	
Other	1	1		
CD4+ T cell counts				
Low (<500)	N/A	7	14	0.03
High (≥500	N/A	6	46	
Missing counts		1		
BMI				
Underweight	0	1	2	ns
Healthy	28	6	22	
Overweight	16	1	16	
Obese	11	5	17	
Missing calculation	3	1	3	

Methods





The intestinal mucosa is a critical environment for HIV viral replication and CD4+ T cell depletion. Dysbiosis in persons with HIV is associated with CD4+ T cell counts. HIV-associated changes in the enteric microbiota lead to systemic inflammation and microbial translocation. ART restores certain microbial communitites to a healthy state (*Prevotella*, *Rikenellaceae*) and in others overshoots its effect (*Coriobacteriaceae*, *Bacteroidaceae*, among others).

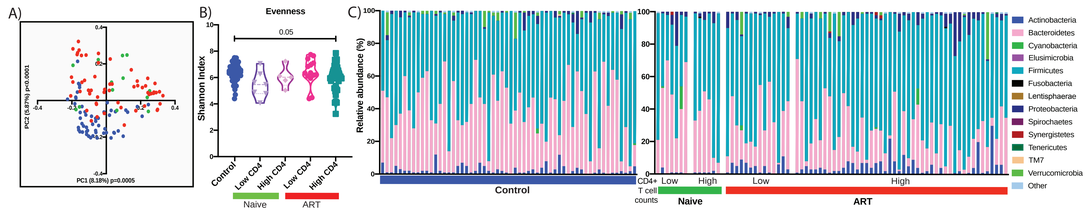


Figure 1. Decreased bacterial diversity of HIV+ Naive is reversed by ART. A) Principal component analysis B) Shannon index C) Microbial composition at phylum level between control, HIV+ naive, and ART-treated individuals separated by CD4+ T cell counts: Low (<500) and High (≥500).

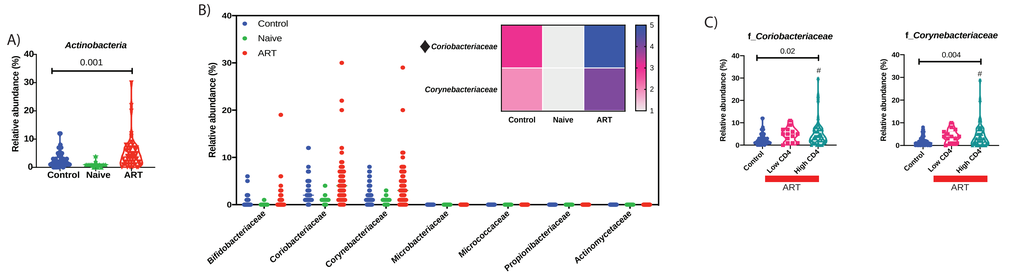


Figure 2. Increased *Actinobacteria* in HIV+ ART-treated individuals with high CD4+ T cells counts. A) Relative abundance of *Actinobacteria* phylum among control (blue), HIV+ Naive (green) and ART (red). B) *Actinobacteria* families detected in the cohorts. Heatmap shows average relative abundance of families that were statistically significant among the cohorts. ♦Designates statistically significant differences in genus abundance (data not shown) C) *Actinobacteria* families in HIV+ ART-treated individuals with low and high CD4+ T cell counts (# driver of the effect). Statistical analysis: One-way ANOVA with Tukey correction.

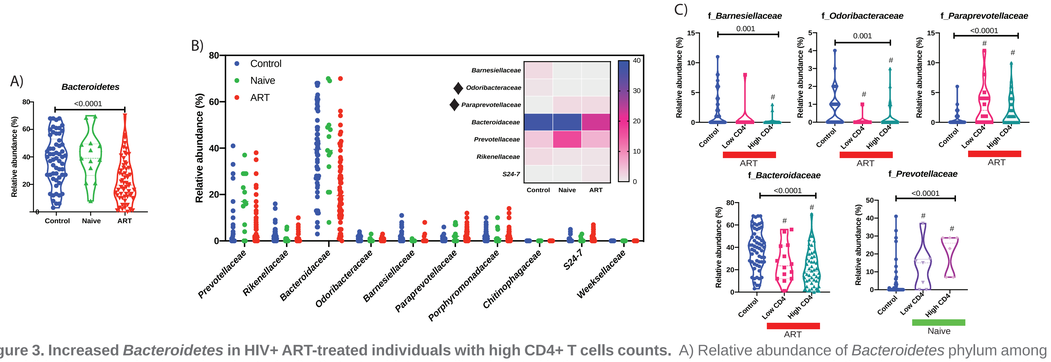


Figure 3. Increased *Bacteroidetes* in HIV+ ART-treated individuals with high CD4+ T cells counts. A) Relative abundance of *Bacteroidetes* phylum among control (blue), HIV+ Naive (green) and ART (red). B) *Bacteroidetes* families detected in the cohorts. Heatmap shows average relative abundance of families that were statistically significant among the cohorts. ♦Designates statistically significant differences in genus abundance (data not shown) C) *Bacteroidetes* families in HIV+ ART-treated individuals with low and high CD4+ T cell counts (# driver of the effect). Statistical analysis: One-way ANOVA with Tukey correction.

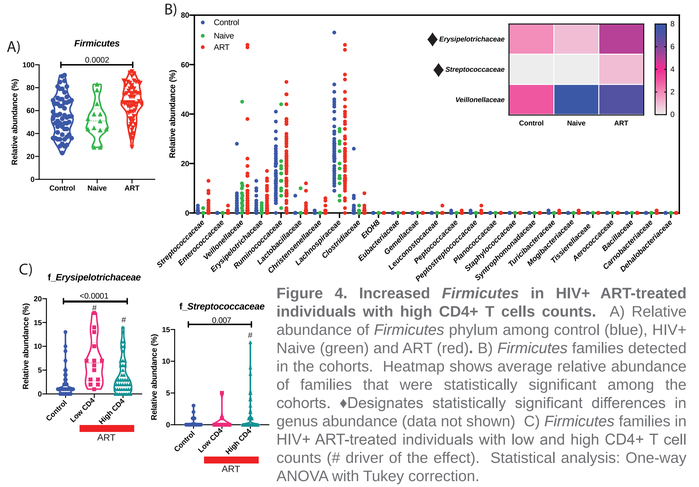


Figure 4. Increased *Firmicutes* in HIV+ ART-treated individuals with high CD4+ T cells counts. A) Relative abundance of *Firmicutes* phylum among control (blue), HIV+ Naive (green) and ART (red). B) *Firmicutes* families detected in the cohorts. Heatmap shows average relative abundance of families that were statistically significant among the cohorts. ♦Designates statistically significant differences in genus abundance (data not shown) C) *Firmicutes* families in HIV+ ART-treated individuals with low and high CD4+ T cell counts (# driver of the effect). Statistical analysis: One-way ANOVA with Tukey correction.

Table 2. Immune mediators

Median (range)	Control	Naive	ART	Statistics
IL-6 (pg/mL)	2.01 (0.2-35)	2.29 (1-5)	2.27 (0.8-8)	
VCAM (ng/mL)	829.93 (327-5292)	843.02 (737-2843)	829.93 (382-1941)	0.0004
TNFRi (pg/mL)	1047.17 (351-1733)	1073.58 (805-1540)	1042.83 (125-4669)	*0.0488
TNFRii (pg/mL)	2797.47 (1314-4678)	2838.21 (1608-5045)	2797.47 (1096-5013)	<0.0001
CRP (ng/mL)	1363.42 (79-31436)	1523.38 (476-25798)	1363.42 (96-61642)	
D-dimer (ng/mL)	339.66 (79-51425)	352.83 (123-2681)	339.66 (179-27337)	
IFAB (pg/mL)	1737.02 (290-8427)	1901.41 (748-5259)	1876.64 (666-10665)	<0.0001
LBP (ng/mL)	13830.91 (6295-45828)	13633.3 (10673-27453)	13830.91 (1485-25676)	*0.0397
sCD14 (ng/mL)	1574.94 (792-2326)	1584.83 (1377-3642)	1587.68 (831-4725)	<0.0001

*Statistically significant with analysis based on CD4 levels

Summary:

- Decreased bacterial diversity (evenness) found in HIV+ Naïve individuals is restored by ART.
- The significant increase in *Actinobacteria* and its families correlates with high CD4+ T cells counts in HIV+ ART-treated individuals.
- The increase in *Firmicutes* is due to an increase in *Erysipelotrichaceae* & *Streptococcaceae* families and is associated with CD4+ T cell counts in HIV+ ART-treated individuals.
- Decreased *Bacteroidetes* is driven by a decrease in *Barnesiellaceae* & *Odoribacteraceae* families. *Prevotella* is increased in HIV+ Naïve individuals and ART restores *Prevotella* levels to control independently of CD4+ T cell counts.
- VCAM, TNFRII, IFABP, and sCD14 are increased in HIV+ individuals independently of CD4+ T cell counts, TNFRI is increased and LBP is decreased in HIV+ ART-treated patients with low CD4 counts.

Acknowledgments: We thank Dr. Jonathan Karn's laboratory for the use of the Qubit instrument, and the Genomics Core Facility of the CWRU School of Medicine's Genetics and Genome Sciences Department. Lastly, we want to thank all the donors that participated in the study.

Funding: National Institute on Drug Abuse (NIDA) [DP1 DA-037997], and [3DP1 DA-037997-04S1], National Institute of Allergy and Infectious Disease (NIAID) Centers for AIDS Research (CFAR) [P30 AI-036219].