ELEVATED FECAL INFLAMMATORY BIOMARKERS IN HIV-/+ MSM ASSOCIATE TO GUT MICROBIOTA

Poster # 264

Jennifer M. Schneider, Sam Li, Janet Seibert, Charles P. Neff,
Nichole Nusbacher, Nancy Moreno-Huizar, Catherine Lozupone, and Brent E. Palmer
Division of Allergy and Clinical Immunology, Department of Medicine, University of Colorado, Aurora, CO

Abstract

Background: HIV replication and bacterial translocation associate with inflammatory biomarkers in the blood, however less is known about the gut. To more directly assess the effect of HIV and gut microbiota on GI inflammation and barrier integrity we measured inflammatory biomarkers in feces. Recent studies have shown pronounced differences in gut microbiome composition between HIV$^-$ men who have sex with women (MSW) and HIV$^-$ men who have sex with men (MSM), but the effects of this altered gut microbiome in MSM on gut inflammation are not understood. Using MSM as a control allows for investigation of contributions of HIV and/or the HIV-associated gut microbiome to gut inflammation by eliminating differences driven by sexual behavior.
Methods: Fecal samples were collected from 72 subjects from: HIV$^-$ low risk (HIV$^-$ LR), high risk (HIV$^-$ HR-MSM), HIV$^+$ ART treated (HIV$^+$ART$^+$) and HIV$^+$ ART naïve (HIV$^+$ART$^-$) subjects. 30 biomarkers of inflammation, innate cell activation and intestinal barrier integrity were measured by ELISA either from the feces itself or water extracted from 2 grams of stool.
Results: There were no significant differences between any of the biomarkers measured in HIV$^-$ HR-MSM and HIV$^+$ subjects. However, 12 biomarkers, primarily inflammatory cytokines (e.g. IL-1b (p=0.0006), IL-12/p23p40 (p<0.0001)) and calprotectin (p=0.009), were elevated in HIV$^+$ART$^-$ compared to HIV$^-$ LR subjects. Except for ICAM-1 (p=0.03) and CRP (p=0.0029), there were no significant differences between HIV$^+$ subjects on or off ART. Seven biomarkers were elevated in HIV$^-$ HR-MSM such as IL-12/23p40 (p=0.001), ICAM-1 (p=0.0003) and sCD14 (p=0.008) compared to HIV$^-$ LR subjects. Fecal IL-12/23p40 (p=0.001) and ICAM-1 (p=0.0005) were elevated in HR-MSM and further increased in HIV$^+$ART$^-$ subjects (p<0.0001, p<0.0001) which correlated to viral load (IL-12/23p40, r=0.42, p=0.015) and CD8$^+$/HLA-DR$^+$/CD38$^+$ T-cells (ICAM, r=0.48, p=0.003) in peripheral blood. We found that in HIV$^-$ LR subjects IL-1α negatively correlated to Bacteroides and positively correlated to Blautia genera. In HIV$^+$ART$^-$ subjects Prevotella correlated with fecal GM-CSF and VEGF, while this effect was not seen in HIV$^-$ HR-MSM suggesting this relationship could be due more to HIV itself.
Conclusions: These data show that gut inflammation is elevated in MSM, and by some measurements exacerbated with HIV infection. Similar to gut microbiome phenotypes, these inflammatory phenotypes are primarily associated with HR-MSM, suggesting that gut inflammation is more impacted by sexual behavior and associated gut microbiome differences than by HIV infection itself. These data highlight the importance of including HIV$^-$ MSM controls in GI inflammation studies.

Background

- The gut microbiome of HIV seronegative subjects in the United States (US) and Europe are typically Bacteroides rich/Prevotella poor while HIV-infected subjects with and without ART are typically Bacteroides poor/Prevotella rich[1].
- Subjects who engage in MSM behavior have a similar gut microbiome as HIV-infected subjects[2].
- The majority of HIV-infected subjects in the US and Europe are MSM, thus the change in microbiome in HIV-infected subjects may not solely be driven by infection[2].
- Gastrointestinal (GI) complications are common among HIV-infected subjects, and they persist with ART[3].
- The affect of an altered gut microbiome composition on GI inflammation is poorly understood.
- Currently, few methods directly quantify intestinal inflammation.

Methods

- Fecal matter was collected from HIV-seronegative at low risk (LR) (20) or MSM at high risk (HR) (18) for infection, or from HIV-infected subjects with (19) or without ART (15).
- Fecal matter was resuspended in a saline solution, homogenized and subjected to ultracentrifugation to remove the insoluble fraction.
- The soluble fraction was filtered and enumerated for levels of biomarkers by ELISA and MesoScale Discovery V-plex kits.
- Analysis was performed using Graph Pad Version 7, Qiime, and Volare.
- Statistical significance was determined using ANOVA Kruskal-Wallis multiple comparisons tests.
  * = p < 0.05, ** = p < 0.005, *** = p < 0.0005.

Results

16s rRNA Taxa Bar Chart of Whole Stool

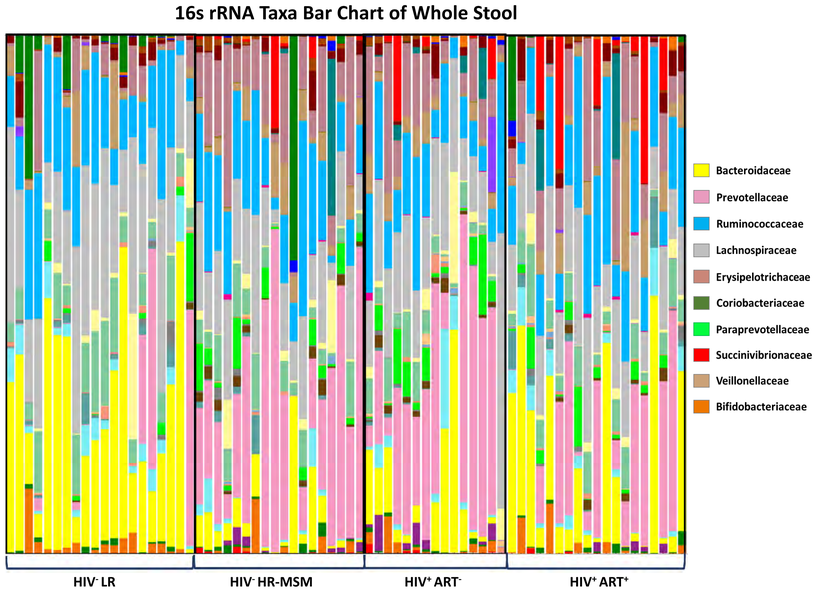

Fig 1. 16s rRNA sequences (V4 region) were generated from stool using the MiSeq platform. The composition of the bacterial communities from HIV$^-$ LR, HIV$^-$ HR-MSM, HIV$^+$ART$^-$, and HIV$^+$ART$^+$ were analyzed using QIIME. Samples were rarified at 13733 sequences, and OTUs not present in at least 20% of samples were filtered out.

HIV$^-$ HR-MSM and HIV-Infected Subjects Cluster Together and Apart From HIV$^-$ LR Subjects

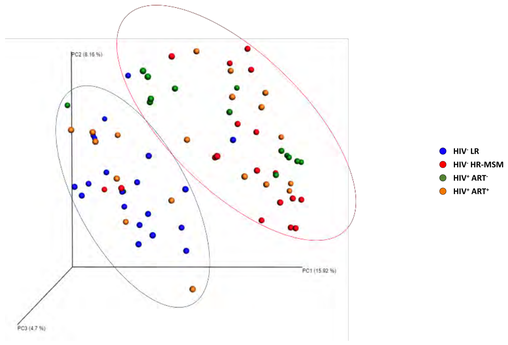

Fig 2. Unweighted Principal Coordinates Analysis (PCoA) of 16s rRNA sequences generated from stool of HIV$^-$ LR, HIV$^-$ HR-MSM, HIV$^+$ART$^-$, and HIV$^+$ART$^+$ subjects.

Cell Adhesion Molecules, ICAM-1 and VCAM-1, and the Growth Factor VEGF Are Increased in HIV$^-$ HR-MSM and HIV$^+$ Subjects and Correlate With CD4$^+$ T-cell Count

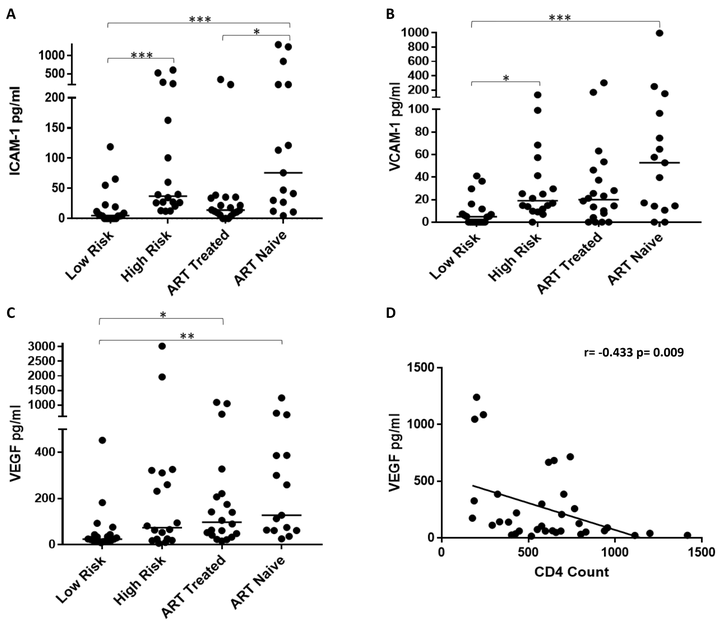

Fig 3. Levels of adhesion molecules, ICAM-1 **(A)** VCAM-1 **(B),** in fecal water from HIV-infected and uninfected subjects. **(C)** Levels of the growth factor VEGF in fecal water from HIV-infected and uninfected subjects. **(D)** Peripheral CD4$^+$ T-cell counts in HIV-infected subjects correlated to levels of fecal VEGF.

Levels of Inflammatory Biomarkers in Fecal Water Samples

Cytokines	HIV$^-$ LR vs HR	HIV$^-$LR vs HIV$^+$ART$^+$	HIV$^-$LR vs HIV$^+$ART$^-$	HIV$^-$HR vs HIV$^+$ART$^-$ or ART$^+$	HIV$^+$ART$^-$ vs ART$^+$
IL-1β			***		
IL-7	**	*			
IL-12/23p40	***	**	****		
IL-15		**	***		
TNF-β	*	***	**		
Chemokines					
GM-CSF	*	***	***		
IL-8			*		
IL-16		***	*		
Inflammatory Products					
sCD14	**				
Calprotectin			**		
CRP					**
Protective					
IL-22		*	*		

Fig 4. Fecal water was analyzed using ELISA and MesoScale Discovery V-plex kits. Comparisons between high risk vs ART naïve and high risk vs ART treated were ns. All biomarkers tested were elevated in comparison to HIV$^-$ LR with the exception of IL-22 which decreased in HIV$^+$ subjects. Statistical significance was determined by Kruskal-Wallis multiple comparisons test (6 comparisons). * <0.05 ** <0.01 *** <0.001 **** <0.0001

Fecal Microbiome Genera Correlate To IL-1α Levels in HIV$^-$ LR Subjects

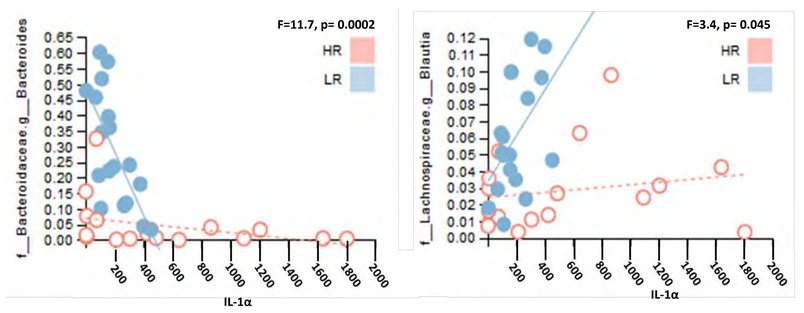

Fig. 5. HIV seronegative LR subjects show increased relative abundance of Bacteroides genus and is associated with lower fecal IL-1α, while seronegative HR-MSM subjects show less of this effect **(Left)**. HIV seronegative LR subjects show increased relative abundance of Blautia and is associated with higher fecal IL-1α, while seronegative HR-MSM subjects show less of this effect **(Right)**.

Fecal Microbiome Genera Correlate To GM-CSF and VEGF Levels in HIV$^+$ART$^-$ Subjects

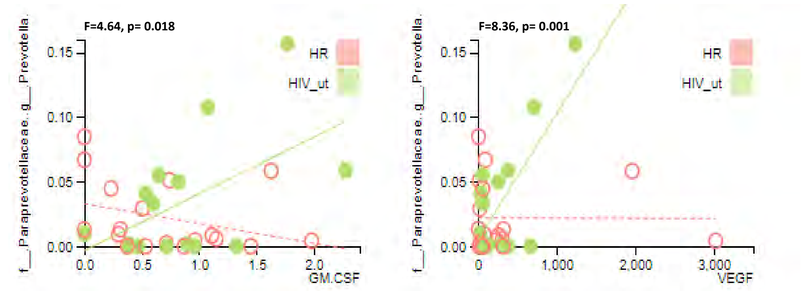

Fig. 6. HIV$^+$ ART$^-$ subjects show increased relative abundance of Prevotella genus and is associated with higher fecal GM-CSF, while seronegative HR-MSM subjects show the opposite of this effect **(Left).** Prevotella genus in HIV$^+$ART$^-$ subjects is associated with fecal VEGF, while seronegative HR-MSM subjects do not show this effect **(Right).**

Conclusions

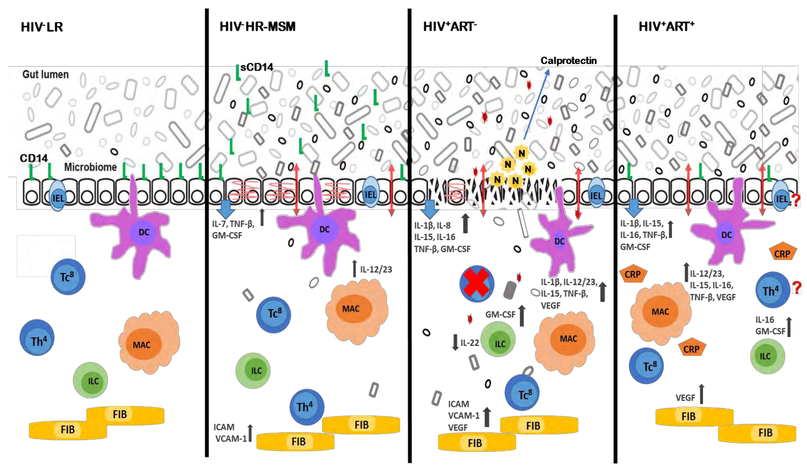

Fig 7. Schematic of inflammatory biomarkers in the gastrointestinal system of our four cohorts.

- HIV$^-$ HR-MSM have increased inflammatory biomarkers similar to HIV$^+$ subjects with or without ART, but are distinguished in that sCD14 shedding was only detected in this group.
- HIV-infected subjects have a marked increase in inflammatory biomarkers that are not entirely resolved with ART therapy. Calprotectin is a distinguishing factor between ART-/+ subjects while CRP was only detected in ART$^+$ subjects indicating therapy may be inflammatory in and of itself.
- Fecal inflammatory markers are correlated to specific bacteria genera, illustrating the impact of dysbiosis on immune activation.

References

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