# Dysbiotic Bacteria Translocate in Progressive SIV Infection

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## INTRODUCTION

HIV infection in humans and pathogenic SIV infection in Asian macaques lead to the development of chronic inflammation and immune activation. This chronic activation of the immune system is associated with disease progression and non-viral comorbidities that include cardiovascular disease, neurologic disorders and increased mortality even in ARV-treated individuals. Indeed, immune activation has been shown to be a better predictor of disease outcome then either peripheral blood CD4+ T-cell count or viral load.

The mechanisms that drive immune activation in HIV/SIV infection are still poorly understood, but one cause is microbial translocation. The gastro-intestinal tract represents the largest mucosal immune organ in the body and carries a very large percentage of the body's immune cells. This concentration of immune defenses at the gut mucosa is likely due to the need to contain and potentially respond to the large microbial mass within the gut. In HIV or SIV infection the earliest site of viral replication and CD4+ T-cell depletion is in the gut. Viral replication in the gut correlates with increasing damage to the tight junctions of the intestinal epithelium. This damage to the barrier, which separates the intestinal microbiome from the body, allows translocation of microbial products. The presence of bacterial products in the blood is significantly increased in HIV and SIV infection.

The intestinal microbiome is a complex and multifaceted community of bacteria. It is made up of over 200 species of bacteria and has roughly the same metabolic output as the liver. The makeup of this bacterial community varies along the length of the intestines and changes in its makeup have been associated with various disease states. Although some work has been done trying to evaluate HIV or SIV driven changes in the gut microbiome, a clear picture of how the virus affects the gut is still elusive. Single time point examinations of the gut microbiome in SIV infected Asian macaques suggest little to no changes in the microbiome due to SIV infection. However, several studies have shown alterations of the microbiome in HIV infection in humans.

In this study we utilize experimental infection of Asian macaques (both pigtail and rhesus) with pathogenic SIV to query changes in the gut microbiome over time and in response to treatment with anti-retroviral drugs. We then further explore the question of what bacteria escape from the gut and account for the observed microbial products in the periphery by sequencing bacterial 16S DNA isolated from different anatomical sites.

## RESULTS

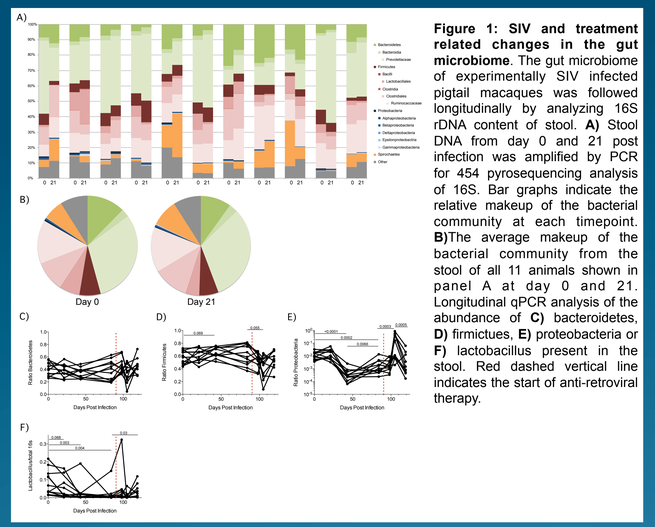



**Figure 1: SIV and treatment related changes in the gut microbiome**. The gut microbiome of experimentally SIV infected pigtail macaques was followed longitudinally by analyzing 16S rDNA content of stool. **A)** Stool DNA from day 0 and 21 post infection was amplified by PCR for 454 pyrosequencing analysis of 16S. Bar graphs indicate the relative makeup of the bacterial community at each timepoint. **B)**The average makeup of the bacterial community from the stool of all 11 animals shown in panel A at day 0 and 21. Longitudinal qPCR analysis of the abundance of **C)** bacteroidetes, **D)** firmictues, **E)** proteobacteria or **F)** lactobacillus present in the stool. Red dashed vertical line indicates the start of anti-retroviral therapy.

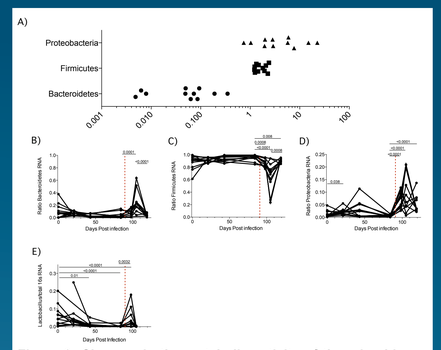



**Figure 2: Changes in the metabolic activity of the microbiome.** The metabolic activity of the gut microbiome of experimentally SIV infected pigtail macaques was followed longitudinally by analyzing 16S RNA content of stool via RT-qPCR. **A)** The ratio of RNA/DNA for each of the three major phyla (bacteroidetes, firmicutes and proteobacteria) for all animals prior to infection. Longitudinal RT-qPCR analysis of the relative metabolic activity of **B)** bacteroidetes, **C)** firmictues, **D)** proteobacteria or **E)** lactobacillus present in the stool as determined by measuring RNA. Red dashed vertical line indicates the start of anti-retroviral therapy.

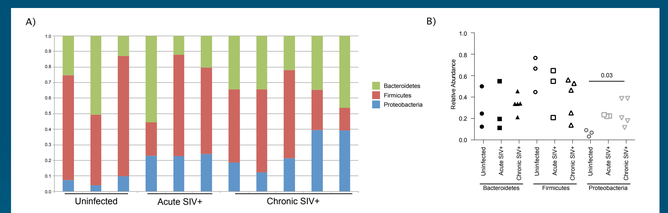



**Figure 3: Analysis of bacteria present in the mesenteric lymph node of SIV infected Rhesus macaques.** DNA was extracted from the mesenteric lymph nodes of rhesus macaques characterized as uninfected, acutely SIV infected or chronically SIV infected. The relative abundance of bacteroidetes, firmicutes and proteobacteria was determined by qPCR for 16s rDNA sequence. **A)** Bar graph showing the community structure of bacteria from the mesenteric lymph node for each of the animals. **B)** Dot plot showing the relative abundance of each phyla as in panel A.

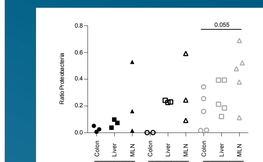



**Figure 4: Abundance of tissue-associated proteobacteria is different than the colon.** The relative abundance of proteobacteria in the colon, liver and mesenteric lymh nodes (MLN) of uninfected, acutely SIV infected or chronically SIV infected Rhesus macaques was determined by qPCR for proteobacteria 16s rDNA.

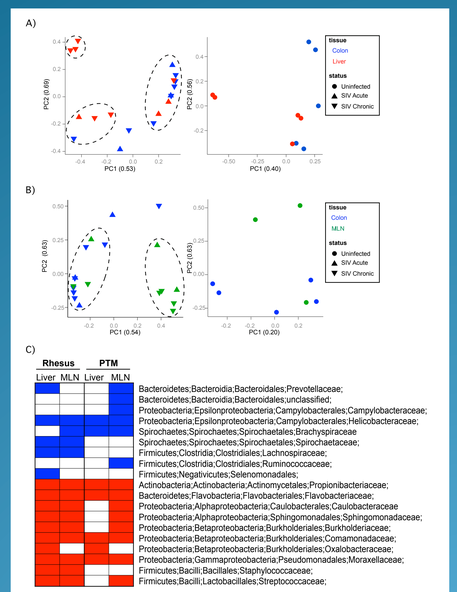



**Figure 5: Specific families of proteobacteria translocate from the gut.** DNA extracted from the colon, liver and mesenteric lymph nodes of uninfected and experimentally SIV infected Rhesus macaques was amplified using bacterial 16s primers tagged for 454 pyro-sequencing analysis. **A)** Principal component analysis showing differences in the bacterial communities of the colon (blue) and liver (red) in SIV infected (left panel) and uninfected (right panel) animals. **B)** Principal component analysis showing differences in the bacterial communities of the colon (blue) and mesenteric lymph node (green) in SIV infected (left panel) and uninfected (right panel) animals. **C)** A metastat analysis was performed to determine which bacteria were present at significantly different abundance in either the liver or mesenteric lymph node compared to the colon of all Rhesus and Pigtail macaques used in the study. The graph shows bacterial families that are significantly under represented (blue) or over represented (red) in the tissues of each animal group.

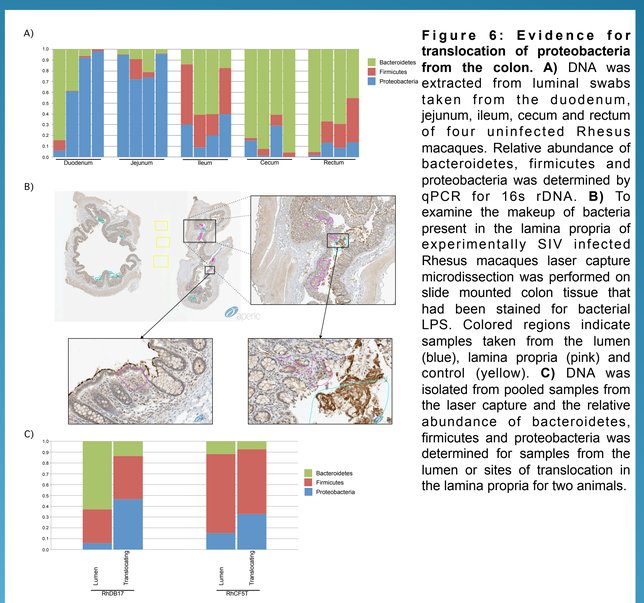



**Figure 6: Evidence for translocation of proteobacteria from the colon. A)** DNA was extracted from luminal swabs taken from the duodenum, jejunum, ileum, cecum and rectum of four uninfected Rhesus macaques. Relative abundance of bacteroidetes, firmicutes and proteobacteria was determined by qPCR for 16s rDNA. **B)** To examine the makeup of bacteria present in the lamina propria of experimentally SIV infected Rhesus macaques laser capture microdissection was performed on slide mounted colon tissue that had been stained for bacterial LPS. Colored regions indicate samples taken from the lumen (blue), lamina propria (pink) and control (yellow). **C)** DNA was isolated from pooled samples from the laser capture and the relative abundance of bacteroidetes, firmicutes and proteobacteria was determined for samples from the lumen or sites of translocation in the lamina propria for two animals.

## CONCLUSIONS

- No significant changes in the gut microbiome were observed in acutely SIV infected macaques.
- Longitudinal analysis of of the gut microbiome revealed changes in the abundance of the phyla proteobacteria and the genus lactobacillus after SIV infection.
- Treatment of animals with anti-retrovirals (20-30 mg/kg PMPA i.v., 30mg/kg FTC i.v., 240 mg L'812 – daily) induced marked changes in the gut microbiome.
- Proteobacteria is the most metabolically active bacteria in the feces, followed by firmicutes and the bacteroidetes.
- SIV infection increased the abundance of the proteobacteria phyla found in the mesenteric lymph node.
- Pyro-sequencing analysis confirmed that the bacterial community in the liver and mesenteric lymph node of SIV infected macaques was different from the colon.
- Increase in the abundance of proteobacteria in the peripheral tissues of SIV infected animals appears to be due to translocation of specific families of bacteria.
- Laser capture micro-dissection revealed that the bacteria present in the colonic lamina propria of SIV infected animals already showed a preference for proteobacteria.

***These results suggest that not all gut bacteria translocate from the gut after damage to the epithelial barrier in SIV infection. Instead, specific bacteria are more capable of expanding into the new niche available after the loss of membrane integrity.***