# Enema use minimally impacts immunity and does not affect susceptibility to low dose intrarectal SIV. #00364

Alexandra M. Ortiz[1], Fabiola Castello Casta[1], Brandon F. Keele[2], and Jason M. Brenchley[1].

[1]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD, USA, [2]AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

## Introduction

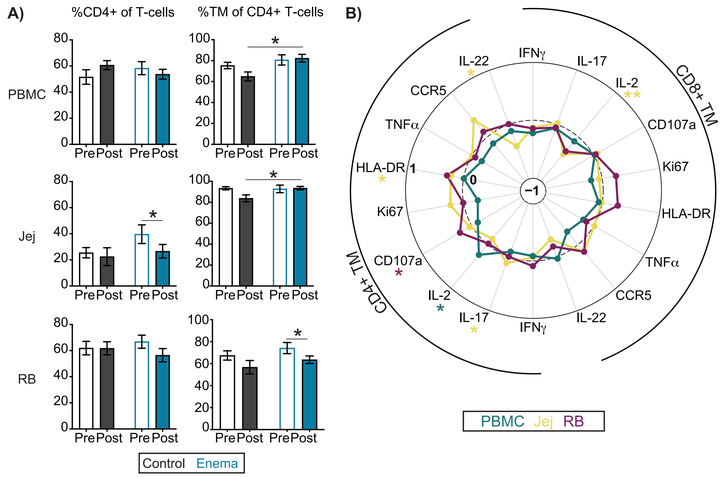
- Men who have sex with men (MSM) are disproportionately affected by HIV.
- Increased susceptibility to HIV in MSM is attributed to high frequencies of susceptible CD4+ T-cells, gastrointestinal epithelial barrier disruption, and dysbiosis of the intestinal microbiome.
- Although colorectal epithelial barrier damage is due, in part, to inflammation resulting from receptive anal intercourse, rectal douching has also been proposed as a significant contributor.
- **The effects of repeated enema use on the composition of the GI tract microbiome, intestinal immunity, and susceptibility to rectal HIV acquisition have not been empirically assessed.**

## Methods

- We administered enemas (Normosol-R) to rhesus macaques (Control/Enema, n=6/6) thrice-weekly, prior to repeated low-dose intra-rectal SIVmac239X challenge.
- Before and 28 days after enema treatment initiation (n=9 enemas) we assessed the fecal microbiome by 16S Illumina sequencing and surveyed intestinal and systemic immunity by flow cytometry, multiplex immunologic transcript quantification (NanoString), and ELISA.
- Fifty-six days after enema initiation (n=24 enemas) we challenged animals with 4 TCID50 SIVmac239X until infection was confirmed.

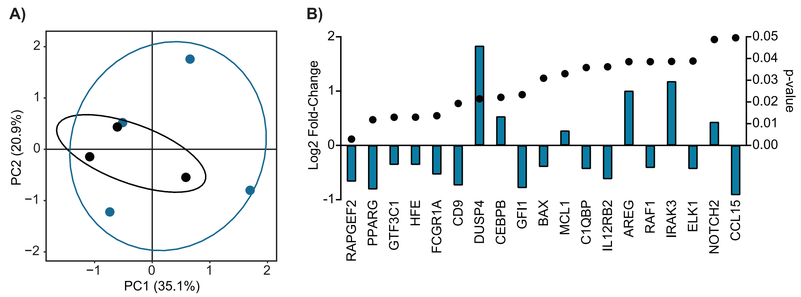
## Results

**Figure 1. Enema use in macaques is associated with modestly altered CD4+ and CD8+ T-cell phenotype and function.**




**A)** CD4+ (left) and CD4+ memory T-cell (TM) frequencies in PBMCs, and jejunal (Jej) and rectal biopsies (RB) of Control and Enema-treated rhesus macaques before (Pre) and 28 days into treatment (Post). **B)** log2 fold-change from baseline expression of cytokines and markers of activation in PBMC, Jej, and RB CD4+ and CD8+ TM from Enema-treated macques. Bar heights and data lines represent means. n=4-6 biological replicates. Significance was assessed by two-way, unpaired or paired t-test as appropriate.* p<0.05, ** p<0.01

**Figure 2. Enema use in macaques minimally alters the rectal immunologic transcriptome.**



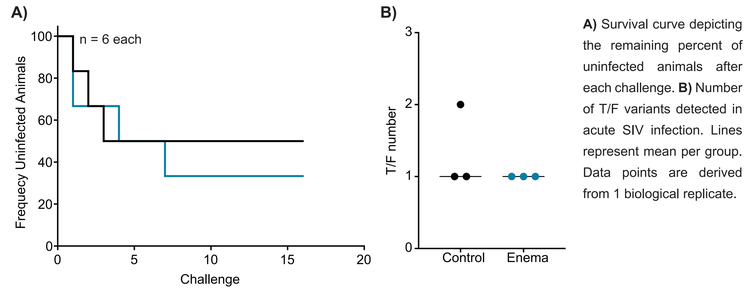

**A)** PCA analysis considering NanoString-enumerated transcripts from Control and Enema-treated rectal homogenates 28 days into treatment. Samples are colored as in Figure 1. **B)** Differentially abundant transcripts identified by NanoString in Enema-treated animals (Post:Pre-treatment). Transcripts are ordered by ascending p-value, with significance values shown in circles, aligned to the right axis. Mean transcript expression fold-change values are shown by bars, aligned to the left axis. n=3-5 biological replicates.

**Figure 3. Enema use in macaques perturbs the intestinal fecal bacterial microbiome.**




**A)** Relative frequencies of fecal amplicon sequence variants (ASVs) identified as differentially abundant by ANCOM-BC, between Control and Enema-treated macaques 56 days into treatment. Data show Enema:Control frequency log fold-change, color-grouped by phylum and individually shaded by family. **B)** Principal coordinate analysis (PCoA) of weighted UniFrac bacteria β-diversity 56 days into treatment in Control and Enema-treated macaques. **C)** Fecal Prevotella frequencies (left) and Bacteroides:Prevotella ratios (right) in Enema-treated macaques before and 56 days into treatment. n=3 biological replicates.

**Figure 4. Enema treatment in macaques does not significantly increase susceptibility to low-dose intrarectal SIV infection.**




**A)** Survival curve depicting the remaining percent of uninfected animals after each challenge. **B)** Number of T/F variants detected in acute SIV infection. Lines represent mean per group. Data points are derived from 1 biological replicate.

## Conclusions and Future Directions

- **Routine enema use in macaques minimally alters intestinal T-cell frequency, phenotype, and function.**
- **Enema use in macaques is associated with modest perturbations of the fecal microbiome, including taxa previously associated with susceptibility to rectal HIV acquisition.**
- **Routine enema use is not associated with increased susceptibilty to low-dose intra-rectal SIV acquisition.**
- **Insight gained from our comprehensive study will inform the causes and consequences of repeated enema usage in MSM and will inform the design of improved bowel-clearing preparations for sexual and surgical use.**

National Institutes of Health