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Uracil treatment does not increase susceptibility to rectal SIV infection.

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Introduction

- The intestinal bacterial microbiome of men who have sex with men (MSM) is distinct as compared to non-MSM and is suggested to increase susceptibility to rectal HIV infection.
- We recently demonstrated that bacterial dysbiosis-associated disruptions in host antimicrobial immunity increase susceptibility to rectal SIVmac239X in rhesus macaques (RMs; *macaca mulatta*).
- Central to the dysbiosis-associated disruptions in host antimicrobial immunity was DUOX2 – a NADPH oxidase that catalyzes reactive oxygen species (ROS) production upon sensing bacterial pathobiont-derived uracil.

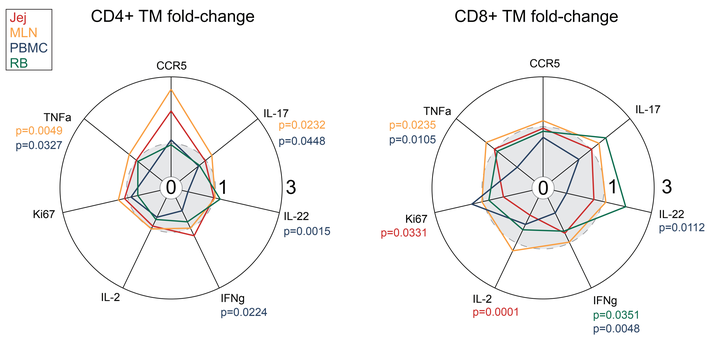
We hypothesize that luminal uracil bioavailability may increase susceptibility to rectal HIV.

Methods

- RMs were treated daily with (n=6) or without (n=8) oral uracil at 250 mg/m^2.
- Blood, mesenteric lymph nodes (MLN), stool, and intestinal biopsies were taken before and 28 days into treatment.
- Animals were challenged intra-rectally with 4 TCID50 of SIVmac239X 56 days into treatment and every 2 weeks thereafter.
- We assessed intestinal immunity by flow cytometry and high throughput quantitative RT-PCR (NanoString), the intestinal microbiome by 16S Illumina sequencing, and fecal uracil concentrations by LC-MS.

Results

Figure 1. Oral uracil treatment induces significant alterations in systemic, adaptive immunity.
- Changes in cytokine production are widespread in the the periphery but limited in intestinal tissue.



Radar plots depicting fold-change from baseline (Day 28 post- versus pre-uracil) expression of peripheral blood (PBMC), MLN, jejunal (Jej), or rectal biopsy (RB) CD4+ TM (left) and CD8+ TM (right) activation markers and PMA/ionomycin stimulation-induced cytokines. Significance refers to pre- versus post-uracil marker expression, color-coded by tissue.

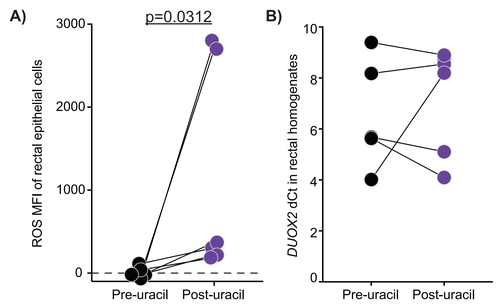
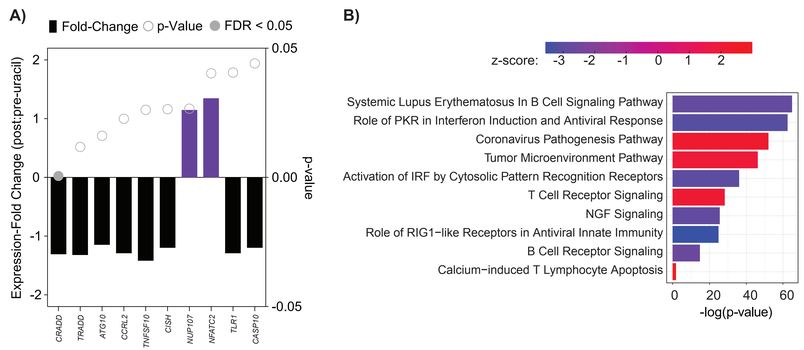


Figure 2. Rectal, epithelial ROS production is significantly increased in response to oral uracil treatment.
- Rectal *DUOX2* transcripts remain unaltered.

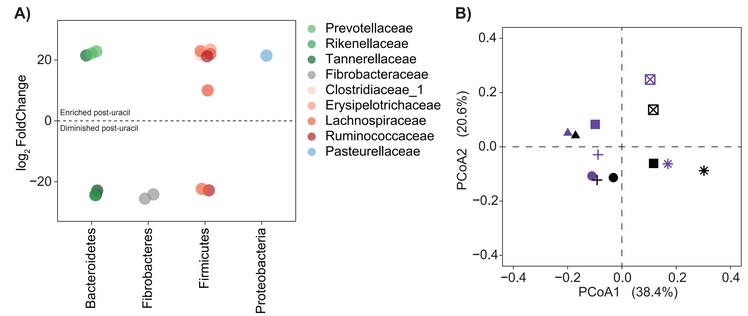
A) Median fluoresence intensity of ROS (CellRox™) in stained rectal epithelial cells. Epithelial cells defined by flow cytometery as Live, CD45-CD31-EPCAM+ cells. **B)** dCT of *DUOX2* in rectal homogenates assessed by quantitative RT-PCR. Ct values normalized to housekeeping gene *mGusB*.

Figure 3. Oral uracil treatment induces modest changes in the rectal transcriptome.
- These changes include reductions in apoptosis-signaling transcripts and decreases in antiviral pathway activation.



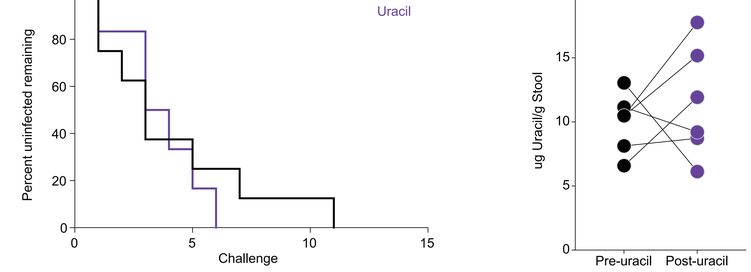
A) Fold-change expression (left axis) and p-values (right-axis) of rectal immunological transcripts identified as differentially abundant in response to uracil treatment, as quantified by Nanostring. Bars are color-coded by enriched group's treatment status as in Figure 2. **B)** Ingenuity pathway analysis of canonical pathways enriched in post-uracil rectal homogenates, relative to pre-treatment. Data include top 10 differentially regulated pathways with p<0.05 and z-score ≥ |±2|. Pathways are color-coded by z-score as shown.

Figure 4. Oral uracil treatment induces modest changes in the fecal microbiome.



A) Fold-change frequencies of ASVs identified as differentially abundant in response to uracil treatment. Data show post-uracil:pre-uracil frequency fold-change, color-grouped by phylum and individually shaded by family as indicated. **B)** Weighted UniFrac beta diversity estimates for pre- and post-uracil. Samples are color-coded by treatment status as in Figure 2, with each symbol representing an individual animal.

Figure 5. Oral uracil treatment does not increase susceptibility to rectal SIV infection.
- Fecal uracil concentrations do not increase after oral uracil treatment.



A) Kaplan-Meier curve depicting the percent of uninfected macaques relative to challenge number. **B)** Fecal uracil concentrations as determined by LC-MS. Samples are color-coded by treatment status as in Figure 2

Conclusions and Future Directions

- Oral uracil treatment induces significant shifts in systemic T-cell activation, with limited evidence of perturbed intestinal T-cell activation.
- Oral uracil treatment significantly increases rectal epithelial ROS production, but only modestly perturbs the intestinal transcriptome and microbiome.
- Oral uracil treatment does not increase susceptibility to low-dose, rectal SIV challenge.
- **Uracil bioavailability remains low in the intestinal lumen following oral treatment. Alternate delivery methods are necessary to assess the effect of intestinal uracil bioavailability on intestinal immunity, the intestinal microbiome, and susceptibility to rectal SIV infection.**