Compared with men who do not engage in anal intercourse, the rectal mucosa of MSM engaging in CRAI showed increased abundance of neutrophils in the lamina propria and increased proliferation of the crypt epithelium. These findings were not clearly associated with the Prevotella rich microbiota seen among MSM.

**RESULTS:**

**Figure 2.** In order to further investigate the cellular source of the IL-17 and FOXP3 expression, we conducted dual staining by IHC of these markers with CD4. Representative images are shown demonstrating co-localization of FOXP3 and CD4 but no co-localization of CD4 and IL-17. We conclude that our FOXP3 staining does represent Treg cells (black arrows) and the IL-17 staining does not represent Th17 cells but rather other IL-17 producing cells, possibly innate immune cells, in rectal mucosa.

**Figure 3.** The distributions of the 5 biomarkers in the crypt epithelium (Ki67 and e-cadherin) or adjacent lamina propria (MPO, FOXP3, IL-17) are shown for MSM engaging in CRAI (red) and men who never engaged in AI (controls; black). Overall, expression of Ki67 in the crypt epithelium and MPO in the lamina propria was significantly higher among MSM engaging in CRAI compared to controls in linear mixed effects models controlling for age, race, and visit number. There were no differences between visit 1 and 2 for MSM engaging in CRAI suggesting that timing of CRAI did not influence these results.

**Figure 4.** We have previously reported enrichment for Prevotellaceae over Bacteroidaceae among MSM engaging in CRAI. However, we did not detect associations between the global microbiota or individual genera and the 5 biomarkers using LDM models controlling for multiple comparisons.

**CONCLUSIONS:**

- The increased epithelial proliferation and abundance of neutrophils in the rectal mucosa seen in this study likely represents an injury response to microtrauma, and/or semen exposure, and/or product use (e.g. lubricants, enemas, etc.) during CRAI, expanding upon our previous work.
- We hypothesize that this unique immune environment in the rectal mucosa of MSM engaging in CRAI could influence HIV transmission or immune responses to candidate HIV vaccine.
- Future research will be needed to understand rectal mucosal interactions with the microbiota among MSM and any potential effects of the mucosal injury response to CRAI on HIV transmission.

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