Condomless Receptive Anal Intercourse is Associated with Markers of Mucosal Injury

Poster 0213

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BACKGROUND:

- The risk of HIV transmission per exposure event for receptive anal intercourse is 1.38%, more than 18-fold higher than other routes of sexual transmission.
- We have previously demonstrated a unique rectal mucosal immune environment among men who have sex with men (MSM) engaging in condomless receptive anal intercourse (CRAI) compared to men who do not engage in anal intercourse typified by:
  - Increased abundance of Th 17 cells, proliferating CD8 cells, and pro-inflammatory cytokine production by CD8 cells
  - mRNA gene signatures associated with mucosal injury/repair and neutrophil activity
  - A microbiota enriched for *Prevotellaceae* over *Bacteroidaceae*
- Our prior flow cytometry approaches were unable to capture the tissue distribution of the cells or examine the crypt epithelium of the rectal mucosa, both of which could be important determinants of HIV transmission.

METHODS:

- We enrolled a cohort of 41 HIV negative, healthy MSM engaging in CRAI and 21 men who never engaged in AI for rectal mucosal sampling over 2 study visits. MSM engaging in CRAI were asked to abstain from CRAI for 72 hours prior to visit 1 and to engage in CRAI within 24 hours of visit 2.
- Expression of MPO (neutrophils), IL-17 (mucosal inflammatory cells), and FOXP3 (T_{regs}) in the lamina propria and Ki67 (proliferation) and e-cadherin (adherens junction) of the crypt epithelium were measured by standardized, quantitative immunohistochemistry and image analysis (qIHC/qIA).
- The microbiota was characterized by 16s rRNA sequencing.
- Linear effects models were used to examine differences in biomarker expression between study groups over time. A linear decomposition model was constructed to examine associations between the biomarkers and microbiota.

Characteristic	MSM engaging in CRAI (n=41)	Men never engaged in AI (n=21)	P-value
Median age in yrs (25^{th},75^{th})	28 (26, 34)	24 (24, 30)	0.02
Race n (%)			
White	33 (80.5)	14 (66.7)	-
Black	6 (14.6)	2 (9.5)	-
Other	2 (4.9)	5 (23.8)	0.11
Lubricant use n(%)	39 (95.1)	NA	-
Enema use n(%)	18 (43.9)	NA	-
Median CRAI episodes in previous month (25^{th}, 75^{th})	5.00 (5.00, 8.00)	NA	-

*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 propia** and **increased proliferation of the crypt epithelium**. These findings were not clearly associated with the Prevotella rich microbiota seen among MSM.*

RESULTS:

Figure 1. Tissue was embedded in paraffin blocks and stained with antibodies for the 5 biomarkers by qIHC/qIA. A precise scoring method was developed for colonic hemicrypts (epithelial markers) or the area adjacent to the hemicrypt (lamina propria markers). A minimum of 3 hemicrypts were scored per participant visit. The optical density of the biomarkers was measured by custom software along the length of the scored area.

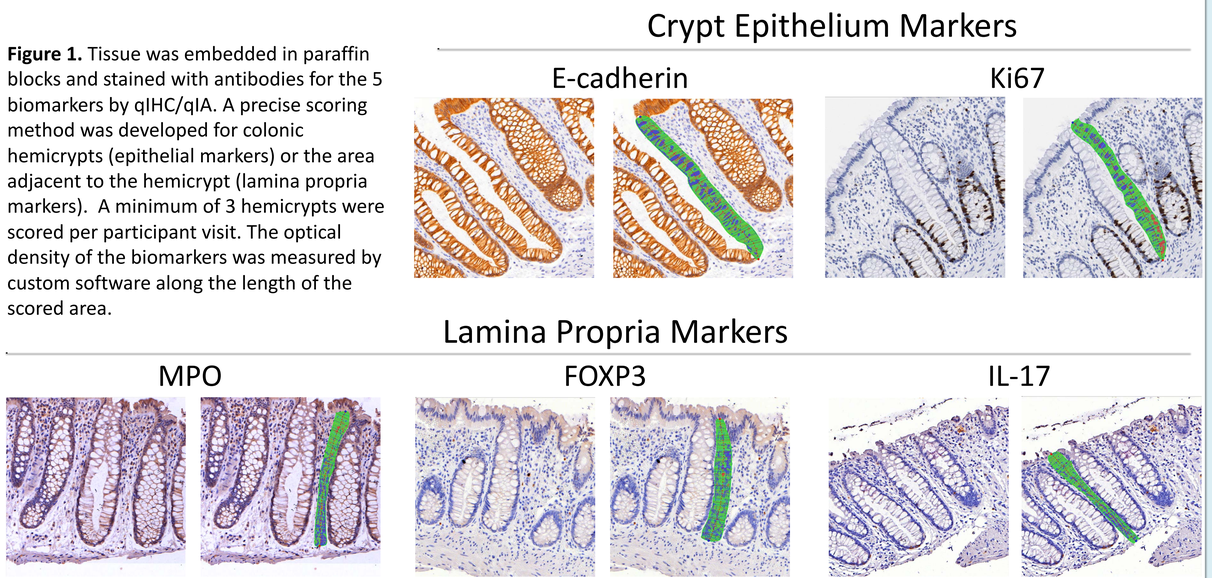
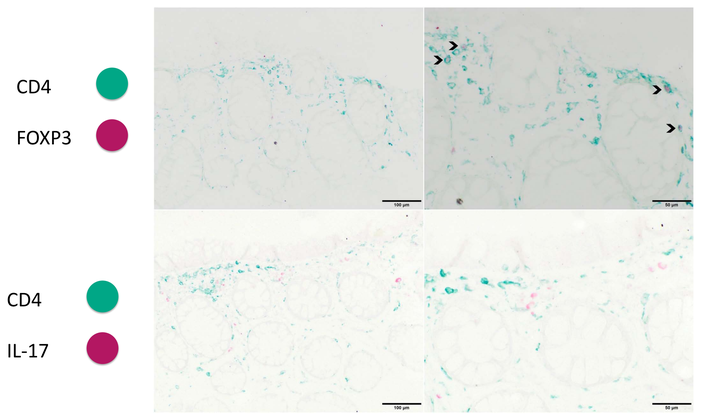


Figure 2. In order to further investigate the cellular source of the IL-17 and FOXP3 expression, we conducted dual staining by IHC of these markers with CD4. Representative images are shown demonstrating co-localization of FOXP3 and CD4 but no co-localization of CD4 and IL-17. We conclude that our FOXP3 staining does represent T_{regs} (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.



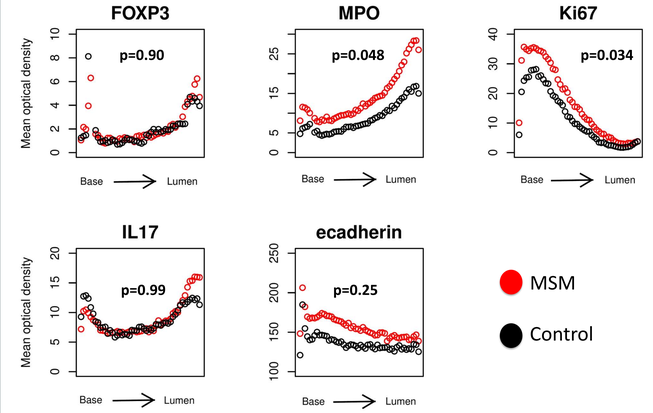


Figure 3. The distributions of the 5 biomarkers in the crypt epithelium (Ki67 and e-cadherin) or adjacent lamina propria (MPO, FOXP3, IL-17) are shown for MSM engaging in CRAI (red) and men who never engaged in AI (controls; black). Overall, expression of Ki67 in the crypt epithelium and MPO in the lamina 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.

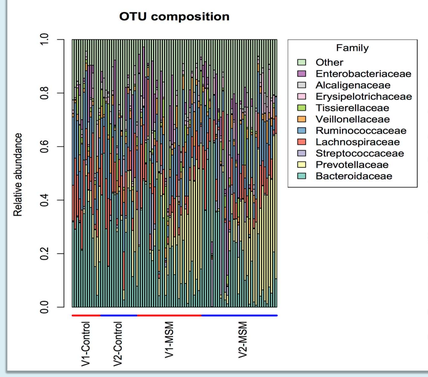


Figure 4. We have previously reported enrichment for *Prevotellaceae* over *Bacteroidaceae* among MSM engaging in CRAI. However, we did not detect associations between the global microbiota or individual genera and the 5 biomarkers using LDM models controlling for multiple comparisons.

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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