Defining Interactions of Human Immunodeficiency Virus -1 (HIV) with Rectal Epithelial Barriers

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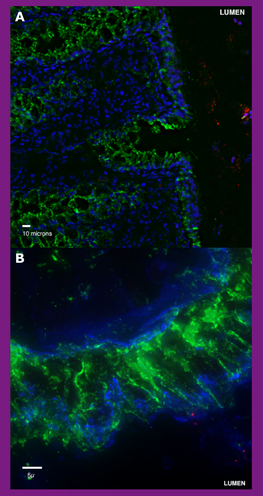
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Background

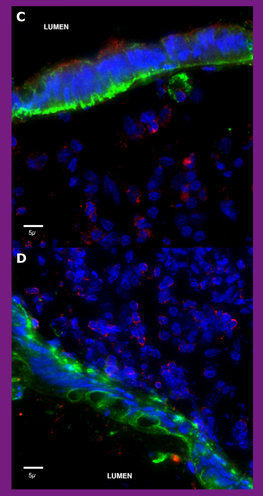
A major mode of HIV transmission is through receptive anal intercourse with an infected individual. How HIV interacts with the rectal mucosal barriers composed of columnar epithelium covered with a protective layer of mucus has yet to be defined. Therapies that affect the rectal mucosal barrier may play an important role in HIV prevention strategies. We sought to investigate how HIV interacts with the rectal epithelium and the role rectal mucus plays in these interactions using a human rectal biopsy model, in vivo challenges in rhesus macaques, and studies of rectal mucus.

Methods

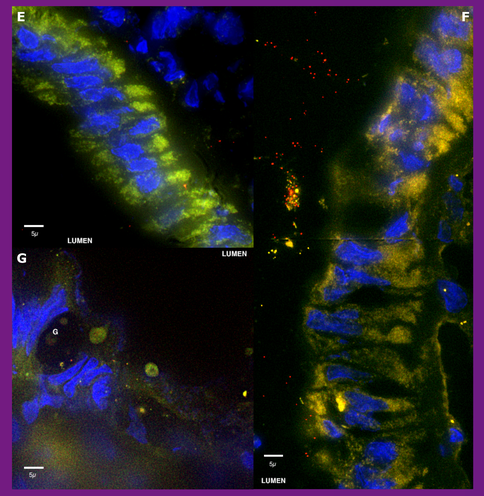
We enrolled 10 HIV seronegative adults undergoing routine screening colonoscopy. 4 rectal biopsies were obtained per patient and transferred to our lab. Tissues were inoculated with a photoactivatable (PA) Green fluorescent protein (GFP) HIV-1 for 1 and 2 hours. Samples were also treated with a mucolytic enzyme, neuraminidase (NA). In vivo challenges in rhesus macaques used the same virus. Animals were necropsied 4 hours post exposure. Human and animal tissues were similarly snap frozen in optimal cutting temperature (OCT) compound, sectioned, and stained with fluorescent antibodies for CD209, CD4, or other cellular markers. Epifluorescent deconvolution images were taken with a Delta Vision RT system and analyzed with SoftWorx software.



Fluorescent nanobead trapping in the rectal mucus: Fluorescent beads applied intrarectally in vivo to Rhesus macaques. (A) Nanobeads (red) seen in the lumen of the rectal compartment, generally above areas protected by adherens junctions (E-cadherin, green). Cell nuclei in blue, 20x. (B) Nanobeads (red) seen trapped in mucus (stained with wheat germ agglutinin (WGA, blue). E-cadherins shown in green. 100x.



Target cell staining in the human rectal compartment: (C) CD209+ dendritic cells (red) seen in close proximity to the epithelial surface. (D) CD4 cells (red) also abundantly seen. E-cadherin (green) and cell nuclei (blue). Images taken at 40x



PA GFP HIV -1 Interactions within the rectal compartment. (E) HIV-1 virions (red) seen penetrating rectal columnar epithelium in *ex vivo* human tissue cultures. (F) Virions (red) in a panel image are seen trapped in cellular debris above the human rectal compartment. (G) Goblet cells are associated with delivery of intraluminal antigen to underlying dendritic cells. Virion (red) seen here inside a goblet cell (labeled with G) in our *in vivo* macaque rectal tissue experiments. In all images, tissue background is shown in yellow (green and red overlay), and cell nuclei is shown in blue, 100X.

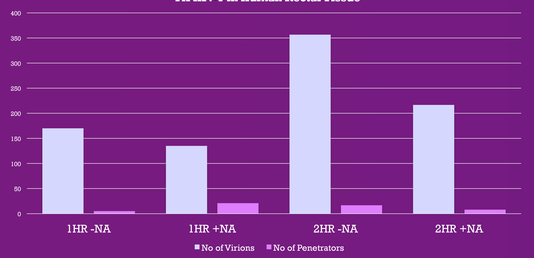
Results

Human rectal biopsy tissues incubated at 1 and 2 hours with an intact mucus layer were associated with the highest number of virions, most of which were seen trapped in overlying mucus. Accordingly, explants treated with NA (which decreased the amount of mucus at the surface) did not show the associated trapping of HIV. HIV penetration of the rectal mucosal barrier was observed as early as after 1 hour of incubation. Penetrators were defined as virions entering more than 1µm into the epithelium. Penetration was more commonly seen in areas where epithelial integrity was apparently compromised. Similar results were seen after *in vivo* challenges in rhesus macaques, validating the explant studies.

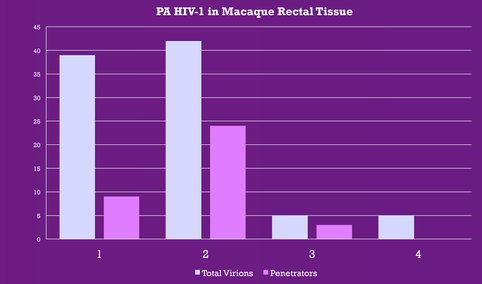
Conclusions

These findings suggest that the mucosal barrier of the rectal compartment can influence the ability of HIV to penetrate this barrier to reach underlying target cells. The protective layer of mucus clearly plays an important role in barrier function. The disruption of the barrier allows increased interaction with underlying target cells. Inflammatory conditions that increase transmission are likely influencing the mucus barrier. A better understanding to the interaction of HIV with the rectal mucosal barrier will facilitate future prevention strategies to decrease HIV acquisition.

Condition	No. of Z scans	No of Virions	No of Virions/ Z scan	No. of Penetrators	No. of Pentrators/ Z scan	Avg. Depth
1HR -NA	3360	170	0.05	5	0.0015	21.21
1HR +NA	3020	135	0.04	21	0.0070	24.66
2HR -NA	2920	357	0.12	17	0.0058	4.96
2HR +NA	3600	217	0.06	8	0.0022	14.23



Animal	No. of Z Scans	No. of Virions	No. of Virions/ Zscan	No. of Penetrators	No. of Penetrators Z scan	Avg. Depth
1	2500	39	0.015	9	0.003	6.388
2	2500	42	0.016	24	0.009	2.876
3	2500	5	0.002	3	0.001	5.52
4	2500	5	0.002	0	0	0



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