OPPOSING ASSOCIATIONS OF NK AND MZ B CELLS IN RECTAL EXPLANT MODEL OF HIV INFECTION

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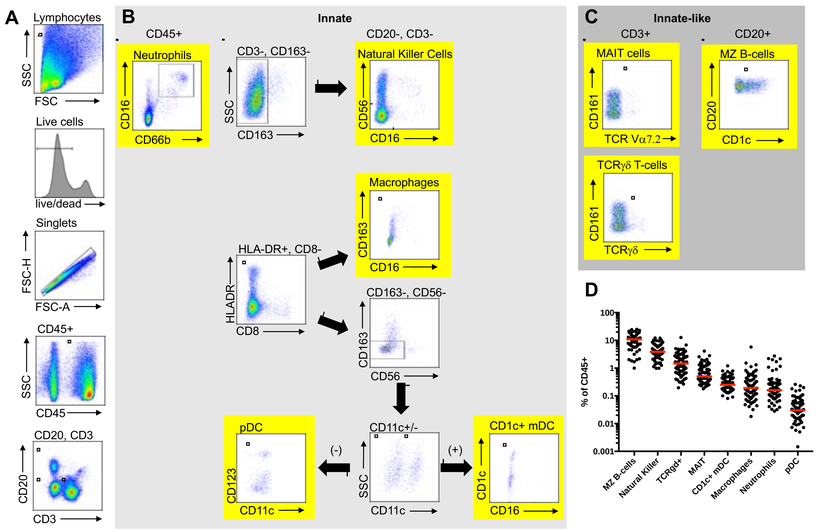
Background: Our understanding of innate immune cells in human rectal mucosal tissues (RM) and their contributions to promoting or restricting HIV transmission is limited.

Defining the RM immune environment at the time of HIV-1 exposure and identifying the immune cell subsets restricting or contributing to HIV replication within the RM could inform strategies that seek to strengthen the barriers to HIV-1 transmission within this tissue.

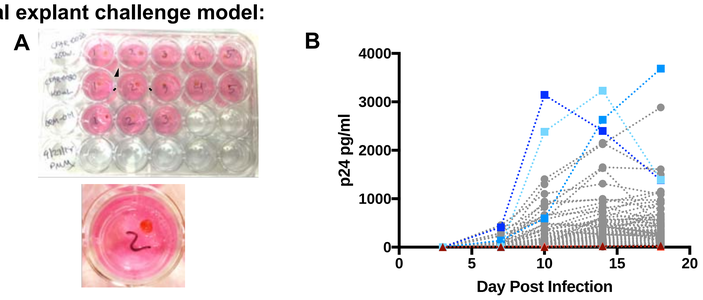
To that end, we sought to define many of the innate and innate-like cell subsets within human RM via flow cytometry (n=85, all male cohort). We then examined potential relationships between these subsets and HIV-1 replication in the *ex vivo* rectal explant model of infection.

Flow Cytometry:

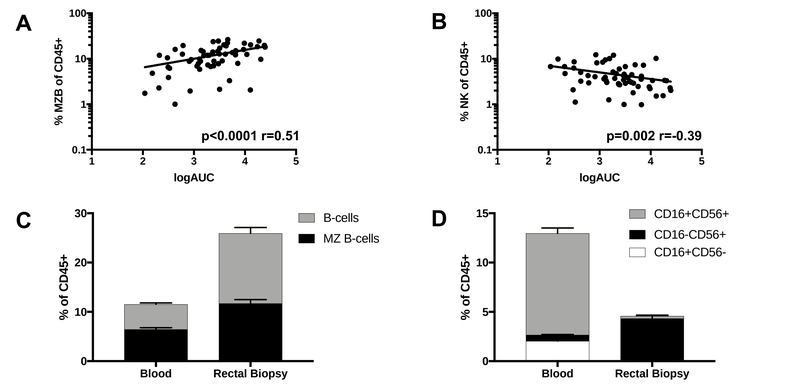


Quantification of innate and innate-like cellular subsets within the rectal mucosa. A) Representative gating strategy for rectal mucosal **B)** innate and **C)** innate-like subsets of CD45+ cells. **D)** Median (red bar) percentage of cell subsets as a percentage of CD45+ cells within rectal mucosal biopsies (n=85 gd T cells and MAIT, n=69 all other subsets). pDCs were the least abundant cell subset analyzed (Kruskal-Wallis, p<0.0002). Both MZB (p<0.0001) and NK (p<0.009) were the more abundant innate cell types quantified.

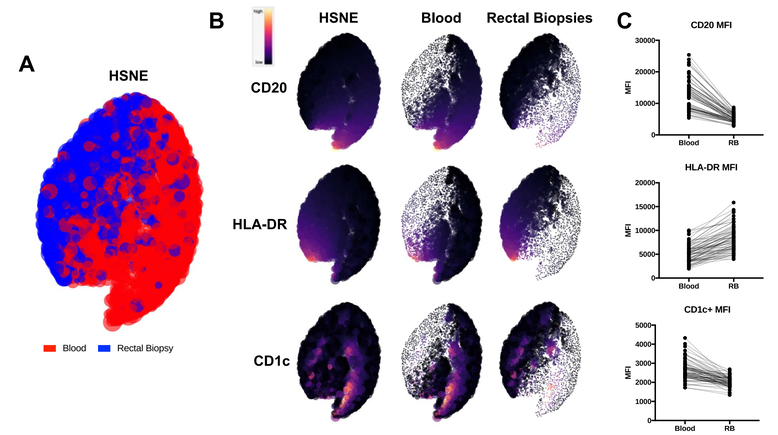
Rectal explant challenge model:



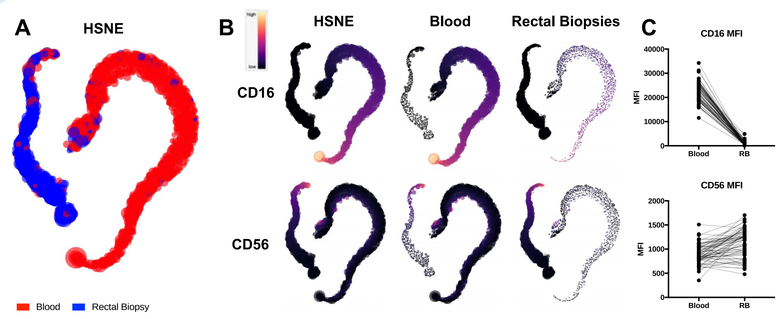
***Ex* vivo model of HIV-1 infection. A)** Rectal biopsies were infected with HIV-1 BaL, placed on collagen rafts, with supernatant collection/media exchanges over 18 days. P24 production was quantified via ELISA and normalized to biopsy weight. **B**) Median p24 varied substantially between participants.



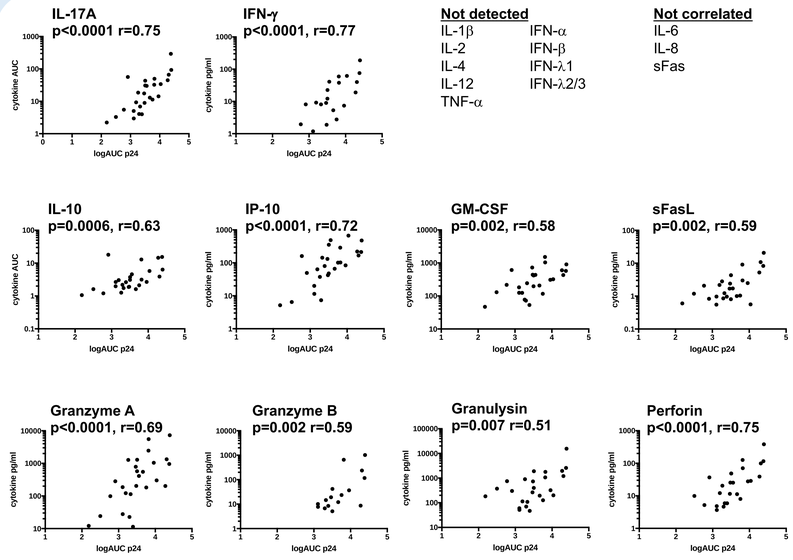
Pre-infection RM-MZB and NK cells are associated with HIV replication in the rectal explant model. Correlations between median log area under the curve (AUC) of p24 and innate cell subsets revealed opposing associations between both RM-MZB (**A**) and RM-NK (**B**) and HIV replication in the rectal explant model of infection. No correlations emerged with blood-derived MZB or NK. B and MZB cell populations differed between the blood and RM (2way ANOVA, p<0.0001) (**C**), as did the NK populations (p<0.0001) (**D**).



HSNE analysis of blood- and RM-MZB cells. A) Polarization in clustering of of blood (red) and RM (blue) MZB cells (CD45+, CD3-, CD20+, HLA-DR+, CD1c+). **B**) Polarization was the result of higher CD20 and CD1c expression on blood-MZB, with increased HLA-DR expression on RM-MZB on HSNE heat-maps, and confirmed by MFI (**C**) (Wilcoxon test, p<0.0001, all markers).



HSNE analysis of blood- and RM-NK cells. A) Discrete populations of blood (red) and RM (blue) NK cells (CD45+, Lin-, CD14-/CD163-, CD16+/-, CD56+/-). **B**) Heatmap analysis illustrates a near absence of CD16 on RM-NK, confirmed by MFI **C**) (Wilcoxon test, p<0.0001, all markers).



Associations between cytokine production and p24 production within the rectal explant model of infection. Cytokine production was quantified, in a subset of rectal explant supernatants (n=26). Correlations emerged between log p24 AUC (x axis) and AUC of IL-17A, IFN-g, IL-10, IP-10, GM-CSF, sFasL, GZA, GZB, Granulysin, and Perforin AUC (y axis). Spearman p and r values indicated. Cytokines assayed, but not detected within explant supernatants are indicated, as are cytokines detected but not correlated with p24 production.

CONCLUSIONS

- Innate immune cell subsets, as a whole, are a minority population of CD45+ cells within the RM, yet some are potentially influencing HIV production within the RM.
- The replicative capacity of HIV-1 BaL within the rectal explant model negatively correlated with the percentage of NK cells quantified within the rectal mucosa. This is in contrast with a positive association between MZB cells and p24 production.
- The MZB and NK populations found within the blood are quantitatively different from MZB and NK cells found within the rectal mucosa in both frequency and surface marker expression.
- Diverse innate anti-viral cytokines and effector molecules are secreted in the *ex vivo* rectal explant challenge model and correlate with productive HIV-1 infection.
- These observations would not have been possible without this novel use of human RM and the rectal explant model of HIV infection, due to the tissue-specific and potentially species-specific features of MZB cells, NK cells, and IL-17A.

SUPPORT

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