Characterization of the gastrointestinal-resident latent HIV-1 reservoir with single-cell RNA seq

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Background:

The gastrointestinal (GI) tract is an important immune site and location of HIV pathogenesis

- As a major site of host-pathogen interaction, gastrointestinal (GI) tissue is a major nexus of immune cell activity.
- Tissue resident immune cells contain gene-expression programs distinct from those in the blood and often persist in the same tissue for the entire lifespan.
- HIV/SIV replicate robustly in immune cells in the GI tissue, leading to a disruption in tissue integrity, translocation of bacterial material, and a broad host inflammatory state associated with infection.

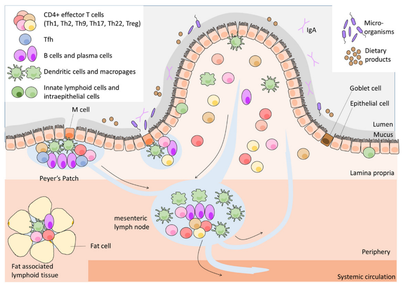
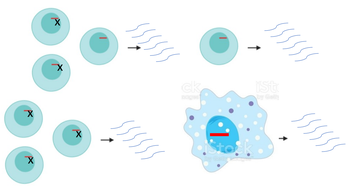


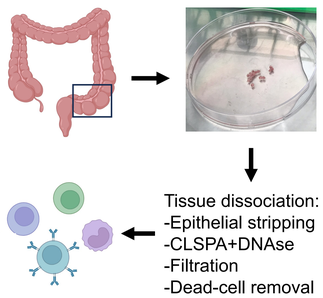
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HIV-1 DNA and RNA persists in GI tissue

Where does viral RNA come from?
What cell-types produce viral RNA in GI tissue?
What is the frequency of viral RNA+ GI cells?



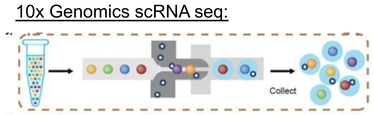
Dissociation of lymphoid tissue from recto-sigmoid GI biopsies

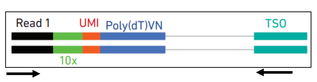


Tissue dissociation:
-Epithelial stripping
-CLSPA+DNAse
-Filtration
-Dead-cell removal

Methods:

10x Genomics scRNA seq:

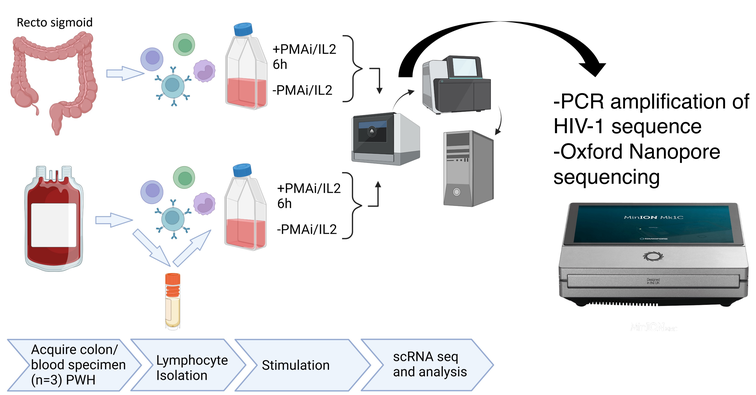




10x Genomics 11-cycle bulking cDNA amplification [PCR1] creates copies of each cDNA molecule

25% of this cDNA is used in Illumina library prep

Remaining 75% of cDNA is available for other molecular assays



- scRNAseq profiling of GI cells from 11 PWH on ART
- Identified HIV infected cells by the presence of vRNA
- Specific clusters of cells are enriched for vRNA+ cells
- vRNA+ cells express a distinct set of genes compared to vRNA- cells

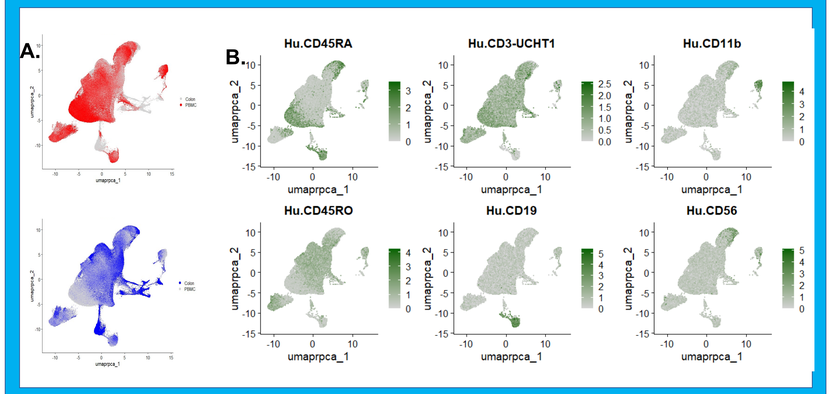


Figure 1: scRNA seq profiles of GI and peripheral blood immune cells:
A) UMAP plot of scRNA seq dataset from cells from 11 donors colored by tissue of origin (blood=red, colon=blue) B) UMAP plot of scRNA seq dataset with expression of the indicated immune cell markers superimposed on the data.

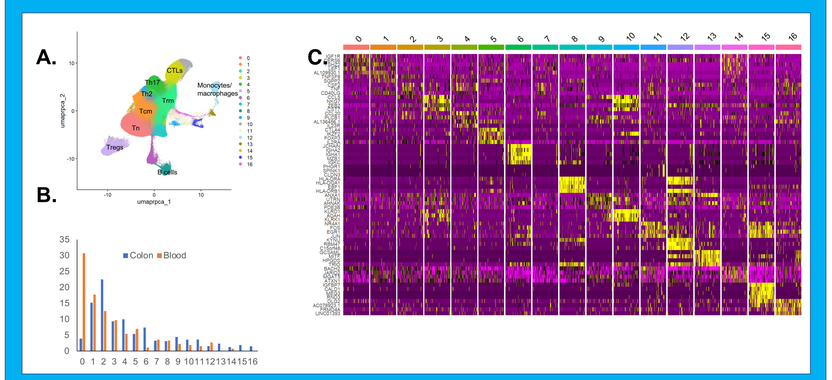


Figure 2: Identification of immune cell clusters. **A.**UMAP plot of colon and blood cells from eleven PWH, with clusters indicated by color. **B.** Abundance of cells in each cluster by tissue type. **C.** Heat map of the top differentially expressed genes per cluster

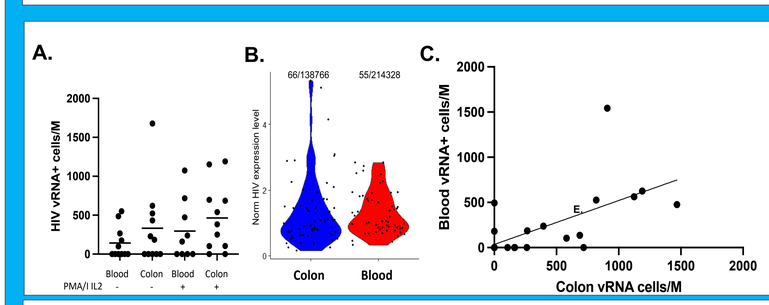


Figure 3: Detection of HIV-1 viral sequences. Cells with HIV mapping reads were identified for the dataset by alignment to a consensus Clade B virus sequence. A. Frequency of HIV vRNA+ cells in each sample, divided by tissue origin and condition. B. HIV Expression level for each vRNA+ cell divided by tissue of origin. C. Correlation between the frequency of vRNA+ cells in blood vs colon for each study participant.

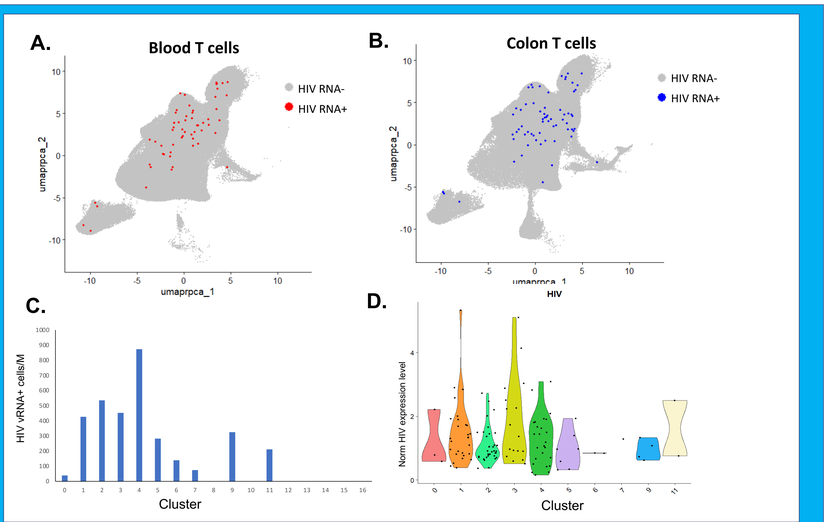


Figure 4: Distribution of vRNA+ cells across transcriptomic clusters
Visualization of vRNA+ cells across the UMAP projection for (**A**) blood and (**B**) colon cells. **C.** Frequency of vRNA+ cells for each cluster. **D.** Expression level for HIV within each cluster.

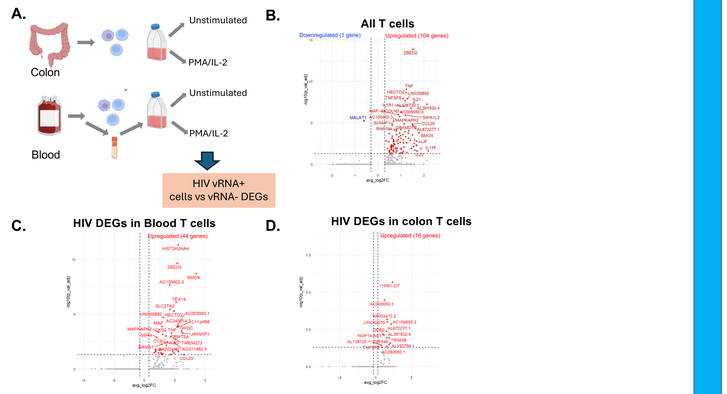


Figure 5: Differentially expressed genes in HIV RNA+ cells in the blood and colon. A. HIV RNA+ cells were compared to vRNA- cells in the same condition. **B-D.** Volcano plots showing differentially expressed genes in vRNA+ cells for different tissues.

Future Directions:

- Expansion of GI pilot study with larger clinical cohort and increased sampling
  - Will recruit up to 35 patients over the next 3-4 years (17 men, 18 women)
  - Additional molecular assays including RNA and DNA ddPCR measurements

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