Single-cell multiomics reveals trafficking and enrichment of HIV RNA+ CD4+ T cells in gut Th17

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ABSTRACT

Persistence of HIV RNA+ CD4+ T cells under ART is a major barrier to cure. We examined T cell polarization, clonal expansion, and migration of HIV RNA+ cells between the blood and gut.

We used ECCITE-seq to examine transcriptome, 131 surface protein, T cell receptor (TCR), and HIV RNA in the same single cells using paired blood and gut samples from 11 ART suppressed people with HIV, before and after 20 weeks of interferon alpha2b (Peg-IFN-α2b). Plasma viral load was undetectable at both sampling points, with no change in the size of the reservoir as measured by IPDA. Blood and gut samples from 4 uninfected individuals served as negative controls.

We analyzed 205,694 CD4+ T cells from blood and 60,317 from the gut. We found that cytotoxic CD4+ T cells (CD4-CTL) were more abundant in the blood (4.9%) compared to the gut (0.9%)(p=0.019), while Th17 cells were more abundant in the gut (17.4%) than in the blood (8.1%) (p=0.001, paired Wilcoxon rank sum test). Similar distributions were observed in control participants. We detected 75 HIV RNA+ CD4+ cells (mean 452/million) in the blood and 33 HIV RNA+ CD4+ T cells (mean 578/million) in the gut. HIV RNA+ cells were enriched in blood Th17 (22 cells, mean 1639/million blood Th17) and the gut Th17 (14 cells, 1317/million gut Th17). There were more clonal cells in the gut (24.1%) than the blood (16.9%), however, blood clones were more highly expanded. Of the top 100 largest T cell clones, 72 were only in the blood, 11 were only in the gut, and 17 were identified in both blood and gut. The top 10 blood-only T cell clones were almost exclusively cytotoxic CD4+ T cells (96.8%), consistent with our previous finding. Notably, T cell clones in the gut were highly heterogeneous within clones and between clones, including a mixture of Th1 (42.7%), Trm (15.8%), and Th17 (11.1%) in the top 10 gut-only T cell clones, and Th1 (44.4.%), CTL (40.4%) and Th17 (5.4%) in the top 10 clones found in blood and gut. HIV+ T cell clones were enriched in Th17 compared to HIV- clones.

While expansion within CD4-CTL clones contributes to the persistence of the HIV reservoir in the blood, the gut showcases a diverse landscape of CD4+ T cells, especially Th17 cells. This distinction between blood and gut emphasizes the gut's unique role in HIV persistence and highlights the necessity for specialized therapeutic approaches for each reservoir.

BACKGROUND

CD4+ T cells are highly heterogeneous with a diversity of differentiation, polarization and exhaustion states, and dynamic responses to antigenic recognition, activation and the local microenvironment. This heterogeneity must be resolved to understand the mechanisms underlying HIV persistence over time in different tissues. Investigation of key processes such as CD4+ T cell migration and proliferation is facilitated by single cell T cell repertoire analysis. Each CD4+ T cell has a unique T cell receptor (TCR) sequence which dictates its antigen specificity. T cell clones which proliferated from one cell can be identified by shared TCR sequences and clones characterized by both frequency and distribution across sampled tissues. The same antigen and immune program that drives the proliferation of a T cell clone also drives the proliferation of HIV-1⁺ cells among them. T cells can migrate between blood and gut and may drive seeding or maintenance of tissue reservoirs.

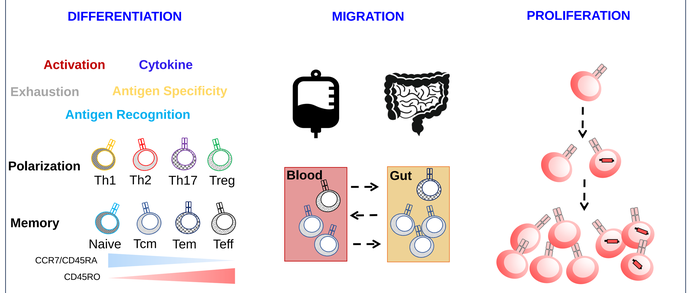

Fig. 1. The HIV CD4+ T cell reservoir is highly heterogenous. Heterogeneity of the HIV reservoir arises from the CD4+ T cell differentiation heterogeneity (polarization, memory, activation, exhaustion, antigen specificity, chemokine/cytokine response), migration into different tissues and proliferation.

METHODS

We used ECCITE-seq to examine transcriptome, 131 surface protein, T cell receptor (TCR), and HIV RNA in the same single cells using paired blood and gut samples from 11 ART suppressed people with HIV, before and after 20 weeks of interferon alpha2b (Peg-IFN-α2b). Plasma viral load was undetectable at both sampling points, with no change in the size of the reservoir as measured by IPDA. Blood and gut samples from 4 uninfected individuals served as negative controls. Cryopreserved PBMC and single cell suspensions from gut biopsies were thawed and dead cells removed. CD4+ T cells were isolated from PBMC by magnetic bead negative selection. Cells were stained with TotalSeq C antibody cocktail and oligo-hashtag antibodies, then partitioning and library preparation performed using the 10x Genomics Platform. Sequencing data was aligned to both the human and a compendium of clade B HIV genomes. Sequencing data was processed by grouping cells from each participant into PBMC, CD4+ T cells isolated from PBMC, and gut. Low quality cells (mitochondrial genes > 10%, expression of < 200 different genes, > 50,000 counts of RNA) and doublets (identified by assignment of multiple hashtags, scDblFinder, and expression of mutually exclusive markers) were removed. Normalization of RNA and protein data, and batch integration (Harmony) was also performed, and cell type was manually annotated. Identified CD4+ T cells were then pooled for analysis and batch integrated (Harmony). Dimensional reduction was applied to both RNA and protein data to generate a UMAP visualization plot and cell subtype was manually annotated.

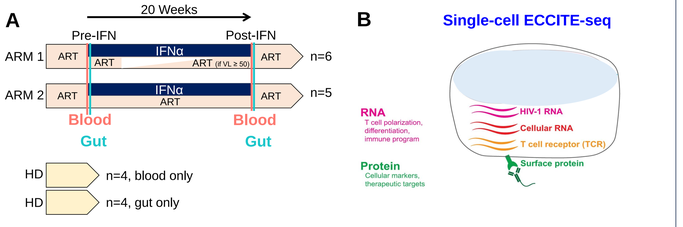

Fig. 2 Study design. To identify rare (<0.1%) HIV-infected cells, examine T cell differentiation and proliferation programs and track their migration between blood and tissues, paired blood and gut samples from people living with HIV on suppressive ART, before and after 20-week IFNα treatment with (n=6) or without (n=5) an analytic treatment interruption (A), were analysed using ECCITEseq (B). Exclusion criteria include age ≥ 18, suppressive ART ≥ 1 year (VL < 50 c/mL), CD4 > 450 cells/μL. Blood (n=4) and gut (colon biopsy, n=4) samples from HIV– donors served as negative controls.

ADDITIONAL KEY INFORMATION

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Acknowledgements
We thank all study participants. This work is supported by NIH R01 AI141009, NIH R61/R33 DA047037, NIH R01 AI174863, NIH P01 AI169768, NIH R37 AI147868, NIH R01 DA051906, NIH R01AI145164, NIH UM1 DA051410, NIH U01 DA053628, NIH CHEETAH P50 AI150464, NIH REACH Martin Delaney Collaboratory UM1 AI164565, NIH BEAT-HIV Martin Delaney Collaboratory UM1 AI164570.

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RESULTS

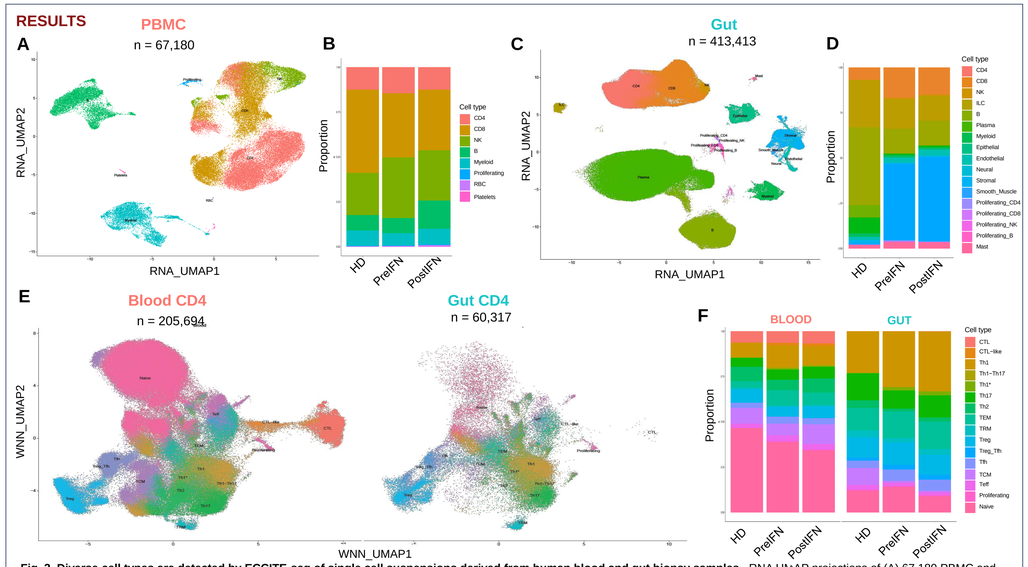


Fig. 3. Diverse cell types are detected by ECCITE-seq of single cell suspensions derived from human blood and gut biopsy samples. RNA UMAP projections of (A) 67,180 PBMC and (C) 413,413 cells from the gut integrated from 11 PWH and 4 HIV- donors. The frequency of identified cell types in blood (B) and gut (D) are shown. (E) WNN UMAP projection of 311,011 CD4+ T cells from blood (205,694) and gut (60,317). The frequency of T cell subsets identified in blood and gut are shown .

Identifying the rare HIV RNA+ CD4+ T cells in blood and gut during suppressive ART

HIV RNA+ cells are enriched in Th17 in the gut

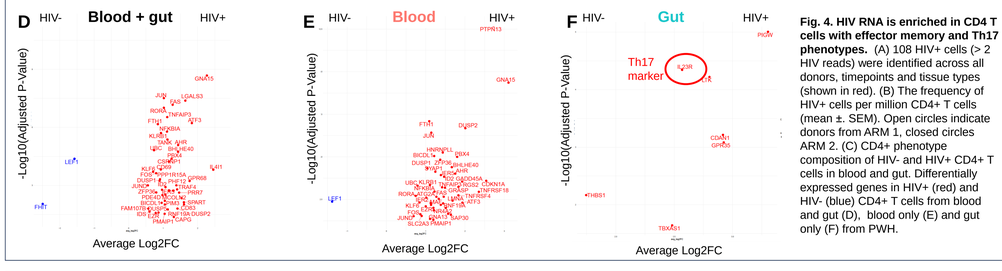


Fig. 4. HIV RNA is enriched in CD4 T cells with effector memory and Th17 phenotypes. (A) 108 HIV+ cells (> 2 HIV reads) were identified across all donors, timepoints and tissue types (shown in red). (B) The frequency of HIV+ cells per million CD4+ T cells (mean ±. SEM). Open circles indicate donors from ARM 1, closed circles ARM 2. (C) CD4+ phenotype composition of HIV- and HIV+ CD4+ T cells in blood and gut. Differentially expressed genes in HIV+ (red) and HIV- (blue) CD4+ T cells from blood and gut (D), blood only (E) and gut only (F) from PWH.

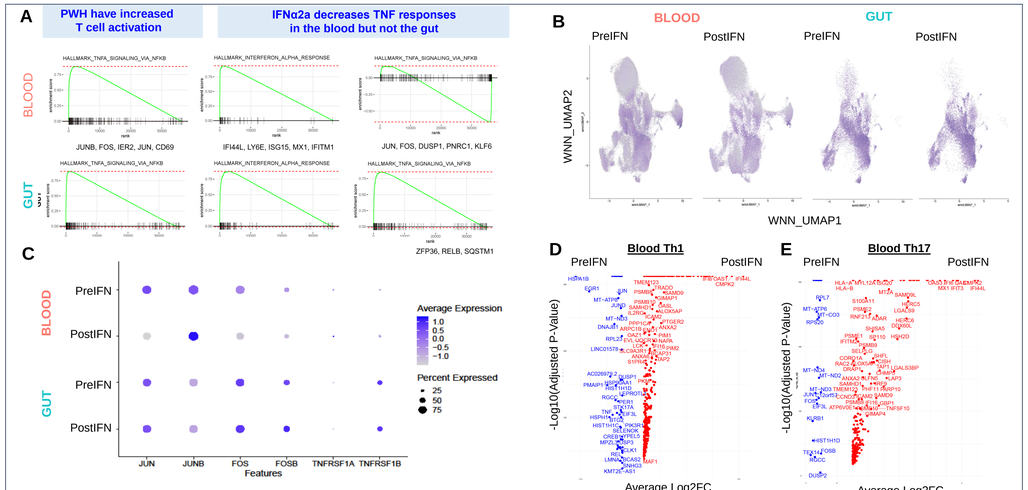
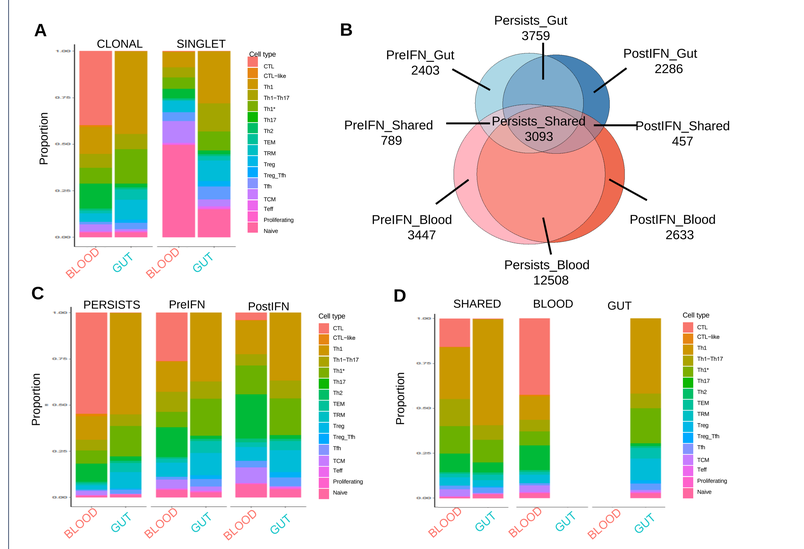
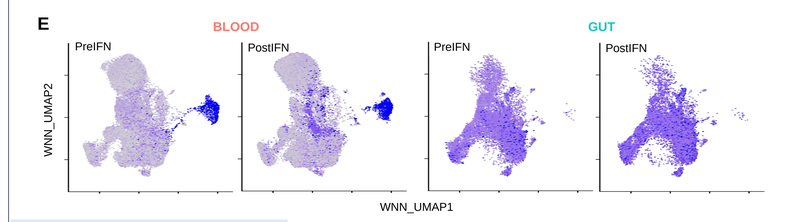


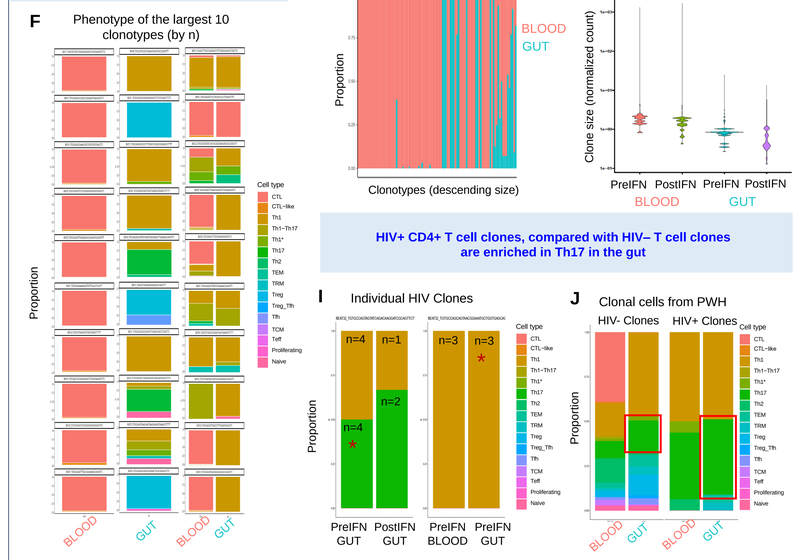
Fig. 5. IFNα treatment differentially immunomodulates blood-derived and gut-derived CD4+ T cells. (A). GSEA analysis in CD4+ T cells shows association of increased TNF signaling responses in both blood and gut HIV+ individuals prior to IFNα treatment compared to HIV– controls. Interferon alpha responses are induced by IFNa treatment, however while TNF signaling responses are elevated post IFNa treatment in the gut, blood CD4+ T cells are express less of the TNF signaling gene set. (B) Module scoring of CD4+ T cells for the Hallmark TNFα response gene set in CD4+ T cells in blood and gut, before and after IFNα treatment. (C) Dotplot of key genes involved in TNF signaling. Differentially expressed genes in CD4+ T cells post-IFN treatment (red) and preIFN treatment (blue) in Th1 (D) and Th17 (E) CD4+ T cells from blood of PWH. Note that JunB represses c-Jun/

The major clonally expanded cells are cytotoxic CD4+ T cells in the blood but Th1 in the gut





Large CD4+ T cell clones are universally cytotoxic CD4s in the blood but highly diverse in the gut



HIV+ CD4+ T cell clones, compared with HIV– T cell clones are enriched in Th17 in the gut

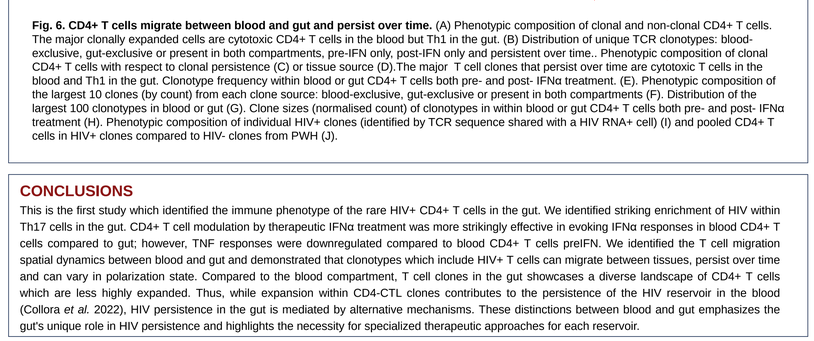


Fig. 6. CD4+ T cells migrate between blood and gut and persist over time. (A) Phenotypic composition of clonal and non-clonal CD4+ T cells. The major clonally expanded cells are cytotoxic CD4+ T cells in the blood but Th1 in the gut. (B) Distribution of unique TCR clonotypes: blood-exclusive, gut-exclusive or present in both compartments, pre-IFN only, post-IFN only and persistent over time.. Phenotypic composition of clonal CD4+ T cells with respect to clonal persistence (C) or tissue source (D).The major T cell clones that persist over time are cytotoxic T cells in the blood and Th1 in the gut. Clonotype frequency within blood or gut CD4+ T cells both pre- and post- IFNα treatment. (E). Phenotypic composition of the largest 10 clones (by count) from each clone source: blood-exclusive, gut-exclusive or present in both compartments (F). Distribution of the largest 100 clonotypes in blood or gut (G). Clone sizes (normalised count) of clonotypes in within blood or gut CD4+ T cells both pre- and post- IFNα treatment (H). Phenotypic composition of individual HIV+ clones (identified by TCR sequence shared with a HIV RNA+ cell) (I) and pooled CD4+ T cells in HIV+ clones compared to HIV- clones from PWH (J).

CONCLUSIONS

This is the first study which identified the immune phenotype of the rare HIV+ CD4+ T cells in the gut. We identified striking enrichment of HIV within Th17 cells in the gut. CD4+ T cell modulation by therapeutic IFNα treatment was more strikingly effective in evoking IFNα responses in blood CD4+ T cells compared to gut; however, TNF responses were downregulated compared to blood CD4+ T cells preIFN. We identified the T cell migration spatial dynamics between blood and gut and demonstrated that clonotypes which include HIV+ T cells can migrate between tissues, persist over time and can vary in polarization state. Compared to the blood compartment, T cell clones in the gut showcases a diverse landscape of CD4+ T cells which are less highly expanded. Thus, while expansion within CD4-CTL clones contributes to the persistence of the HIV reservoir in the blood (Collora *et al.* 2022), HIV persistence in the gut is mediated by alternative mechanisms. These distinctions between blood and gut emphasizes the gut's unique role in HIV persistence and highlights the necessity for specialized therapeutic approaches for each reservoir.