HIV Can Persist in Multiple Subsets of SARS-CoV-2-Specific CD4+ T Cells in Tissues During ART

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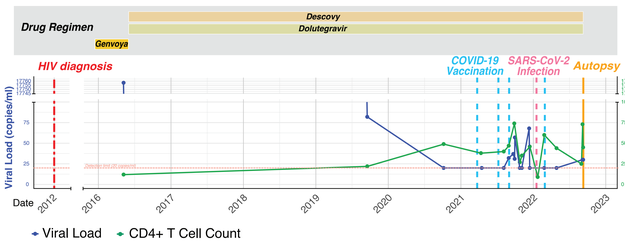
BACKGROUND

Transcriptionally-active cells (TACs) that produce HIV RNA persist during ART contribute to chronic inflammation and co-morbidities, and can lead to viral rebound upon ART interruption. However, our understanding of these cells – particularly within tissues – is limited. We implemented HIV-seq†, a technique we established to efficiently identify HIV+ cells by scRNAseq, to characterize the transcriptional profiles and antigen specificities of TACs from tissues.

METHODS

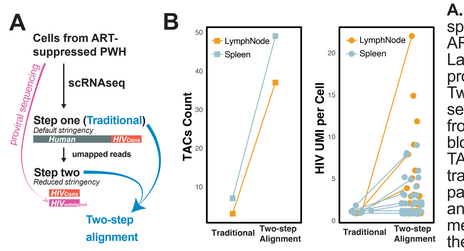
Our case study focused on participant LG34 enrolled in the Last Gift rapid research autopsy program. LG34 initiated ART during chronic HIV in 2016 and remained on ART with inconsistent intake until his death in 2022. Between 2021 and 2022, he experienced multiple low-level HIV blips (<75cp/ml), received four COVID-19 vaccinations, and had a breakthrough SARS-CoV-2 infection. We hypothesized that blipping during active SARS-CoV-2-specific immune responses (from vaccination and breakthrough infection) could seed an HIV reservoir within SARS-CoV-2-specific CD4+ T cells. To investigate this, we processed LG34's spleen and lymph nodes post-mortem, and performed multiomic HIV-seq to characterize HIV RNA+ cells through scRNAseq and VDJ-seq to evaluate SARS-CoV-2 specificities.

Figure 1. Timeline of clinical events, antiviral therapy, HIV viral load, and CD4+ T cell count of Last Gift participant LG34



The top panel shows the history of antiretroviral drug treatment. The bottom panel depicts viral load (blue) and CD4+ T cell count (green) from the time of HIV diagnosis in 2012 until death in 2022. LG34 exhibited multiple viral blips over the course of treatment. The timing of four COVID-19 vaccination events (blue), one SARS-CoV-2 breakthrough infection (pink), and autopsy (orange) are indicated.

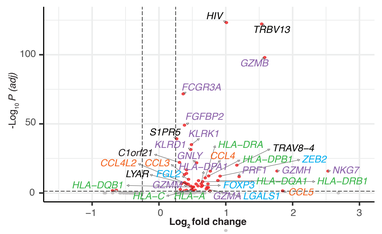
Figure 2. Enhanced HIV detection using Two-step Alignment implementing autologous HIV reference sequences and reduced stringency for alignment



A. CD4+ T cells from the spleen and lymph node of an ART-suppressed PWH (LG34 Last Gift cohort) were processed for scRNAseq and Two-step Alignment. Proviral sequences were generated from participant-matched blood. **B.** Dot plots show the TAC count (left panel) and HIV transcript UMI per cell (right panel) detected by traditional and Two-step Alignment methods. TACs or UMIs from the same tissue or cell are connected by lines.

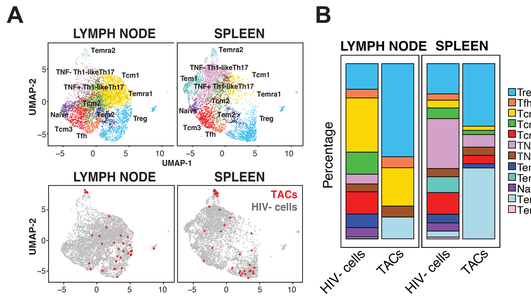
RESULTS

Figure 3. TACs express higher levels of cytolytic markers, MHC molecules, Treg-related immune suppression factors, and chemokines



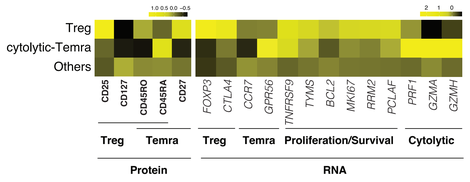
Volcano plot showing differentially expressed genes (DEGs) with a $\log_2$ fold change > 0.25 in HIV transcriptionally active cells (TACs) as compared to HIV- CD4+ T cells from the lymph node and spleen of ART-treated Last Gift participant LG34. Genes with an adjusted p-value ≤ 0.05 (Wilcoxon rank-sum test with bonferroni correction) are labeled with red circles. Select transcripts that have been labeled include those corresponding to cytolytic markers (purple), MHC molecules (green), Treg-related genes (blue), and chemokines (orange).

Figure 4. TACs are highly enriched in Treg and Temra CD4+ T subsets in both lymph node and spleen from ART-suppressed PWH



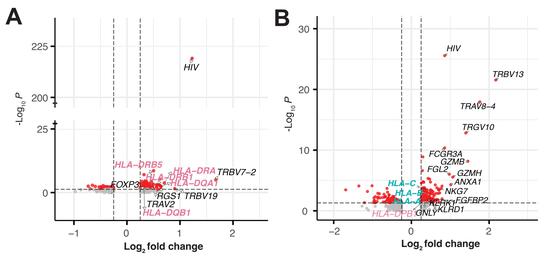
A. UMAP plots of total CD4+ T cells from the lymph node and spleen of LG34. The top panels depicted the annotated clusters, while the bottom panel highlights TACs (red) overlaid onto HIV- CD4+ T cells (gray). **B.** Enrichment of TACs was observed among the Treg and Temra2 clusters. Stacked bar graphs depicting the percentages of each annotated cell cluster among HIV- cells as compared to TACs in lymph node and spleen of LG34.

Figure 5. Treg cells from LG34 preferentially express higher proliferation and survival markers while Temra2 cells preferentially express cytolytic markers



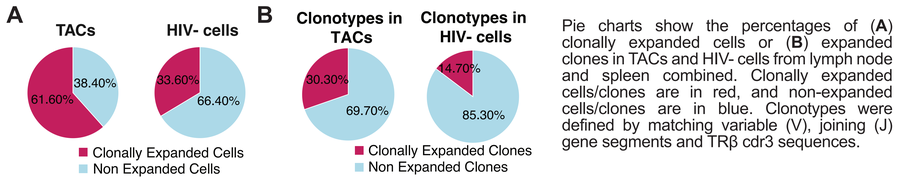
Heatmap displaying expression levels of protein (**bold**) and transcripts (*italics*) used to annotate the Treg (**CD45RO+ CD45RA- CD25+ CD127-** *FOXP3+ CTLA4+*) and Temra2 (**CD45RO- CD45RA+ CD27-** *CCR7- GPR56+*) clusters. Depicted also are transcripts linked proliferation/survival (*TNFRSF9, TYMS, MKI67, RRM2, BCL2*) preferentially expressed in the Treg cluster, and cytolytic markers (*PRF1, GZMA, GZMH*) preferentially expressed in the Temra2 cluster.

Figure 6. Treg TACs express elevated levels of MHC class II molecules relative to uninfected Treg cells, while Temra2 TACs express elevated levels of MHC class I molecules relative to uninfected Temra2 cells



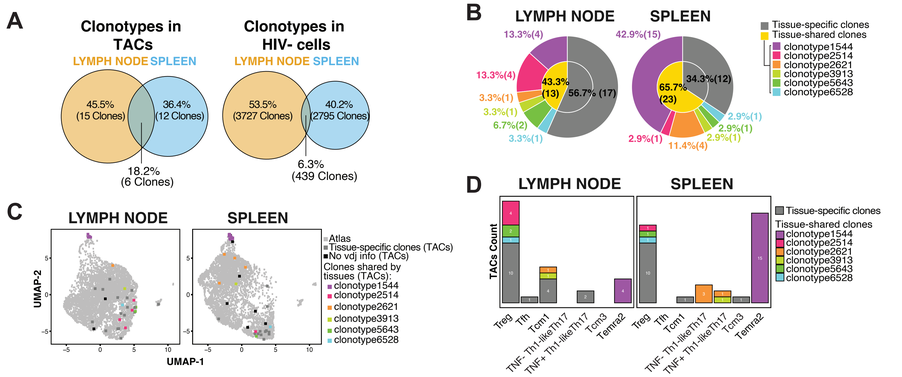
Volcano plots showing differentially expressed genes (DEGs) with a $\log_2$ fold change > 0.25 in TACs compared to HIV- cells within distinct T cell clusters. Genes with a p-value ≤ 0.05 (Wilcoxon rank-sum test) are highlighted in red. (**A**) In the Treg cluster, MHC class II molecules (pink) were upregulated in TACs, whereas MHC class I molecules were not. Additionally, genes essential for Treg-related immune suppressive function (*FOXP3, RGS1*) were elevated among the TACs. (**B**) In the Temra2 cluster, all MHC class I molecules (cyan) were upregulated in cytolytic-Temra2 TACs, along with the MHC class II molecule transcript HLA-DPB1 (pink). Cytolytic markers (*GZMB, GZMH, NKG7, FGL2, GNLY, KLRD1, KLRK1, FGFBP2, FCGR3A*) were also elevated among the TACs.

Figure 7. TACs are more clonally expanded than HIV- cells



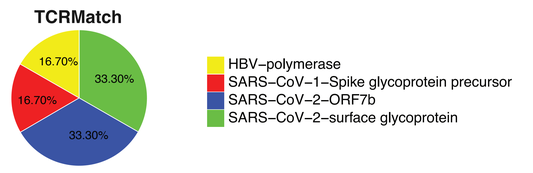
Pie charts show the percentages of (**A**) clonally expanded cells or (**B**) expanded clones in TACs and HIV- cells from lymph node and spleen combined. Clonally expanded cells/clones are in red, and non-expanded cells/clones are in blue. Clonotypes were defined by matching variable (V), joining (J) gene segments and TRβ cdr3 sequences.

Figure 8. Clonotypes of highly-expanded TACs are dispersed across tissues and share cellular phenotypes



A. Venn diagrams showing shared clonotypes in TACs and HIV- cells among lymph node (orange) and spleen (blue). **B.** Nested pie charts illustrating the distribution of tissue-specific clones (gray) and tissue-shared clones (inner circle: yellow; outer circle: colored by specific clonotypes). **C.** TACs colored by tissue-shared clones and tissue-specific clones (intermediate gray) overlaid on top of HIV- CD4+ T cells (light gray). Cells for which no VDJ information could be obtained are depicted in black. Tissue-shared clonotypes are depicted in no-gray colors. **D.** Stacked bar graph showing cell subset distribution of TACs among unique vs. tissue-shared clonotypes.

Figure 9. TACs include CD4+ T cells with predicted SARS-CoV-2 specificities



TACs with public TCR sequences are predicted to recognize SARS-CoV-2 and HBV epitopes. TCR specificities were determined based on TRβ CDR3 sequence matches identified through TCRMatch, which predicts TCR specificity by comparing sequence similarity to previously characterized receptors in the Immune Epitope Database (IEDB).

CONCLUSIONS

HIV-seq enabled detection of high numbers of TACs from tissues of LG34 under ART. These TACs were predominantly Tregs and Temras. Infected Tregs upregulated MHC-II which may and facilitate recognition by CD4+ T cells. Infected Temras express cytolytic molecules, and their increased MHC-I expression may facilitate recognition by CD8+ T cells. TACs exhibit shared clonotypes across tissues, and include CD4+ T cells with specificities for SARS-CoV-2 antigens.

† Frouard, J., et al. (2024). HIV-SEQ Reveals Global Host Gene Expression Differences Between HIV-Transcribing Cells from Viremic and Suppressed People with HIV. bioRxiv. https://doi.org/10.1101/2024.12.17.629023