Single-cell atlas and clonal expansion dynamics of CD4⁺ T cells during HIV-1 infection

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Abstract

Background. Despite effective antiretroviral therapy (ART), HIV-1 infection induces chronic immune activation. Further, HIV-1-infected CD4⁺ T cells undergo clonal expansion. We propose to examine the immune exhaustion phenotypes and clonal expansion dynamics in CD4⁺ T cells from HIV-infected individuals during acute HIV-1 infection and after viral suppression in the native cellular status without ex vivo stimulation.

Methods. We obtained paired CD4⁺ T cells from six HIV-1-infected individuals from the Sabes cohort during acute infection and after suppressive ART. CD4⁺ T cells from three uninfected individuals were obtained as negative controls. Using ECCITEseq, we captured surface protein expression (memory phenotypes, activation status and exhaustion markers), transcriptome, HIV-1 RNA and T cell clonality by T cell receptor sequences in the same single cells.

Results. We captured an average of 8,223 single cells and 1,504 genes mapped to human genome per sample. To examine how HIV-1 infection affects CD4⁺ T cell immune phenotypes, we used a combination of unbiased gene module identification and gene set enrichment analysis to identify key pathways. We found that CD4⁺ T cell subsets which upregulate interferon (IFN)-response genes during acute viremia downregulate IFN-response genes to baseline levels after ART. In contrast, CD4⁺ T cell subsets which are exhausted during acute viremia do not recover from immune exhaustion after ART. This exhausted subset is enriched for expression of tumor necrosis factor (TNF)-target genes. To examine T cell expansion dynamics, we analyzed T cell clonal abundance, repertoire dynamics and clone tracking. We found that the T cell clone size correlates with antigen response gene expression. To examine the in vivo status of HIV-1 RNA⁺cells, we identified a total of 90 HIV-1 RNA⁺ cells. These cells upregulate chemokines and immune effectors. We found that HIV-1 RNA⁺ cells are enriched in dynamic T cell clones, which expand and contract more dramatically.

Conclusions. The single-cell multi-omic landscape of CD4⁺ T cells identified TNF-target genes as mediators of HIV-1-induced immune exhaustion. HIV-1 RNA⁺ cells upregulate chemokines and reside in subdominal T cell clones during acute infection. These findings provide therapeutic insights in reverting HIV-1-induced immune exhaustion and targeting HIV-1-infected cells.

Methods

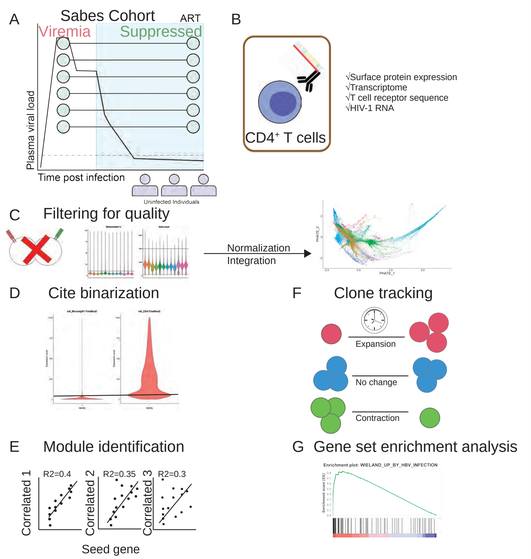

Figure 1. A multi-omics CD4⁺ T cell Atlas of HIV-1 infection. A. CD4⁺ T cells were isolated from the same six individuals from the Sabes cohort at two time points, one during viremia and one after 1 year of suppressive antiretroviral therapy. **B.** Cells were isolated in water-in-oil droplets using the 10X Chromum Controller to capture mutliple modalities from the same single cell. **C.** Following sequencing, cells were filtered to remove cells with fewer than 500 genes or greater than 10% mitochondrial read content, and to remove doublets based on multiple T cell receptor sequences recovered. Remaining cells were normalized using SCtransform (Hafemeister et al. *Biorxiv*, 2019) and integrated to remove batch effect using reciprocl principal component analysis as implemented by Seurat (Stuart et al. *Cell*, 2019). The resulting integrated count matrix was used to calculate dimensionality reduction using PHATE which preserves global relationships between cells better than alternatives such as t-SNE and UMAP (Moon et al. *Nature Biotech*, 2019). **D.** Resulting antibody counts were converted to binary by covnerting any value greater than the 90th percentile of the antibodies isotype control. **E.** Modules were identified using Pearson correlation to identify the top 50 correlated genes with a given marker gene (ex. *PDCD1*). **F.** Clones were defined based on the TCR-β chain as defined by Change-O (Gupta et al. *Bioinformatics*, 2015) . **G.** Gene set enrichment analysis was utilized in preranked mode (Subramanian, Tamayo, et al. *PNAS*, 2005) against the currated human genesets (C2) from MSigDB.

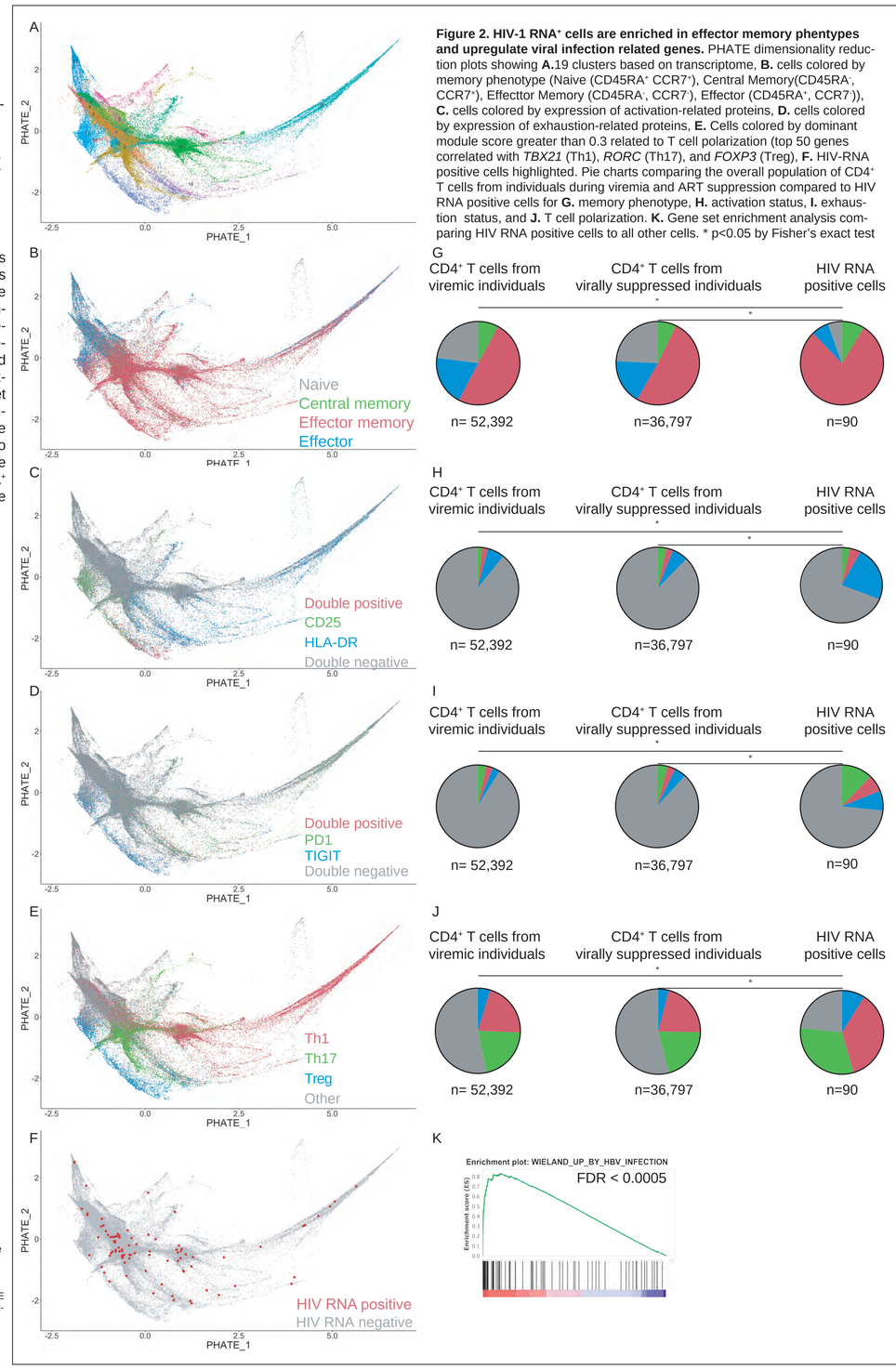

Figure 2. HIV-1 RNA⁺ cells are enriched in effector memory phentypes and upregulate viral infection related genes. PHATE dimensionality reduction plots showing **A.**19 clusters based on transcriptome, **B.** cells colored by memory phenotype (Naive (CD45RA⁺ CCR7⁺), Central Memory(CD45RA⁻, CCR7⁺), Effecttor Memory (CD45RA⁻, CCR7⁻), Effector (CD45RA⁺, CCR7⁻)), **C.** cells colored by expression of activation-related proteins, **D.** cells colored by expression of exhaustion-related proteins, **E.** Cells colored by dominant module score greater than 0.3 related to T cell polarization (top 50 genes correlated with *TBX21* (Th1), *RORC* (Th17), and *FOXP3* (Treg), **F.** HIV-RNA positive cells highlighted. Pie charts comparing the overall population of CD4⁺ T cells from individuals during viremia and ART suppression compared to HIV RNA positive cells for **G.** memory phenotype, **H.** activation status, **I.** exhaustion status, and **J.** T cell polarization. **K.** Gene set enrichment analysis comparing HIV RNA positive cells to all other cells. * p<0.05 by Fisher's exact test

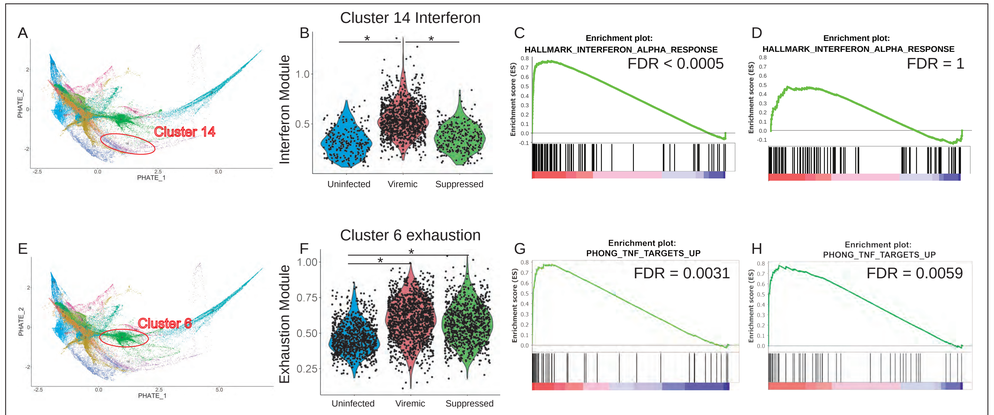

Figure 3. HIV-1 driven immune dysfunction in CD4⁺ T cells is correlated with TNF target gene expression. A. PHATE plot highlighting the location of cluster 14. B. Violin plot comparing the interferon response (as defined by the top 50 genes correlated with ISG15) in uninfected individuals to viremic individuals and virally suppressed individuals. Gene set enrichment analysis results indicating enrichment of interferon alpha response genes C. during viremia, and D. after viral suppression. E. PHATE plot highlighting the location of cluster 6. F. Violin plot comparing T cell exhaustion (as defined by the top 50 genes correlated with PDCD1) in uninfected individuals to viremic individuals and virally suppressed individuals. Gene set enrichment analysis results indicating enrichment of TNF target genes G. during viremia, and H. after viral suppression. * p<0.05 by Mann-Whitney U test.

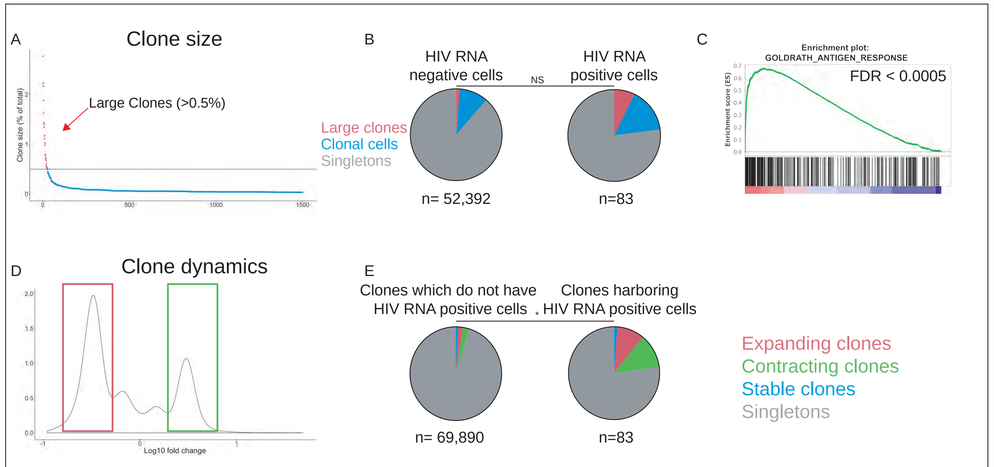

Figure 4. HIV-1 RNA Positive cells are enriched in dynamic, not large clones. A. Size rank plot of top 1,500 clones captured. Clones comprising greater than 0.5% of the total CD4⁺ T cells captured are considered large and colored red. B. Pie charts comparing clone size of cells with or without detected HIV RNA. C. Gene set enrichment analysis using gene correlation with clone size for ranking correlates most strongly with antigen response. D. Clone log10 fold change between viremia and viral supression in infected individuals. E. Pie charts comparing clones with HIV RNA positive cells to those without. * p<0.05 by Fisher's exact test NS not significant.

Conclusions

HIV RNA positive cells are enriched in effector memory cells. Clones contatining an HIV RNA positive cell are more dynamic than average. T cell exaustion may persist on antiretroviral therapy through TNF signaling. An antigen simulation signature is most strongly associated with clone size irrespective of infection status.

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