NF-κB dependent Gene Expression and Plasma IL-1β, TNFα and GCSF drive transcriptomic diversity and CD4:CD8 Ratio in People with HIV on ART

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Background

Despite antiretroviral therapy (ART), people with HIV (PWH) on ART experience higher rates of morbidity and mortality vs. age-matched HIV negative controls, which may be driven by chronic inflammation due to persistent virus. We performed bulk RNA sequencing (RNA-seq) on peripheral CD4+ T cells, as well as quantified plasma immune marker levels from 154 PWH on ART to identify host immune signatures associated with immune recovery (CD4:CD8) and HIV persistence (cell-associated HIV DNA and RNA). Using a novel dimension reduction tool - Pairwise Controlled Manifold Approximation (PaCMAP), we defined three distinct participant transcriptomic clusters. We found that these three clusters were largely defined by differential expression of genes regulated by the transcription factor NF-κB. While clustering was not associated with HIV reservoir size, we observed an association with CD4:CD8 ratio, a marker of immune recovery and prognostic factor for mortality in PWH on ART. Furthermore, distinct patterns of plasma IL-1β, TNF-α and GCSF were also strongly associated with the clusters, suggesting that these immune markers play a key role in CD4+ T cell transcriptomic diversity and immune recovery in PWH on ART. These findings reveal novel subgroups of PWH on ART with distinct immunological characteristics, and define a transcriptional signature associated with clinically significant immune parameters for PWH. A deeper understanding of these subgroups could advance clinical strategies to treat HIV-associated immune dysfunction.

Cohort characteristics

Descriptive characteristic	Total (N=154)
Male (%)	146 (95%)
Age (years)	47 (25-70)
Years of therapy	4.9 (0.6-16.6)
Nadir CD4+ T cell count (cells/mm^3)	336 (2-1500)
Maximum pre-ART HIV RNA (log_{10}copies/mL)	47260 (40-4494698)
HIV total DNA (log_{10}copies/10^6 CD4+ T cells)	13.3 (0-561.4)
HIV unspliced RNA (log_{10}copies/10^6 CD4+ T cells)	828.0 (0-16550.5)

Methods

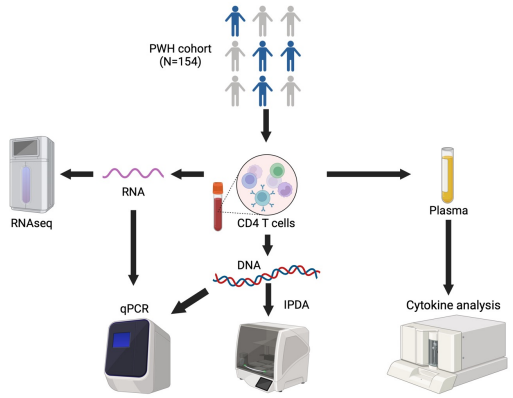


Figure 1: Overview of experimental design. CD4 T cells and plasma were isolated from a cohort of ART-suppressed PWH. Cellular RNA was analyzed by bulk RNAseq and qPCR for viral RNA. Cellular DNA was analyzed by qPCR for viral DNA and for intact proviruses by IPDA. Plasma was analyzed for the concentrations of seven protein markers (IP-10, G-CSF, Pentraxin-3, IL-1β, IL-10, TNF-α and sTLR4)

- A novel dimension reduction tool was used to analyze an RNAseq dataset from 154 PWH on ART.
- Three clusters of PWH were identified that represent low, mid and high levels of NF-κB activity and inflammatory gene expression.
- Plasma cytokine levels for TNFα and IL-1β were highly associated with cluster membership.

Dimension reduction reveals three clusters of PWH on ART

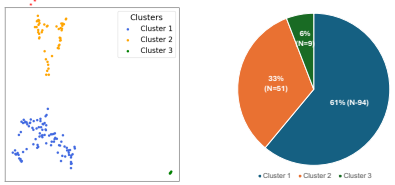


Figure 2: Dimension reduction of RNAseq data from CD4 T cells. PaCMAP was used to project bulk RNAseq data from CD4 T cells from 154 PWH on ART. A. PaCMAP projection with clusters color coded. B. Pie chart showing proportion of PWH in each transcriptomic cluster

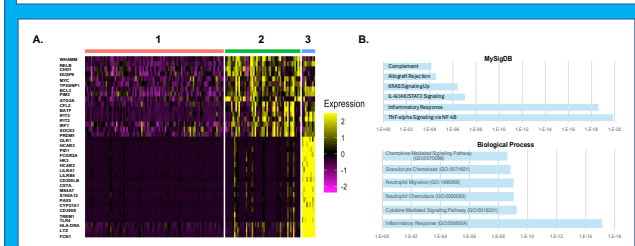


Figure 3: Transcriptomic comparison of PWH clusters. DESeq2 was used to identify differentially expressed genes between each of the clusters of PWH. **A.** Top differentially expressed genes shown. **B.** Pathway enrichment analysis for genes expressed higher in cluster 2 relative to cluster 1.

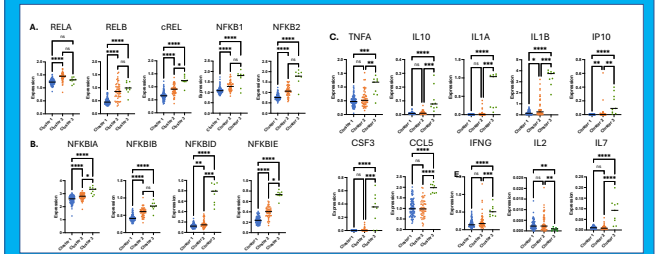


Figure 4: Differential expression of NF-kB pathway genes between clusters of PWH on ART. Scaled and natural log normalized gene expression is shown for NF-κB family member genes (A), genes encoding NF-κB inhibiting I-κB proteins (B), and NF-κB regulated cytokines (C) are shown. Each datapoint represents an individual PWH. Cluster 1 – blue, Cluster 2 – orange, Cluster 3 – green. Asterisk indicates statistical significance (P<0.05, Kruskal-Wallis test). ns = not significant (P>0.05). P < 0.05*, P < 0.01**, P < 0.001***, and P<0.0001****.

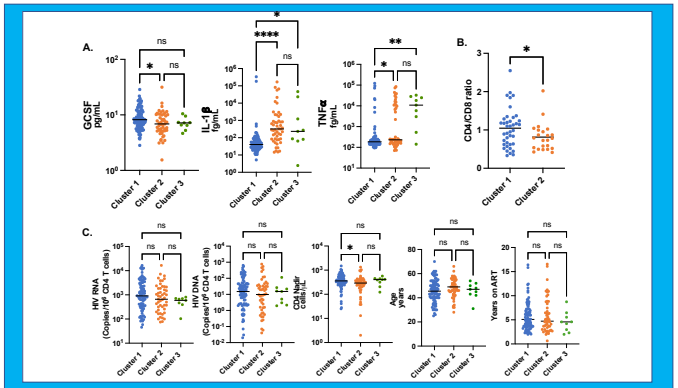


Figure 5: Plasma IL-1β and TNF-α levels and CD4/CD8 ratio are associated with transcriptomic cluster membership. Plasma immune marker concentrations (A), CD4/CD8 T cell ratio (B) and viral reservoir measurements for each member of the cohort (C) are shown, divided by each transcriptomic cluster (Cluster 1 - blue, Cluster 2 - orange, Cluster 3 - green). Each datapoint represents an individual PWH. Asterisk indicates a statistically significant difference between columns (P<0.05, Kruskal-Wallis test). ns = not significant (P>0.05). P < 0.05*, P < 0.01**, P < 0.001*** and P < 0.0001****.

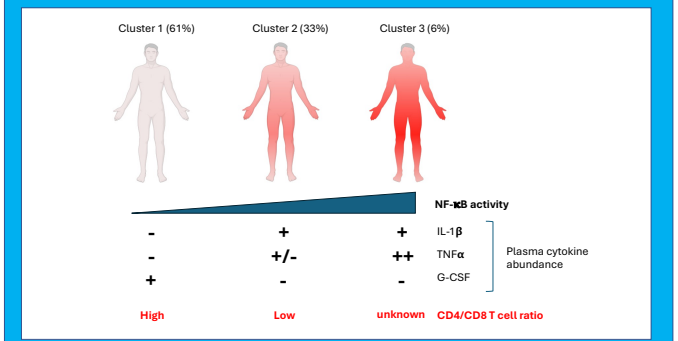


Figure 6: Model for cytokine abundance and CD4 T cell transcriptome clustering. Conceptual model shown for how plasma immune marker abundance and NF-κB activity associates with transcriptomic cluster for CD4 T cells, immune cells and overall inflammation level in people with HIV (PWH). Figure prepared using Biorender.

Conclusions:

- Identified three distinct clusters of PWH in ART using PaCMAP projection of bulk RNAseq data from a cohort of PWH (n=154)
- NF-κB dependent inflammatory gene expression is the primary difference between the clusters
- Plasma IL-1β and TNF-α levels predict cluster membership

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