Inflammatory Monocyte and T Cell Signatures Linked to IL-1β, a Marker of Vascular Risk in HIV

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NIH
- NIH K23GM112526
- NIH R01AI143464
- NIH NIAID Y1-AI-5072
- U.S. DoD HU0001190002

231

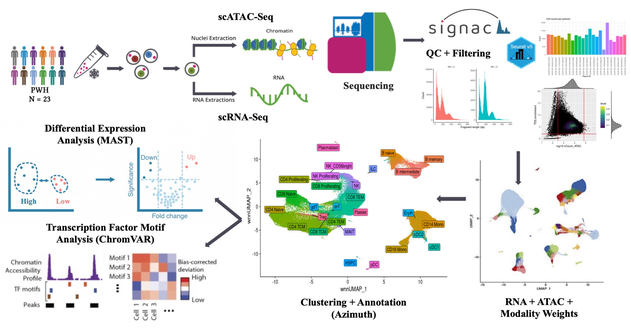
BACKGROUND

- People with HIV (PWH) on antiretroviral therapy (ART) exhibit persistent immune activation despite viral suppression.
- NLRP3 inflammasome activation, which drives IL-1β/IL-18 and downstream IL-6 signaling, has been associated with increased vascular risk[1-3] and mortality[4-5] in PWH on ART.
- However, the cellular and molecular pathways linking elevated IL-1β to cardiovascular risk in ART-suppressed PWH remain incompletely defined.

METHODS

- We analyzed PBMCs from 23 PWH on ART in the U.S. Military HIV Natural History Study,[6] stratified by plasma IL-1β levels (11 high, 12 low). Groups were matched by age, sex, and incident vascular event (atherosclerotic cardiovascular disease [ASCVD] or venous thrombotic event [VTE]).
- Single-cell multiome profiling defined transcriptional and chromatin differences.

Figure 1: Workflow of Multiome (scRNA-seq + scATAC-seq) Analysis



Software used for data processing: CellRanger,[7] Seurat,[8] MACs,[9] Signac,[10] Azimuth, MAST,[11] ClusterProfiler,[12] and ChromVAR[13]

RESULTS

Table 1: Participants' Characteristics

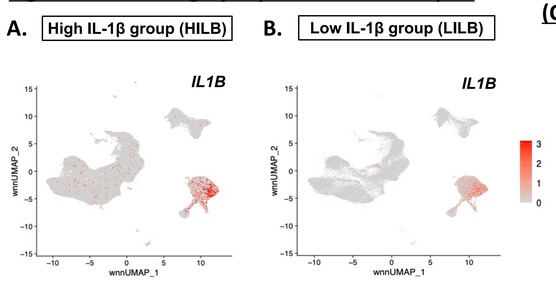
Characteristic	HILB (N = 11)	LILB (N = 12)
Age[a] (years)	44 (41-51)	42 (32-55)
Sex at birth (% male)	6 (54.5%)	6 (55%)
Race/ethnicity (% Caucasian; % Hispanic; % Other)	8 (72.7%); 0 (0%); 3 (27.3%)	2 (16.7%); 3 (25%); 7 (58.3%)
Nadir CD4+ T cell count (cells/mm^3)	355 (237-520)	301 (215-398)
Pre-ART viral load (log$_{10}$ copies/mL)	4.4 (4.3-4.7)	4(3.7-4.5)
ART initiation (years after seroconversion)	1.07 (0.08-1.01)	2.3 (0.13-2.63)
Hyperlipidemia[a]	9 (81.8%)	9 (75%)
Hypertension[a]	7 (63.6%)	6 (50%)
Type II diabetes[a]	1 (9.1%)	3 (25%)
Family history of ASCVD[a]	0 (0%)	0 (0%)
Tobacco use (% ever; % current)[a]	9 (81.8%); 3 (27.3%)	9 (75%); 1 (8.3%)
Alcohol use (% ever; % current)[a]	11 (100%); 8 (72.7%)	9 (75%); 7 (58.3%)
Incident vascular event (% total; % ASCVD; % VTE)[b]	6 (54.5); 4 (36.3%); 6 (54.5%)	6 (50%); 5 (41.66%); 6 (50%)

Continuous variables are presented as medians (interquartile range [IQR]) and categorical variables as counts (percentages) for participants with high IL-16 (HILB; N=11) and low IL-16 (LILB; N=12). [a] Prevalence assessed at the time of sample collection. [b] Incident (occurring after the sample timepoint, beginning at least 1 year post-collection) vascular event, defined as any new diagnosis of ASCVD or VTE. ASCVD (atherosclerotic cardiovascular disease): coronary artery disease (CAD), myocardial infarction (MI), cerebrovascular accident (CVA), or peripheral arterial disease (PAD). VTE (venous thrombotic events): deep vein thrombosis (DVT) or pulmonary embolism (PE). Participants with prior vascular events were excluded, and multiple incident events after sample collection were permitted.

Among PWH on ART, elevated plasma IL-1β was associated with coordinated transcriptional and epigenetic reprogramming across innate and adaptive immune cells, with NF-κB-driven inflammatory activation and metabolic remodeling.

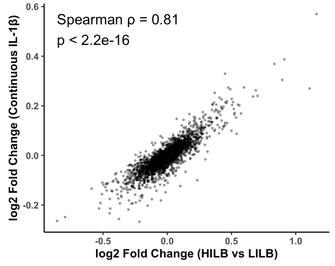
Single-cell RNA-seq and ATAC-seq analyses revealed a persistent, chromatin-accessible inflammatory state in monocytes and T cells consistent with immune priming despite long-term viral suppression.

Figure 2: *IL1B* is Highly Expressed in Monocytes



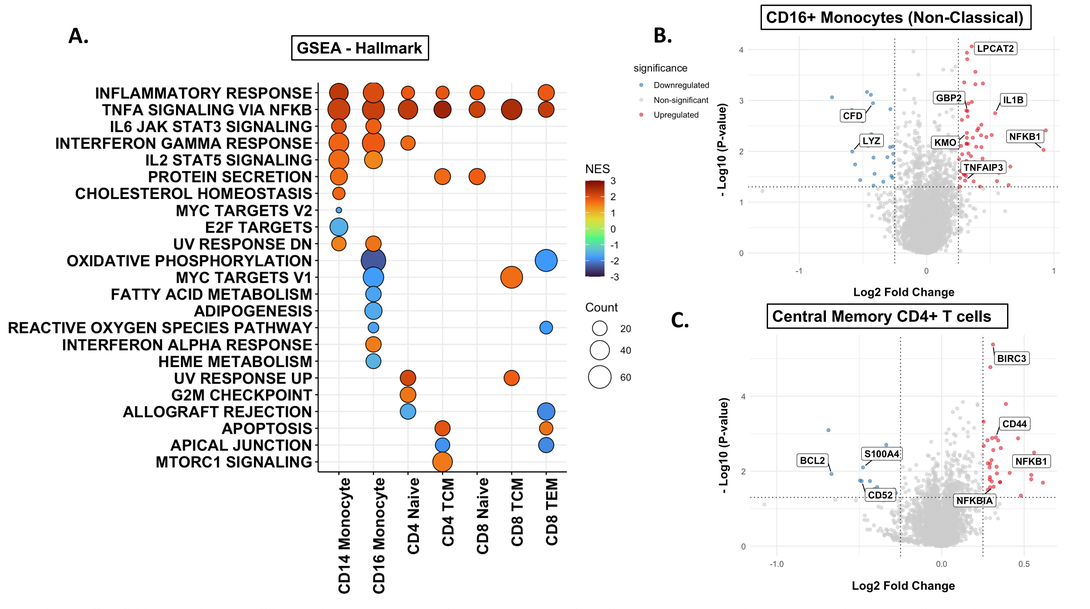
Uniform manifold approximation and projection (UMAP) dimensionality reduction plot demonstrating average IL1B gene expression among HILB (left) and LILB (right) groups. The majority of IL1B expressing cells (red dots) localized to the CD14+ monocyte cluster (shown at the bottom right of the figures).

Figure 3: High Concordance Between Continuous IL-1β (Covariate-Adjusted) and High/Low Group Classification



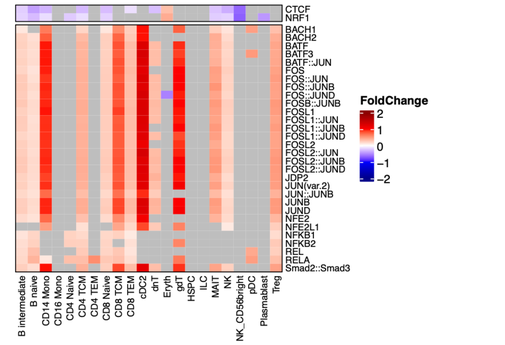
Sensitivity analyses indicate that the HILB and LILB groups - carefully matched for age, sex, and incident vascular event - lie along a continuum of plasma IL-16 levels. Scatterplot shows the correlation between gene expression changes by group (HILB vs. LILB, x-axis) and by continuous plasma IL-16 levels (y-axis, adjusted for age, sex, and incident vascular events). Each point represents a transcript; Spearman ρ indicates correlation strength.

Figure 4: Single-Cell GSEA Reveals Coordinated NF-κB, Interferon, and IL6–JAK–STAT3 Pathway Enrichment with Metabolic Suppression in PBMC Subsets from ART-Suppressed PWH with High Plasma IL-1β



Heatmap of Gene Set Enrichment Analysis (GSEA) results comparing high (HILB) versus low plasma IL-16 (LILB) groups across monocyte and T cell subsets (x-axis) (A). Significantly enriched Hallmark pathways (FDR < 0.05) are shown on the y-axis. Dot color indicates direction of enrichment in the high IL-16 group (red, upregulated; blue, downregulated), and dot size corresponds to the number of leading-edge genes contributing to each pathway. Volcano plots of differential gene expression between HILB and LILB in CD16$^+$ monocytes (B) and CD4$^+$ central memory T cells (C), p<0.05. The x-axis represents log$_2$ fold change, and the y-axis shows −log$_{10}$(unadjusted p value). Points in red and blue denote significantly upregulated and downregulated genes, respectively, in the high IL-16 group.

Figure 5: Increased Accessibility of NF-κB and AP-1 Transcription Factor Motifs in High IL-1β ART-Suppressed PWH



Heatmap of chromVAR transcription factor (TF) motif accessibility scores comparing high IL-16 (HILB) versus low IL-16 (LILB) groups across PBMC subsets. Immune cell types are displayed on the x-axis and TF motifs on the y-axis. Motifs in the upper panel exhibit reduced accessibility in HILB relative to LILB (blue), whereas motifs in the lower panel show increased accessibility in HILB (red), indicating differential chromatin accessibility and altered regulatory potential associated with elevated plasma IL-16.

CONCLUSIONS

- Single-cell RNA-seq revealed broad upregulation of TNFα/NF-κB, interferon, and IL6–JAK–STAT3 signaling in monocyte and T cell subsets from HILB individuals.
- Monocytes in HILB participants showed inflammatory priming with suppressed oxidative phosphorylation, consistent with metabolic reprogramming.
- Adaptive immune cells demonstrated increased NF-κB signaling, MTORC1 activation, and apoptotic signatures, suggesting heightened activation with reduced survival tone.
- scATAC-seq revealed increased accessibility of NF-κB, AP-1 (JUN), REL, and BATF motifs and reduced accessibility of NRF1 and CTCF, indicating stable epigenetic reinforcement of inflammatory circuitry.
- CTCF is a key chromatin architectural regulator implicated in maintenance of HIV latency; reduced CTCF accessibility suggests inflammation may affect HIV latency control.
- Together, these findings link IL-1β-associated inflammation to cardiometabolic risk and HIV persistence in PWH on ART.

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PLAIN LANGUAGE SUMMARY

Even with effective HIV treatment, some people have persistent inflammation. We found that higher IL-1β levels are linked to changes in gene activity in immune cells, which may help explain ongoing immune activation and long-term cardiovascular risk.