Single-Cell Analysis Identifies Broad Immune Alterations Despite ART-Induced Viral Suppression

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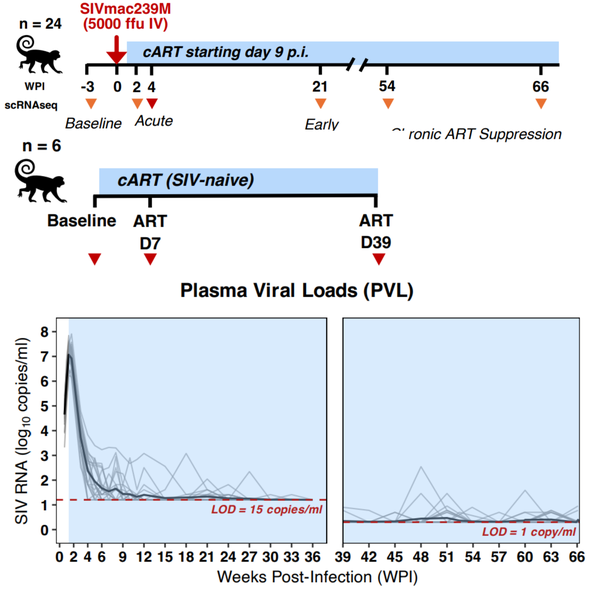
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BACKGROUND

Antiretroviral therapy (ART) has dramatically increased the life expectancy of people living with HIV (PLWH). Despite this, PLWH are at higher risk of many non-AIDS complications, driven at least in part by chronic immune activation from incomplete viral suppression. Precisely characterizing the long-term impact on immune cells is critical for patient care and the development of curative strategies that rely on the host's immune system.

METHODS

- Longitudinal sampling of a rhesus macaque cohort that received SIVmac239 with ART initiation at Day 9
- Performed single-cell RNA-seq (10x) from blood and tissues, and plasma proteomics (NULISA)
- Separate cohort received ART alone



This work was supported by the BMGF HIV Reservoirs Program and the Delaney AIDS Research Enterprise (DARE)

HIV/SIV continues to reprogram immune cells long after ART-mediated viral suppression. Changes can be separated into one program correlated with viremia and distinct set of late-ART changes.

RESULTS

Multiple unsupervised analysis methods identify unique gene programs that emerge in late ART samples, after 30 weeks of near-complete viral suppression

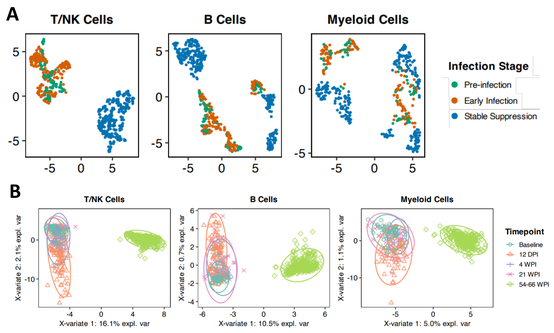


Fig. 1. Unsupervised analysis of scRNA-seq Data. A) PCA/UMAP demonstrates late ART samples are highly distinct from baseline, acute phase and early ART samples. **B)** sPLS-DA analysis identified orthogonal gene programs (components) between acute SIV and late ART samples. This indicates there are significant transcriptional changes that accumulate during ART-suppression.

Interferon-stimulated gene (ISG) module correlates with viral burden, dominates acute phase response, identifies immunosuppressive characteristics of gut-associated lymphoid tissue

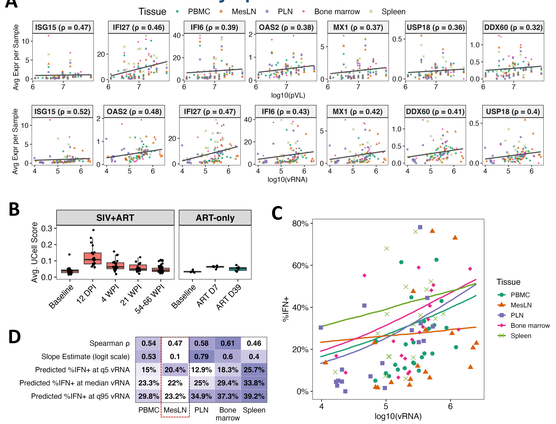


Fig. 2. Dominant gene signatures during acute and early ART phases. A) Top genes correlated with PVL and CA-vRNA at 12 DPI define a viral burden signature, dominated by interferon-stimulated genes (ISGs). **B)** Longitudinal average ISG scores, based on the identified gene signature, in PBMCs by cohort. (**C-D**) In Mes LN at 12 DPI, beta regression shows flatter slope and lower predicted %IFN+, indicating ISG signaling does not scale with increasing CA-vRNA in this tissue. This suggests a mechanism through which gut-associated lymphoid tissue supports enhanced viral persistence.

Plasma proteomics align with scRNA-seq data, identifies significant alterations specific to late-ART phase

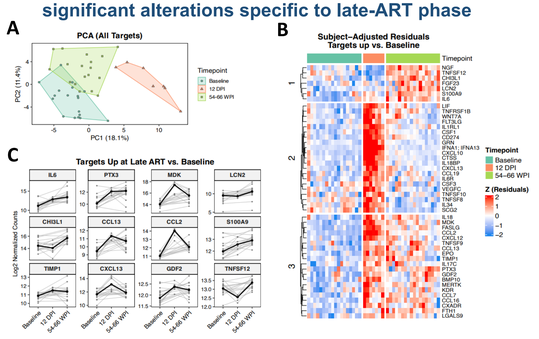


Fig. 3. Plasma proteomics identifies unique proteome alterations between acute SIV and late ART phases. All panels summarize Alamar NULISA plasma proteomics data. **A)** PCA on 119 inflammatory and immune plasma proteins separates acute phase and late ART signatures. **B)** Heatmap of subject-adjusted residuals (scaled within protein) from mixed-effects models for proteins identified as upregulated relative to baseline at acute infection and/or late ART. **C)** Per-subject trajectories (gray lines) and group medians (black line) for representative proteins significantly upregulated at late ART compared with baseline, shown as $\log_2$-normalized counts across baseline, acute infection, and late ART.

Unique change emerge late, after >30 weeks of near-complete viral suppression. Includes bone marrow inflammatory monocytes and broad increases in TGFβ and NF-kB

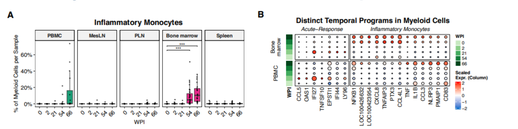


Figure 4. Bone marrow exhibits persistent dysregulation during late ART suppression. A) Proportions of inflammatory monocytes (% of myeloid cells per sample) across tissues over time, highlighting enrichment during late ART, most prominently in bone marrow and PBMC. **B)** Dot plot of scaled mean expression illustrating that the inflammatory monocyte signature is distinct from the acute infection response in myeloid cells, displayed separately for bone marrow and PBMC across WPI.

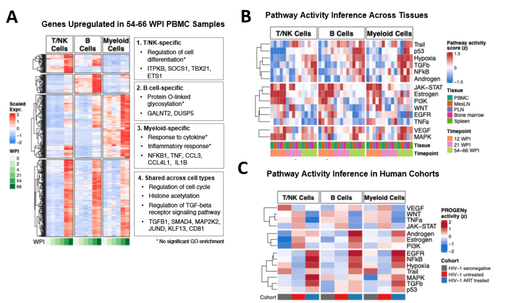


Fig. 5. Differential gene expression and pathway analysis identify TGF-β and NF-kB programs enriched in late ART phase. A) Clustered heatmap of genes upregulated during late ART timepoints relative to pre-infection and early infection, with representative genes and enriched GO terms annotated for each gene cluster. **B)** Pathway activity inference using decoupleR highlighting distinct programs induced during acute infection and late ART, including increased TGFβ, NF-kB, VEGF, and MAPK signaling at 54-66 WPI. **C)** Public scRNA-seq data from an HIV/ART cohort (Pollara et al. 2022) were processed and analyzed similar to **(B)**. The heatmap displays inferred pathway activity between the HIV/ART, HIV negative and HIV untreated groups. Pathways upregulated in the HIV/ART cohort are shared with SIV/Late ART.

CONCLUSIONS

Single-cell transcriptomic and plasma proteomic data demonstrate SIV/ART induced changes can be separated into to two distinct phases.

Temporal Findings:

- Acute and early ART phases characterized by interferon-stimulated programs, JAK-STAT, and TNF. Signatures correlate closely with plasma and cell-associated viral measurements.
  - These pathways decline rapidly as viremia is controlled but remain slightly elevated relative to pre-infection.
- Late ART (54-66 WPI) cells accumulate broad changes distinct from virus-correlated programs.
  - Their transcriptomes are more distinct from baseline than early infection and early ART cells. This includes an increase in the immunosuppressive TGFβ pathway.
  - Analogous pathway findings in HIV/ART cohort.

Tissue Findings:

- Gut-associated lymphoid tissue is a key site of viral rebound. We add to these studies by identifying tissue-specific blunting of the interferon response, suggesting localized immunosuppression.
  - This decrease in cell intrinsic immunity could contribute to a more permissive environment for viral persistence and replication, also providing a target for intervention.
- Bone marrow is the most overtly dysregulated compartment in Late ART, with changes emerging at 54-66 WPI, after ~30 weeks of viral suppression. Inflammatory monocytes expand in bone marrow, are initially absent from blood, with heterogeneity between subjects.
  - This population may be an important driver of the broad immune changes specific to late ART.

Individualized differences in the extent of reprogramming may impact curative strategies that rely on the host immune system for control or clearance of HIV.