HIV Suppression is Linked to Shifting Epigenetic/Transcriptome Features in Monocytes

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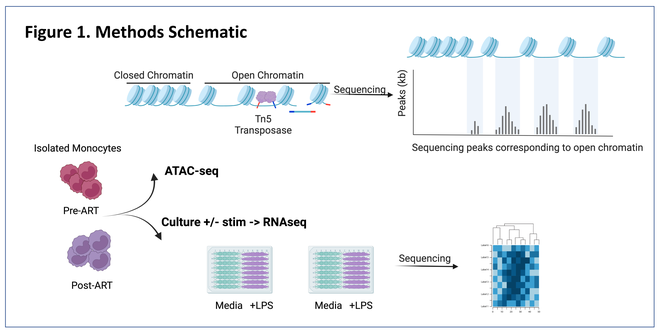
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BACKGROUND

Advanced HIV disease/AIDS is associated with susceptibility to a broad array of pathogens. This susceptibility includes both pathogens classically controlled by adaptive immune responses and those believed to be primarily controlled by innate immunity. In addition, there is immune dysregulation, with abundant production of inflammatory cytokines despite inefficient pathogen control. Notably, some of these deficits improve with viral suppression, prior to CD4 reconstitution. Consistent with this, we have shown enhanced monocyte cytokine production after viral suppression, linked to differences in histone marks[1]. *We hypothesized* that there would be global epigenomic changes in monocytes from pre and post antiretroviral therapy (ART) initiation, and that these changes would be linked to differences in transcriptional responses.

METHODS

Figure 1. Methods Schematic



20 participants (19M, 1F) with paired cryopreserved PBMCs from before and after the initiation of ART were selected. PBMCs were thawed, monocytes were isolated by CD14+ selection and were divided into two analyses (1) transcriptional response (RNAseq) after 24 hours of culture with or without LPS stimulation (2) direct ex vivo analysis of chromatin accessibility with ATACseq[2] (Figure 1). All sequencing was performed on the NovaSeq6000 platform. ATACseq analysis of mapped reads used Genrich for peak finding, Motif-discovery using MEME-chip, visualization of peak regions with ChIPseeker, and DiffBind was used to compute differentially bound sites between the pre and post-ART samples. RNAseq data was analyzed for differential gene expression both at baseline and after LPS stimulation between the pre and post-ART conditions

HIV viral suppression is linked to changes in regions of open chromatin in circulating monocytes. Some of these changes are mirrored in differences in both resting and LPS-induced gene expression.

RESULTS

Participants were predominantly male and 16 of 20 participants were within 6 months of HIV seroconversion (Table 1). Immune status was generally well preserved, with a median pretreatment CD4 count of 492 and a wide range of pretreatment viral loads. At the second time point, all were on treatment and <100 copies/mL with 95% of participants were at <50 copies/mL.

Analysis of the ATAC-seq data from isolated monocytes yielded a median of 26,000 peaks of accessible chromatin per sample. There were a median of 2700 unique pre-ART peaks and 6400 unique post-ART peaks per sample. Samples clustered according to pre- or post-ART status with only 3 exceptions (Figure 2).

Table 1. Participant characteristics

Sex	19 male, 1 female
Age	36.5 years (IQR 29.5, 47)
Median time between samples	11.5 months (IQR 6.4, 15.6)
Median pre-ART viral load	209,000 copies/mL (IQR 48,900; 2,130,000)
Median pre-ART CD4	492 cells/mm^3 (IQR 386, 589)
Median post-ART CD4	716 cells/mm^3 (IQR 485, 867)

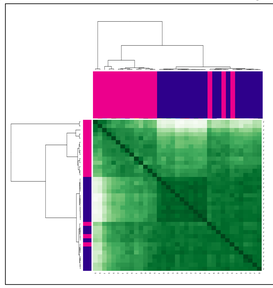


Figure 2. Differential peak analysis across all samples demonstrates that the majority of samples cluster based on pre (pink) or post-ART (deep purple) status.

Across the study there were 88 unique loci at an FDR<0.01 that demonstrated differential regulation based on pre- or post-ART status that were shared across all participants. Among differentially accessible peaks were two related to fatty acid metabolism in the acyl-coA synthetase family (Figure 3A, B) and a peak proximal to *IFI27* (Figure 3C and inset). In paired RNA-seq samples, *IFI27* was among the top differentially regulated genes in both resting and LPS-stimulated samples (log fold change 3.4, FDR<0.01 for both conditions). CCL22 also showed differential regulation in resting and LPS-stimulated conditions (log fold change -2 (media) and -1.8 (LPS-stim), both at FDR<0.01).

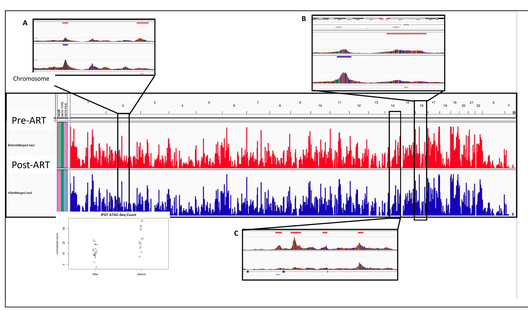


Figure 3. Concatenated ATAC-seq files showing the frequency of peaks across the chromosomes for pre-ART (red) and post-ART (blue) monocytes. Highlighted boxes demonstrate 3 of the 88 loci with differential accessibility based on ART treatment status at FDR<0.01. In the boxes, the red or blue lines indicate regions of significantly differential accessibility. Inset box shows the raw count comparison for the region proximal to *IFI27* in the two sample groups.

CONCLUSIONS

These studies suggest that HIV viremia is associated with differences in the epigenetic features of circulating CD14$^+$ monocytes. The functional significance of these differences is not known, but in both resting and stimulated conditions, one of the gene loci with conserved changes in epigenetic accessibility was also differentially regulated at the gene expression level (*IFI27). IFI27* is a putative adaptor protein in interferon signaling in innate immune cells and has been identified previously as a feature of HIV infection and also as a potential target for regulation of adipocyte energy metabolism[3]. Additional chromatin signals in the acyl-coA synthetase family suggest that innate function may be modulated by metabolic programming. Defining the determinants of innate immune dysfunction in HIV viremia may identify novel therapeutic targets for reducing dysregulated inflammation.

FUTURE DIRECTIONS

Future analysis will query whether there are more changes linked to viremia status obscured by inter-individual variations. Additional studies could explore whether specific innate phenotypes are linked to subsequent risk for development of immune reconstitution inflammatory syndrome after initiation of ART.

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Methods figure created with use of Biorender.