Spatial Mapping of SIV-Infected Rhesus Macaque Lymph Node Environment During Rebound

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BACKGROUND

Lymphoid tissues are important sites of the HIV-1 reservoir that contribute to viral rebound after analytical treatment interruption (ATI), and peripheral blood mononuclear cells may not reflect immune responses occurring in tissues. We hypothesize that different immune cell subsets exhibit distinct immune signatures during early versus late viral rebound in lymph nodes. Understanding these immune signatures and their respective immune cell subsets throughout viral rebound provides mechanisms for immune control.

METHODS

To understand immune responses during SIV rebound in rhesus macaques, we examined the transcriptional landscape in lymph nodes. From 2 female rhesus macaques intravaginally infected with SIVmac239 and treated with suppressive antiretroviral therapy (ART) for 6 months from 4 days post-challenge, we obtained 2 mesenteric lymph nodes each at 4 days and 24 days after ATI. These lymph nodes were SIV$^+$ by targeted PET-CT. To resolve lymphoid cell heterogeneity and their respective immune responses, particularly in the context of spatial interactions, we applied deterministic barcoding in tissues (DBiT-seq) to profile transcriptomes from 10 μm x 10 μm pixels. This state-of-the-art platform allows the closest estimate of single-cell transcriptomes at a genome-wide scale compared with commercially available platforms at 55 μm resolution. RNA-seq reads were mapped to the rhesus macaque genome (MacaM) using STAR and processed downstream using R scripts from DBiT-seq developers and custom scripts to integrate all samples together and determine gene set enrichment in early versus late viral rebound with GSEA.

Study design and regions analyzed

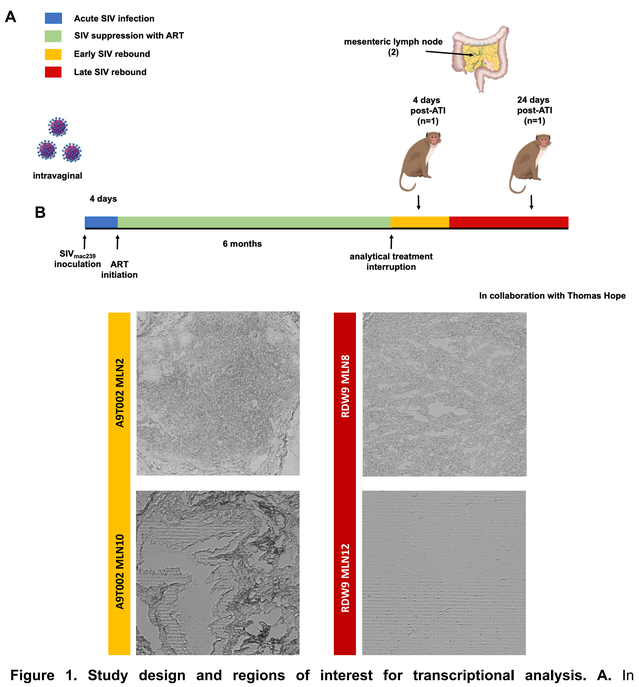


Figure 1. Study design and regions of interest for transcriptional analysis. A. In collaboration with Dr. Thomas Hope, female macaques were infected intravaginally with SIVmac239, initiated on ART on day 4, and removed from ART after 6 months. Lymph nodes (n=2 per individual) were dissected at day 4 post-ATI (n=1 macaque) and day 24 post-ATI (n=1 macaque). **B.** Images were taken at 10X magnification to show regions containing barcoded cDNA collected for processing and analysis. Regions are approximately 5 mm x 5 mm and contain 2500 individual 10 μm x 10 μm pixels. 9822 pixels total contained reads mapping to MacaM and were analyzed with a median of 265 reads and 163 genes per pixel.

Spatial transcriptomics method – DBiT-seq

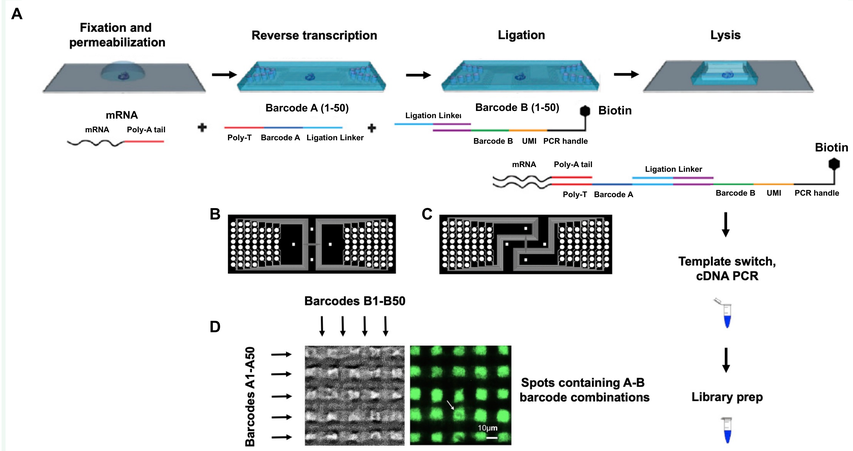


Figure 2. DBiT-seq enables spatial transcriptional profiling. A. Tissues centered on slides are fixed and permeabilized. Reverse transcription is primed with oligos containing barcodes A1-A50 flowed through horizontal channels (**B**) across tissues. Barcodes B1-B50 flowed through vertical channels (**C**) across the tissues are ligated to A barcodes on cDNAs. After tissue lysis, cDNAs are collected, amplified, and prepared in libraries for sequencing. **D.** Example of overlapping horizontal and vertical channels with fluorescence indicating areas with A-B barcode combinations. Figure is adapted from Liu *et al.*, *Cell*, 2021.

Spatial chromatin accessibility method – spatial-ATAC-seq

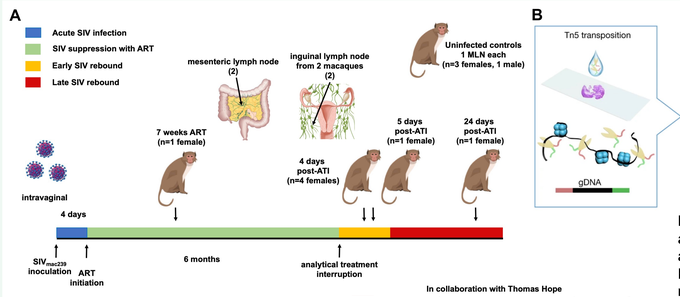


Figure 4. DBiT-seq platform has been adapted to examine chromatin accessibility in spatial context. A. Expanded study design will include lymph nodes from additional individuals at day 4 post-ATI (n=3 new macaques), day 5 post-ATI (n=1), and uninfected controls (n=3 females, 1 male). **B.** Tn5 transposition is performed on the whole tissue to insert ligation linkers into accessible genomic loci. Barcodes A1-A50 are ligated to these ligation linkers, and barcodes B1-B50 are ligated to A barcodes. The resulting gDNA fragments are collected from tissue after lysis, amplified, and prepared in libraries for sequencing. Figure is from Deng *et al.*, *Nature*, 2022. **C.** Consecutive sections from tissues in **Figure 1** were processed and showed the expected ATAC-seq peak pattern with TapeStation QC.

Enriched gene sets expressed in early and late SIV rebound

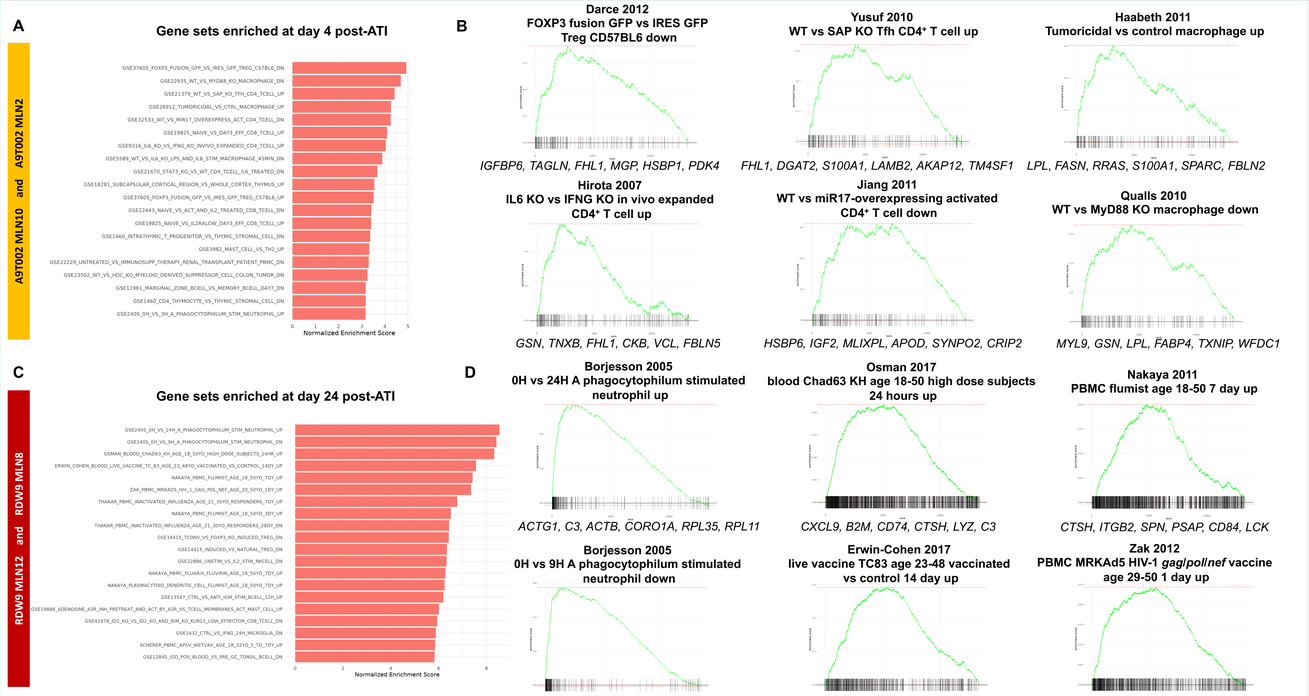


Figure 5. Gene set enrichment analysis reveals early CD4$^+$ T cell and macrophage responses and later immune effector cell responses to SIV rebound. RNA-seq reads from four regions shown in **Figure 1** were filtered, normalized, and integrated in Seurat for GSEA with 13,401 genes. **A.** The top 20 gene sets for day 4 post-ATI with the highest normalized enrichment score (NES) are shown (adjusted p<0.008 for all 20 pathways shown). **B.** Enrichment plots for the top six gene sets for day 4 post-ATI. The top six leading edge genes are listed below each enrichment plot. **C.** The top 20 gene sets for day 24 post-ATI with the highest normalized enrichment score (NES) are shown (adjusted p<0.008 adjusted p<0.008 for all 20 pathways shown). **D.** Enrichment plots for the top six gene sets for day 24 post-ATI. The top six leading edge genes are listed below each enrichment plot.

CONCLUSIONS

- DBiT-seq can reveal transcriptional differences in lymph node tissue at different time points of SIV rebound post-ATI.
- At 4 days post-ATI, we found enrichment in gene signatures for Tregs, cytotoxic macrophages, Tfh cells, and expanding CD4$^+$ T cells in lymph nodes.
- At 24 days post-ATI, we found upregulated gene signatures of stimulated neutrophils and vaccine responses to Leishmania, VEEV, influenza, and HIV-1 in lymph nodes.

Caveats

- Data sparsity from these samples after removing duplicate and unmapped reads hinders the ability to identify spatial locations by cell type. Modifications to the DBiT-seq protocol are being tested to improve data quality. Integration with a reference single cell RNA-seq dataset from rhesus macaque lymph node will further improve cell type identification.
- The limited number of tissue sections and individual rhesus macaques in this work could limit the generalizability of the results. To expand the study size, lymph nodes from additional SIV-infected rhesus macaques at 4 days post-ATI (n=3) and 5 days post-ATI (n=1) will be included.
- Lymph nodes from uninfected rhesus macaques have been processed in DBiT-seq and spatial-ATAC-seq as comparisons for SIV-infected lymph nodes.

Future Directions

- To understand how early SIV rebound affects chromatin accessibility in lymph node tissue with spatial resolution, we will combine DBiT-seq with spatial-ATAC-seq on consecutive tissue sections. This analysis could identify transcription factors that drive immune responses to early SIV rebound in lymph node tissue.

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