Deciphering the Source of Early Viral Rebound Upon ATI in SIV-Infected Rhesus Macaques

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Antonio Solis-Leal[1], Nongthombam Boby[1], Suvadip Mallick[1], Yilun Cheng[2], Fei Wu[1], Grey De La Torre[1], Xavier Alvarez[1], Vinay Shivanna[1], Jeffrey D Lifson[3], Qingsheng Li[2], Brandon F Keele[3], Jason Dufour[4], Binhua Ling[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, USA,
[2]Nebraska Center for Virology & School of Biological Sciences, University of Nebraska-Lincoln, NE, USA,
[3]AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, Maryland, USA
[4]Tulane National Primate Research Center, Covington, LA, USA,

BACKGROUND

An HIV cure is elusive due to the viral persistence in tissue reservoirs. Upon antiretroviral therapy (ART) interruption (ATI), the virus can reemerge from tissue reservoirs and spread through the body[1]. To develop better HIV therapies, a deeper understanding of the tissue and cellular origin of early rebound is critical. This study aims to better understand the sources of early rebound after ATI.

METHODS

The barcoded virus SIVmac239M[2] was used to infect 7 Chinese rhesus macaques. The animals were treated with ART for 42 weeks beginning at 12 weeks post-infection, followed by a 3-week ATI (ATI-1). ART resumed for 9-16 weeks, and a second ATI (ATI-2) was started one week before each individual necropsy (**Figure 1**). To evaluate potential sources of viral rebound, and possible cellular factors contributing to viral reactivation, we used quantitative real-time PCR to measure viral loads along the study. Deep sequencing and single genome amplification to detect viral barcodes in plasma and tissue DNA/RNA. Intact proviral DNA assay to quantify intact provirus in the reservoir. Immunofluorescence analysis, combined CODEX/RNAscope/in situ hybridization (Comb-CODEX-RNAscope) analysis to identify cell subsets producting vRNA, and single-cell RNA sequencing (scRNA-seq) to explore pathway activation.

RESULTS

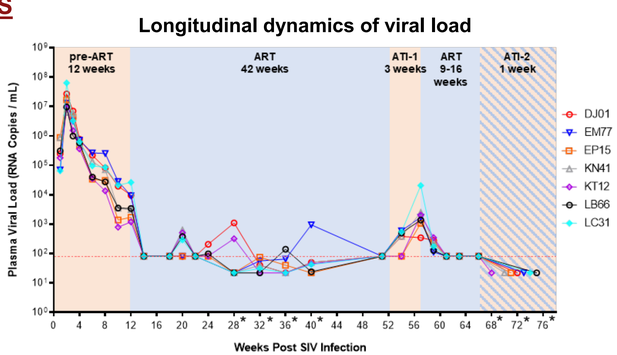


Figure 1: Experimental design and dynamics of SIV plasma viral load by quantitative RT-PCR of vRNA.

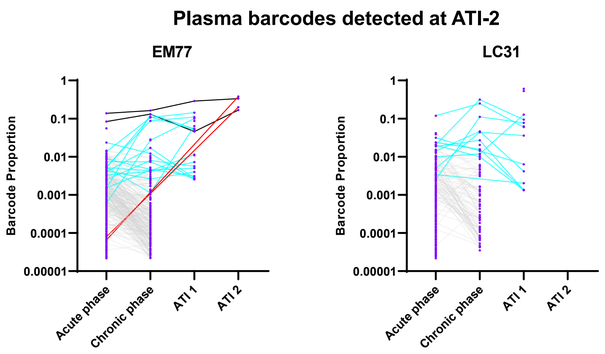


Figure 2: Detection of SIVmac239M barcodes in plasma by deep sequencing.

Despite pVL < 22 copies/ml at necropsy after ATI-2, Eleven viral barcodes were detected in plasma across the 4 animals at ATI-2 (PBDA2 group, **Figure 2**), with mesenteric lymph node (MesLN), inguinal lymph nodes (IngLN), and spleen exhibiting viral barcode clonotype overlapped with plasma viruses, viral barcode clonotypic diversity, a larger intact proviral reservoir size, and the production of vRNA detected by Comb-CODEX-RNAscope[3] (**Figure 3 and 4**). Moreover, scRNA-seq analysis revealed expression of genes related to immune activation, mitochondria damage, and epigenetic modulation in these 4 animals. While the remaining 3 animals (non-PBDA2 group) had no measurable rebound at ATI-2, evidence of virus was also found in lymphoid tissues identified in PBDA2 animals (**Figure 5**).

MesLN, IngLN and Spleen could be sources of plasma rebound after ATI

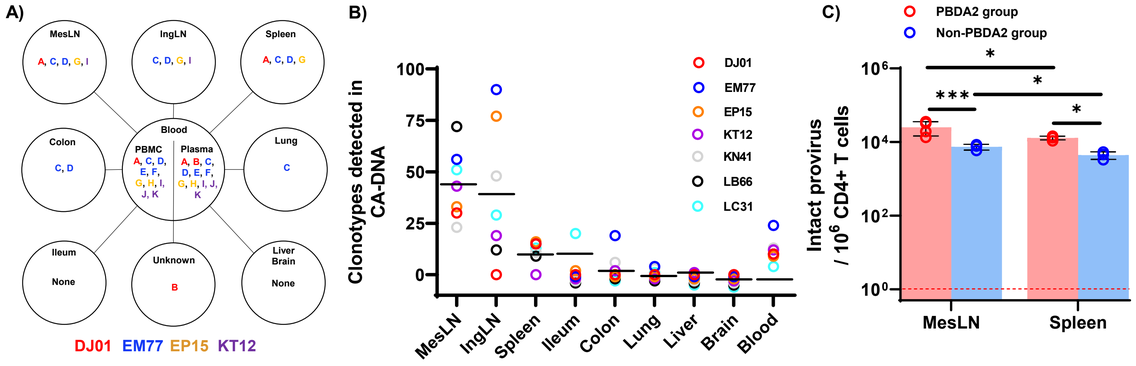


Figure 3: Viral barcodes and reservoir assessment. A) SIVmac239M barcode detection in CA-DNA from 9 tissues corresponding with plasma barcode detection at ATI-1 and ATI-2 in the PBDA2 group **B)** Tissue reservoir diversity from CA-DNA or -RNA at ATI-2. **C)** Distribution of SIV intact provirus in tissues at ATI-2

Higher viral expression detected in T cell zones of MesLNs

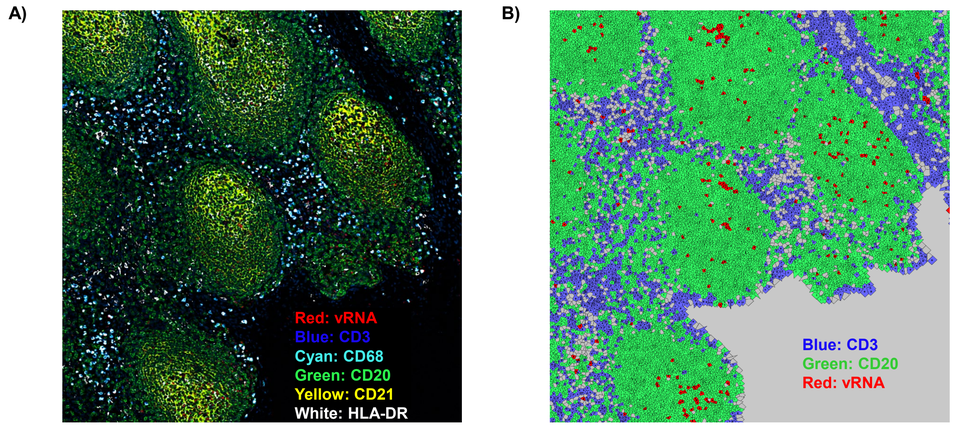


Figure 4. Com-CODEX-RNAscope from MesLN at ATI-2. Images of concurrent detection of multiplexed immunological protein markers and SIV vRNA in MesLN tissues using the Com-CODEX-RNAscope. **A**) A merged image of vRNA, CD68, CD3, CD20, CD21, CD31, HLA-DR from each animal. **B**) A Voronoi plot of the previous figure shows vRNA (red) distribution in the B cell zones (marked by CD20 in green) and T cell zones (Marked by CD3 in blue).

Differential gene expression in MesLNs with larger reservoirs

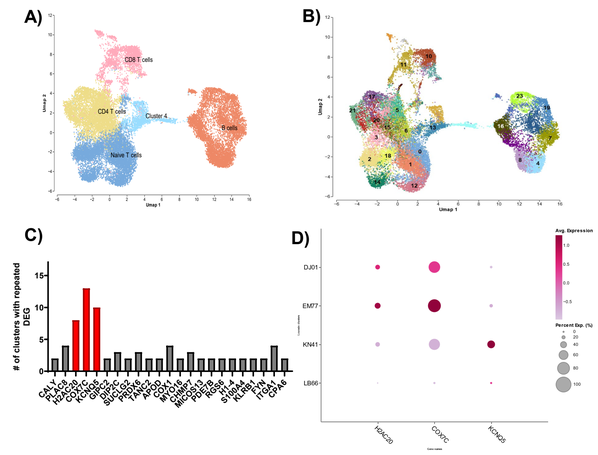


Figure 5. scRNAseq analysis of cells isolated from MesLN at ATI-2. A) Louvin clusters at 0.1 resolution. **B)** Louvin clusters at 2.0 resolution **C)** DEGs repeated across different louvin clusters at 2.0 resolution. **D)** Dot plot of the genes more frequently dysregulated across multiple louvin clusters.

CONCLUSIONS

- Barcoded rebound virus in plasma at the first week after ATI overlapped with virus found in lymphoid tissues, such as MesLN, IngLN, and spleen.
- Lymphoid tissues with higher levels of virus appear to be potential sources of early viral rebound.
- $CD4^+$ T cells in MesLNs and spleen could carry the largest amount of viral reservoir.
- Cells located in the T cell zone could be the leading source in MesLNs.
- Larger reservoirs in MesLN could be related to enhanced activation of cellular pathways related to viral latency and mitochondrial damage.

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AUTHOR CONTACT INFORMATION

Dr. Antonio Solis-Leal
Email: aleal@txbiomed.org Tlf: (210) 258 916