SIV-Producing Macrophages From the CNS Seed Deep Cervical Lymph Nodes After ART Interruption

396

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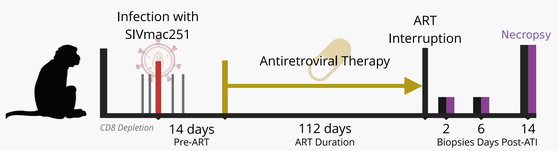
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INTRODUCTION

The cell types that contribute to viral rebound after antiretroviral therapy interruption (ATI) in people living with HIV or experimentally SIV-infected macaques are not well understood. Further, the mechanisms and impact of central nervous system (CNS) reservoirs[1] in viral recrudescence after ATI remain unclear. Lymphoid tissues serve as a key site for the establishment and maintenance of viral reservoirs[2]. Deep cervical lymph nodes (DCLN) serve as a drainage site for CNS meningeal lymphatic vessels. Recent studies have reported the migration of productively SIV-infected perivascular macrophages (PVM), a subset of CNS-associated macrophages, from the CNS via meningeal lymphatics to the DCLN[3]. This migratory pathways outlines a potential CNS-associated mechanism that contributes to the sustained viral persistence. Given these findings, we hypothesize that productively infected macrophages from the CNS contribute to the spread of infection after ATI.

METHODS

- Rhesus macaques were CD8 depleted and intrarectally infected with SIVmac251.
- Animals were ART suppressed for 112 days before treatment interruption, followed by necropsy 14 days later.



RNAScope *in situ* hybridization and immunohistochemistry. Formalin-fixed paraffin-embedded tissue sections (5 µm) were incubated with an SIVmac239-specific RNAscope probe to determine the types of SIV vRNA-producing cells, as previously described[4]. Tissue sections were then stained to detect T cells (CD3) and macrophages (CD68) or CD68$^+$ macrophages and Lyve1-expressing cells. Imaging was performed using a Nikon Eclipse Ti-E inverted microscope, and analysis was conducted in QuPath[5].

SIV-producing cells are primarily CD3$^+$ T cells post-ATI, and virus-producing CD68$^+$ macrophages, including those that can traffic from the CNS to the DCLN, contribute to viral rebound. These findings provide insights into HIV and SIV pathogenesis and may aid in HIV treatment improvement .

RESULTS

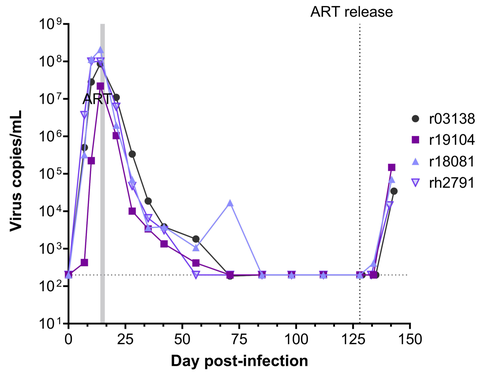


Figure 1. SIV rebound is detectable 14 days post-ATI. Viral copies per mL of plasma following successful ART suppression and viral rebound post-ATI before necropsy at 14 days, as determined by PCR. Dotted line is the minimum level of detection.

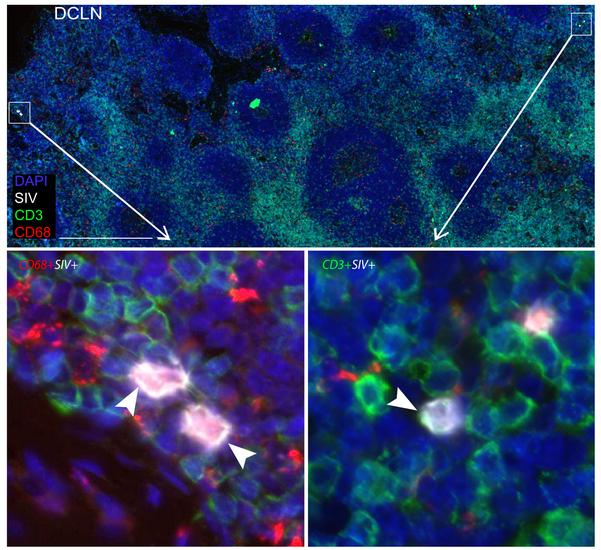


Figure 2. SIV vRNA$^+$ CD68$^+$ macrophages are present at high levels in the DCLN. Representative image from DCLN of animal r19104 with SIV vRNA$^+$ cells (white), CD3$^+$ T cells (green), and CD68$^+$ macrophages (red). White boxes indicate several SIV vRNA$^+$ cells, showing two vRNA$^+$ macrophages (left) and a vRNA$^+$ T cell (right) in close proximity to a vRNA$^+$ macrophage and are enlarged below. Scale bar = 200 µm.

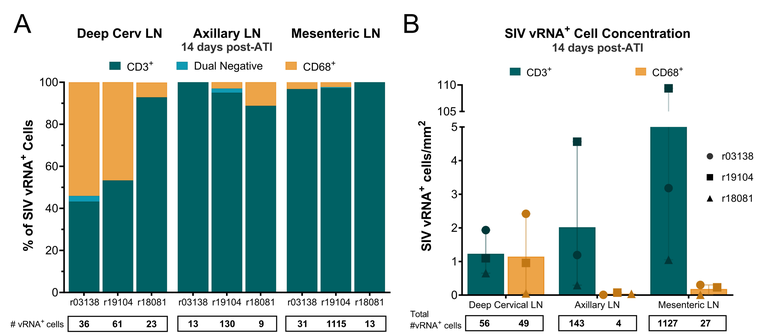


Figure 3. Type of SIV-producing cells in rhesus macaques at 14 days post-ATI varied by lymph node location. (**A**) SIV-producing cell type represented as a percentage of total SIV vRNA$^+$ cells. SIV$^+$ CD68$^+$ macrophages (orange) comprised a median of 46% (7-54%) in DCLN, but just 3.2% (0-3.5%) in AxLN and 1.5% (0-11%) in MesenLN. (**B**) SIV-producing cell type identification shown as the concentration of SIV vRNA$^+$ cells per mm^2. In DCLN, vRNA$^+$ macrophages comprised a median of 0.41 vRNA$^+$ cells/mm^2 (0.3-0.5) and T cells were observed at a similar concentration of 0.42 vRNA$^+$ cells/mm^2 (0.2-0.5). T cells had high levels of detection in AxLN and MesenLN, with concentrations of 0.2 and 4.3 vRNA$^+$ cells/mm^2, respectively. The median concentration of macrophages in AxLN and MesenLN was both zero cells/mm^2.

CONCLUSION

- Following successful viral suppression and treatment interruption, the predominant virus-producing cell type in lymph nodes 14 days post-ATI are CD3$^+$ T cells, and a smaller cohort of CD68$^+$ macrophages, suggesting early viral rebound is driven by T cells.
- A higher concentration of SIV-producing CD68$^+$ macrophages was found in DCLNs compared to non-CNS-associated lymph nodes indicating a portion of this population may migrate from the CNS.

FUTURE DIRECTIONS

- Continue delineating macrophage populations and virus-producing cells in DCLN, AxLN, and MesenLN.
- Repeat experiments in rhesus macaques that underwent 5-month necropsy following ART cessation and compare changes in virus-producing cell populations.
- Explore spatial transcriptomics and RNA-seq to further categorize vRNA$^+$ cells.

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REFERENCES

[1] Mohammadzadeh, N., et.al., (2023). Is the Central Nervous System Reservoir a Hurdle for an HIV Cure? *Viruses*, *15*(12), 2385. https://doi.org/10.3390/v15122385
[2] Connick, E., et.al., (2007). CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue. *Journal of immunology (Baltimore, Md. : 1950)*, *178*(11), 6975–6983. https://doi.org/10.4049/jimmunol.178.11.6975
[3] Alvarez, X., et. al. (2025). Simian Immunodeficiency Virus-Infected Macrophage Traffic Out of the Central Nervous System. *Research square*, rs.3.rs-6435804. https://doi.org/10.21203/rs.3.rs-6435804/v1
[4] Pampusch, M. S et, al., (2022). CAR/CXCR5-T cell immunotherapy is safe and potentially efficacious in promoting sustained remission of SIV infection. *PLoS pathogens*, *18*(2), e1009831. https://doi.org/10.1371/journal.ppat.1009831
[5] Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Scientific Reports* (2017).https://doi.org/10.1038/s41598-017-17204-5

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