# IL-21 and IL-15 Shape Distinct Neuroimmune Responses and Viral Persistence in SIV-Infected Brain

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## BACKGROUND

- Simian immunodeficiency virus (SIV) adapted to human hosts and evolved into highly-pathogenic HIV through extensive mutation and multiple cross-species transmissions.

- SIVmac251 is a strain of SIV originally isolated from rhesus macaques (Macaca mulatta). Native to Asia, they are not natural hosts of the virus and typically develop progressive disease, making SIVmac251 a well-established model for studying HIV pathogenesis in nonhuman primates.

## INTRODUCTION

**Clinical Research and Relevance**

- HIV-associated neurological diseases (HAND) are a consequence of chronic neuroinflammation and remain an unsolved health issue, despite complete control of viral replication with antiretroviral therapy.
- SIV is used extensively in research as a model to study HIV pathogenesis, immune responses, and develop potential treatments.
    - Our previous studies and others have demonstrated that SIVmac251 infection in rhesus macaques recapitulates many features of HIV disease progression in humans, including persistent viremia, immune dysregulation, and central nervous system (CNS) involvement.
- Understanding how immune modulation influences SIV persistence and CNS inflammation is crucial for developing therapeutic strategies targeting HAND.
- This study investigates how cytokine modulation with IL-21 and IL-15 differentially impacts viral persistence and neuroimmune signatures in SIVmac251-infected rhesus macaque brain tissue.

## METHODS

Rhesus macaques were infected intrarectally with a single high-dose ($5\times10^6$ IU) of SIVmac251.
Animals received IL-21 (50 µg/kg, SC), IL-15 (10 µg/kg, SC), or anti-IL-15 (5 mg/kg, IV) at 70- and 84-days post-infection (dpi); n=5 per group. Necropsies were performed 43–64 days after final treatment.

**A** **Immunostaining and imaging:**

- Peripheral viral loads were measured via **RT-qPCR** from longitudinal plasma samples.
- **RNAscope in-situ hybridization** to detect and localize SIVmac251 RNA, including colocalization with immune and vascular markers.
- Immune and inflammatory histopathology was evaluated via **ISH-IHC-IF** staining; area values were normalized relative to DAPI area per region.

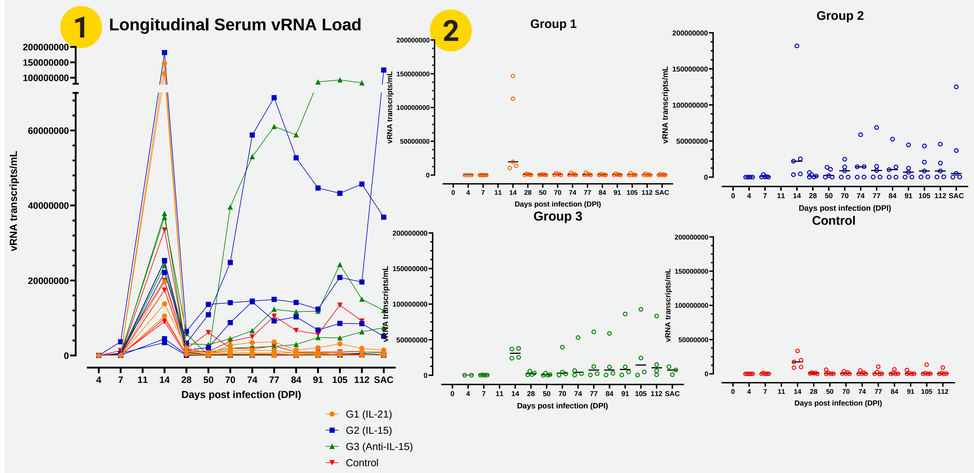
**B** **Analysis:**

- Fluorescence microscopy images were obtained at 40x magnification on a Keyence BZ-X800 system. 25-40 regions per specimen were sampled with consistent imaging settings and evaluated on the corresponding analysis software.
- Images were screened to meet preset quality criteria for analysis.

## RESULTS

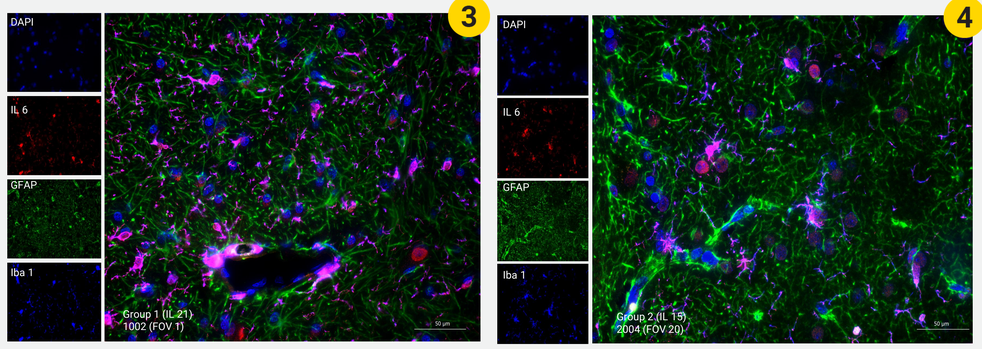
**Time course analysis of plasma viral load following SIVmac251 infection.**

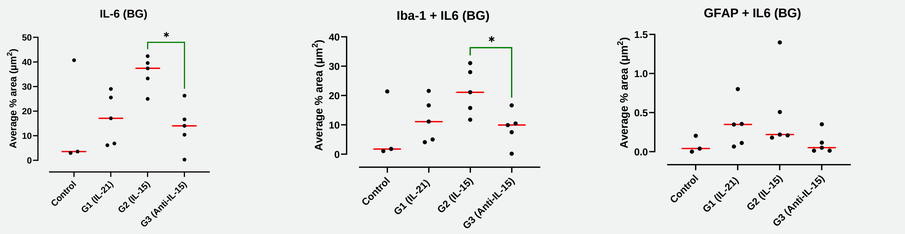
Figures 1, 2. Longitudinal RT-qPCR quantification of plasma SIVmac251 presence.



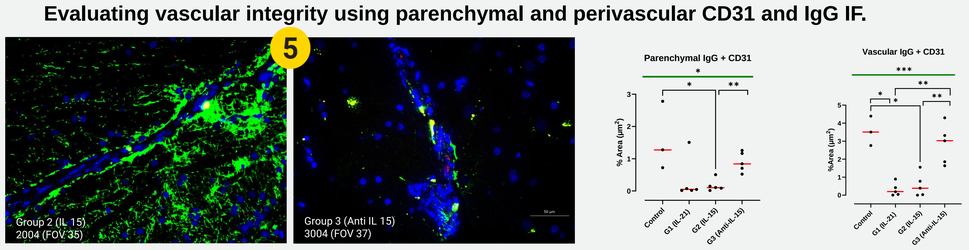

**IL-15 and IL-21 treatments differentially modulate IL-6 expression and microglial activation.**

Figures 3, 4. Immunofluorescence (IF) stain (40x) of IL-6, Iba-1, and GFAP in basal ganglia showed increased IL-6 and IL-6/Iba-1 colocalization in IL-15 treatment group relative to IL-21.




**Evaluating vascular integrity using parenchymal and perivascular CD31 and IgG IF.**




## RESULTS (cont'd)

**T cell (CD3e) and macrophage (CD163/68) vRNA+ area highlight divergent antiviral immune responses induced by IL-15 and IL-21.**

Figures 6, 7. IF stain (40x) of SIVmac251gag RNA transcripts (yellow), CD163/68 (red), and CD3e (green) in thalamic parenchymal regions of IL-21 and IL-15 groups, respectively.

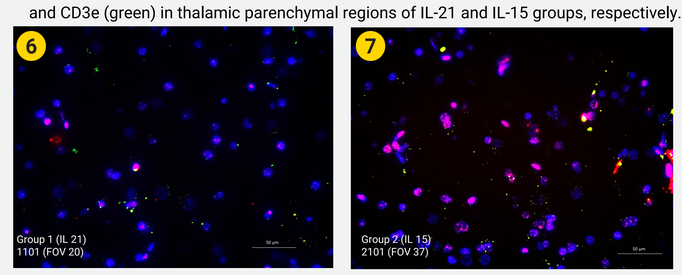
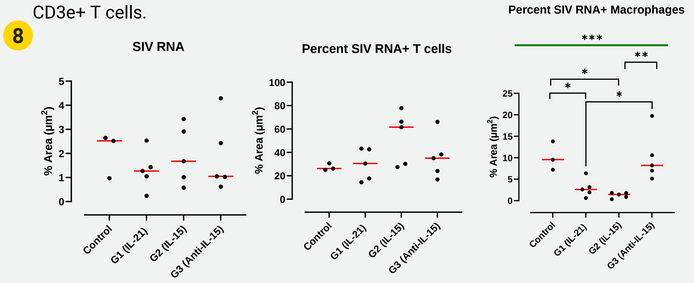



Figure 8. IL-21–treated animals exhibited modestly reduced total viral RNA levels compared to IL-15 and control groups, with a higher percentage of RNA+ area colocalized with CD163/68+ macrophages than IL-15 but lower than control and anti-IL-15, while IL-15 showed the highest percentage of RNA+ area colocalized with CD3e+ T cells.




**Anti-IL-15 treatment led to strong macrophage infiltration and significant TGFβ upregulation, suggesting a dysregulated or compensatory immune response compared to the more controlled environment seen in IL-15 and IL-21-treated brains.** This was evidenced by increased CD163/68+ macrophage area and TGFβ signal intensity in the basal ganglia, as well as notable TGFβ colocalization in the thalamus, indicating heightened immunoregulatory activity in regions with elevated viral burden.

## FINDINGS

- IL-21 significantly reduced plasma viremia (up to 100-fold decrease 14 dpi) and rapid viral control, despite elevated tissue vRNA in thalamus and cervical lymph nodes.
- IL-21 and IL-15 preserved BBB integrity, evidenced by reduced CD31/IgG colocalization in thalamic vasculature, unlike BBB compromise in control and anti-IL-15 groups.
- Anti-IL-15 induced neuroinflammation (increased CD163/68 and TGFβ expression) in basal ganglia and thalamus.
- IL-21 enhanced T-cell targeting of viral reservoirs; highest proportion of SIVmac251+ CD3e T-cells relative to total vRNA in thalamus and basal ganglia. IL-15 elevated glial and astrocytic activation (Iba-1, GFAP); IL-21 showed higher IL-6 and IL-6/Iba-1 colocalization.
- IL-15 and IL-21 shape immune responses and vascular integrity in the SIV-infected CNS, with IL-21 associated with reduced BBB leakage, fewer infected macrophages, and greater control of T cell-associated virus.

## DISCUSSION

Can IL-21 serve as a potential immunotherapeutic strategy for HAND by simultaneously suppressing CNS viral reservoirs and preserving BBB integrity?

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