TLRs Agonists and IL-15 Induce Intrinsic and Extrinsic Innate Anti-viral Immunity in CD4+ T Cells and Limits HIV Persistence

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Background and Hypothesis

1. **HIV persistence despite ART:** Although antiretroviral therapy (ART) effectively suppresses HIV replication, the virus remains hidden in latent reservoirs, such as lymph nodes, making eradication challenging.
2. **Role of interferons in HIV reservoir size:** The size of the HIV reservoir in CD4+ T cells is influenced by restriction factors downstream of type I and II interferons, which limit viral infection and integration into the host genome.
3. **Impact of ISG expression on HIV control:** Our single-cell analysis from human and non-human primate (NHP) studies shows that participants with better HIV control or smaller reservoirs had increased expression of interferon-stimulated genes (ISGs) in CD4+ T cells, linked to enhanced chromatin accessibility and transcriptional activation that restricts HIV integration.
4. **Induction of ISGs by TLR agonists:** We further observed that ISG expression can be induced by pathogen-associated molecular patterns (PAMPs) such as Toll-like receptor (TLR) agonists, specifically TLR3 and TLR7/8.

Hypothesis: We hypothesize that Activation of intrinsic innate antiviral immunity (IIAVI) in CD4+ T cells upregulates interferon response factors (IRFs), enhancing the accessibility and transcription of antiviral interferon-stimulated genes (ISGs). This response creates an innate anti-viral state that confers a poised antiviral response and reduces the likelihood of latent HIV reactivation upon exposure.

In vitro: IL-15 and IFNβ promote H3K27ac of genes involved in anti-viral immunity

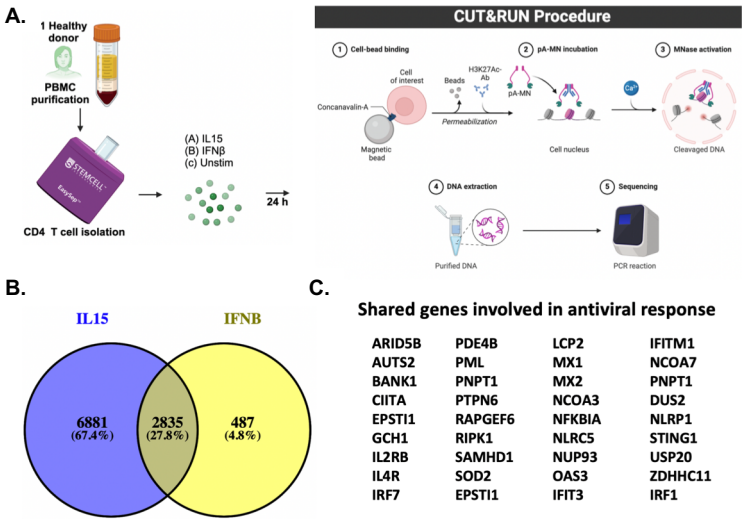


Figure 2. CD4 T cells stimulated with IL-15 and IFNβ for 24 hours promote H3K27 acetylation of a shared set of genes, which are involved in the anti-viral immune response by enhancing chromatin accessibility. (A) Schematic representation of the in vitro stimulation procedure, followed by the CUT&RUN assay protocol to identify histone modifications. (B) Venn diagram illustrating the overlap of genes exhibiting high H3K27ac enrichment between IL-15 and IFNβ stimulations. (C) List of co-regulated genes, demonstrating H3K27ac epigenetic modifications, playing a role in anti-viral immunity by modulating innate and adaptive immune responses.

In vitro: Not all TLRs limits HIV infection

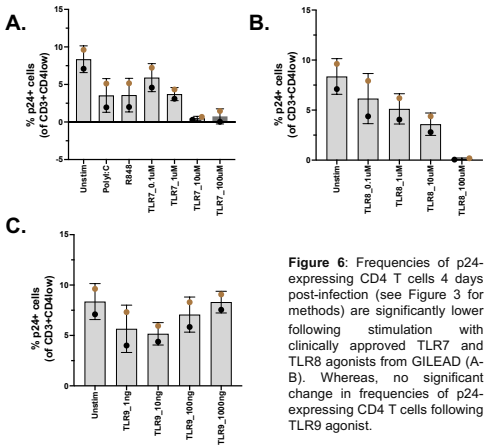
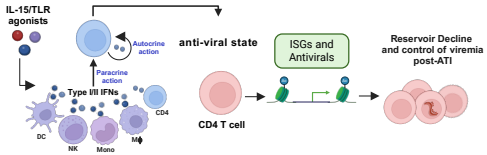


Figure 6: Frequencies of p24-expressing CD4 T cells 4 days post-infection (see Figure 3 for methods) are significantly lower following stimulation with clinically approved TLR7 and TLR8 agonists from GILEAD (A-B). Whereas, no significant change in frequencies of p24-expressing CD4 T cells following TLR9 agonist.

Conclusion and future directions



- Stimulation of CD4 T cells with IL-15 and IFNβ promotes H3K27 acetylation and an epigenetic program that triggers expression of genes involved in the anti-viral immune response.
- Co-culture of memory CD4 T cells with CD8-depleted PBMCs and stimulation with IL-15 and TLR agonists induces an innate anti-viral response by promoting expression of ISGs which protect these cells from HIV infection.
- Ongoing: Multiomic molecular studies are underway to elucidate the mechanisms that epigenetically train CD4 T cells to resist infection.
- Future: Investigating the in vivo use of TLR agonists and IL-15 in prophylactic or therapeutic interventions to target the HIV/SIV reservoir.

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In vivo: IL-15 drives ISG expression

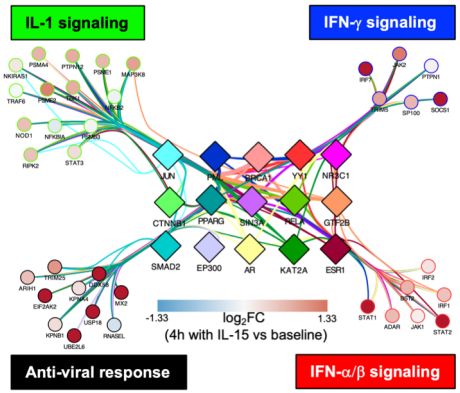


Figure 1: Naïve non-human primates were administered Het-IL-15 intravenously. Whole blood transcriptomics conducted at 0 and 4 hours post-treatment revealed significant upregulation (red circles) of interferon-stimulated genes downstream of both type I and type II interferon response factors (diamonds). This upregulation was observed even after repeated stimulation, with doses up to 32-fold higher.

In vitro: IL-15/TLR stimulation promotes ISG expression & limits HIV infection

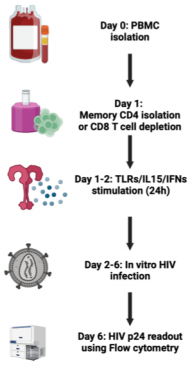


Figure 3: In vitro stimulation and HIV infection protocol.

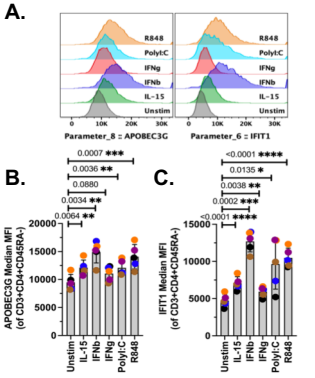


Figure 4: Memory CD4 T cells in CD8 depleted PBMCs stimulated with TLR3 agonist (Poly I:C) and TLR7/8 agonist (R848) for 24h express higher ISGs. (A) Representative flow cytometry plots. Mean fluorescence intensity of APOBEC3G (B) and IFIT1 (C) following stimulation with each agonist.

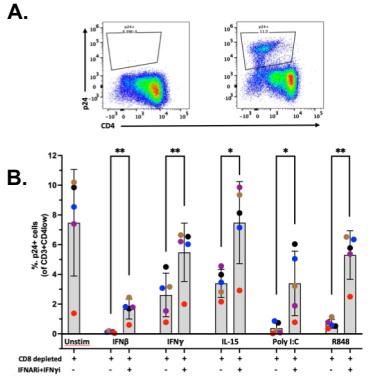


Figure 5: Frequencies of p24-expressing CD4+ T cells at 4 days post-infection (see Figure 3 for methods) are significantly reduced following stimulation with TLR agonists and IL-15 (A-B). This effect is reversed in presence of anti-IFNAR and anti-IFNγ. Representative flow cytometry plots illustrate p24 expression in memory CD4+ T cells cocultured with CD8-depleted autologous PBMCs (A).