EMORY NATIONAL PRIMATE RESEARCH CENTER

Dual IL-15/IL-21 treatment enhances CD8 T and NK cell function, promoting viral control post-ART interruption

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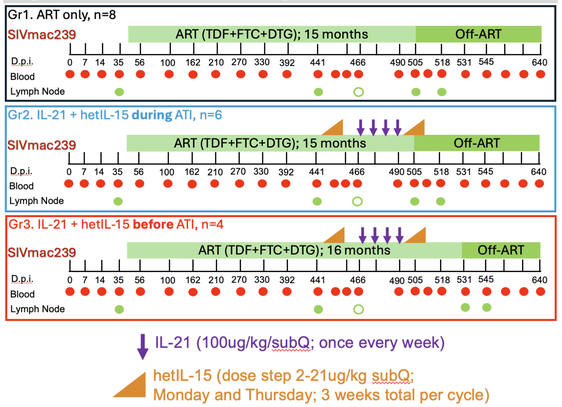
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BACKGROUND

HIV persistence within cellular and anatomical viral reservoirs remains the principal barrier to an HIV cure, with viral rebound occurring in most people with HIV following antiretroviral therapy (ART) interruption (ATI). This highlights the need for novel strategies that can reduce reservoir size while enhancing host immune control. CD8$^+$ T cells and natural killer (NK) cells, key effector populations capable of eliminating infected cells, often exhibit functional impairment and altered differentiation despite long-term suppressive ART. Within lymph nodes (LNs), major anatomical sites of viral persistence, CD8$^+$ T cells are predominantly non-cytolytic, and NK cells are present at low frequencies, limiting their capacity to eliminate infected target cells *in vivo*. Given their complementary functions, we tested a dual heterodimeric IL-15 (hetIL-15) and IL-21-Fc treatment in SIV-infected, ART-suppressed rhesus macaques (RMs) to determine its effects on immune responses, reservoir persistence, and viral control following ATI.

METHODS

Fig. 1. Experimental design

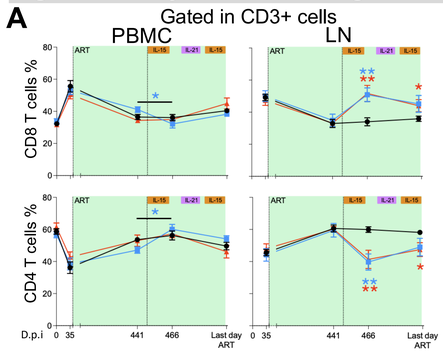


Eighteen RMs infected with SIVmac239 started ART 6 weeks post-infection (p.i.). After 13 months on ART, 10 RMs received two cycles of hetIL-15 (step-dose, 2-21 µg/kg, six subcutaneous [s.c.] injections every 3-4 days) and one cycle of IL-21-Fc (four weekly s.c. injections), after which two treatment groups were established. 6 RMs discontinued ART during the second hetIL-15 cycle (arm 1) and 4 RMs discontinued ART 20 days after its completion (arm 2). 8 RMs remained on ART-only (arm 3). Plasma viral load and intact proviral DNA were quantified. Phenotypic and transcriptional profiles of T and NK cells in blood and LNs were assessed by flow cytometry and single-cell RNA sequencing.

Appropriately timed hetIL-15/IL-21 therapy drives proliferative, cytotoxic CD8 T and NK cell programs, coupled to IFN-I transcriptional signatures, which are associated with reduced lymph node reservoirs and improved viral control after ART interruption.

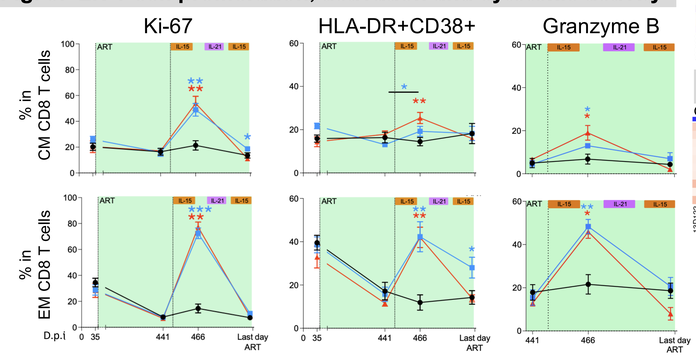
RESULTS

Fig. 2. T cell dynamics following dual IL-15/IL-21 treatment



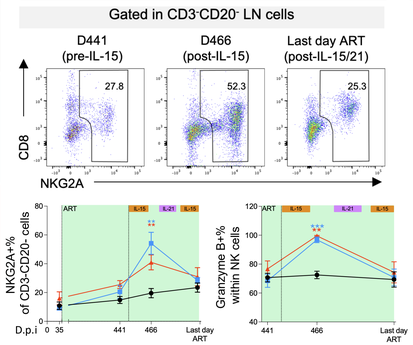
At day 466 p.i., hetIL-15/IL-21 treated RMs displayed a significant increase in LN CD8+ T cell frequency (Fig. 2A). Treatment induced a potent, significant, and sustained increase in LN EM CD8+ T cells as compared to controls (Fig. 2B).

Fig. 3. LN T cell proliferation, activation and cytotoxic activity



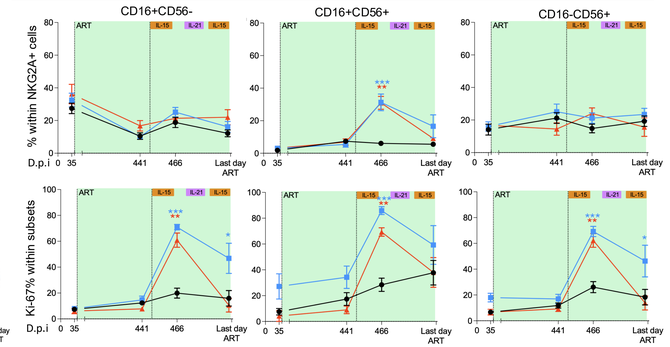
- Transient increase of proliferation (Ki-67+) and activation (HLA-DR+CD38+) of both CM and EM CD8+ T cells; not maintained at the last day of ART.
- Transient increase of cytotoxic (Granzyme B+) CD8+ T cells

Fig. 4. LN NK cell dynamics



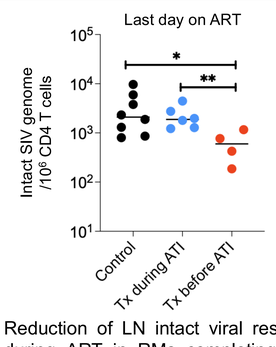
Transient increase in NK cell frequencies and associated cytotoxic potential in LNs.

Fig. 5. LN NK cell subset dynamics and proliferation



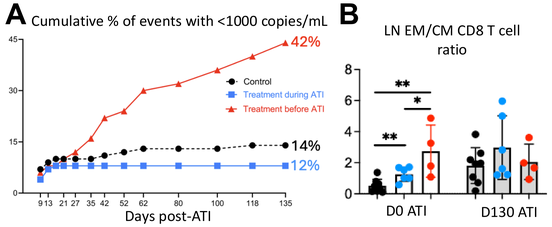
- Notable expansion of CD16+CD56+ subsets following the first hetIL-15 cycle
- Ki-67 upregulation across all NK subsets, persisting in arm 2 animals

Fig. 6. LN viral reservoir size



Reduction of LN intact viral reservoirs during ART in RMs completing hetIL-15/IL-21 before ATI (arm 3), but not with treatment extended after ATI (arm 2).

Fig. 7. Post-ATI virological control associated with higher LN EM CD8 T cells



Post-ATI controllers were distinguished by higher pre-ATI effector CD8 T cells.

Fig. 8. LN single-cell RNAseq from post-ATI controllers vs non-controllers

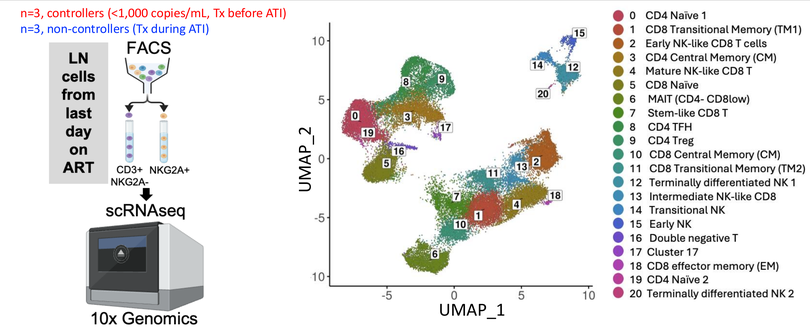


Figure 9. Interferon-associated gene expression

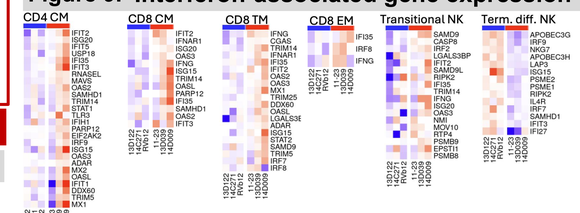
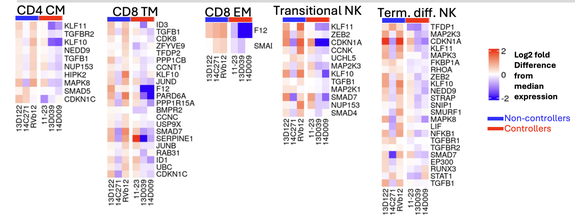


Figure 10. TGFβ-associated gene expression



At the last day of ART (D0 ATI), controllers are characterized by a multi-lineage interferon-stimulated transcriptional program (Fig. 9), whereas non-controllers display a TGF-β-driven immunosuppressive signature (Fig. 10), a profile that may favor viral persistence.

CONCLUSION

These findings demonstrate that hetIL-15 and IL-21 administration potently enhances antiviral immunity in lymphoid tissues, identify IFN-driven responses as critical for HIV control, and provide a mechanistic support for cytokine-based strategies toward durable HIV remission.

ACKNOWLEDGMENTS

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PLAIN LANGUAGE SUMMARY

Pairing two immune-boosting molecules strengthens key virus-fighting immune cells and helps to control the virus after ART is stopped. The timing matters: giving this boost before stopping ART led to better viral control.

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