ScRNA-seq to characterize NK-like CD8 T and NK cell subsets in lymph nodes of IL-15/IL-21 treated, ART-suppressed SIV-infected macaques

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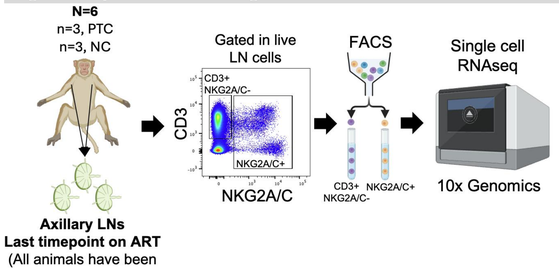
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BACKGROUND

In HIV/SIV infection, CD8 T and natural killer (NK) cells are critical antiviral effectors, yet the exact mechanisms for their inability to mediate viral control remains unclear and a key challenge for curative strategies. We used single-cell transcriptomics to dissect the heterogeneity of CD8 T and NK cells within lymph nodes (LNs) of SIV-infected, ART-treated rhesus macaques (RMs). A detailed definition of effector transcriptional programs in LNs, a key anatomic site for HIV persistence, is needed to better understand their contribution to HIV control and for implementing therapeutic strategies promoting the ability of those cells to control HIV in absence of ART.

METHODS

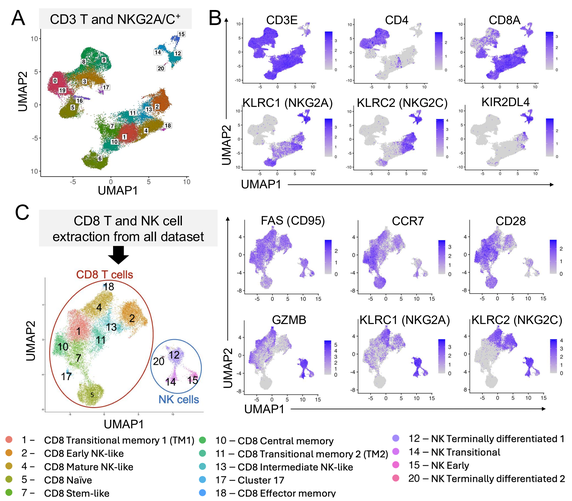
Fig. 1. Experimental design



We characterized transcriptional signatures of CD8 T and NK cell subsets in LN samples from ART-suppressed, SIV-infected RMs dual treated with IL-15+IL-21, in which post-treatment controllers (PTC; n=3; VL<1,000 copies/mL) and non-controllers (NC; n=3) emerged after ART interruption (**see poster #310**). Single-cell RNA sequencing was performed on CD3 T (CD3$^+$NKG2A/C$^-$) and NKG2A/C$^+$ cells FACS-sorted from LNs collected at the final day of ART.

RESULTS

Fig. 2. LN CD8 T and NK cell subsets identification

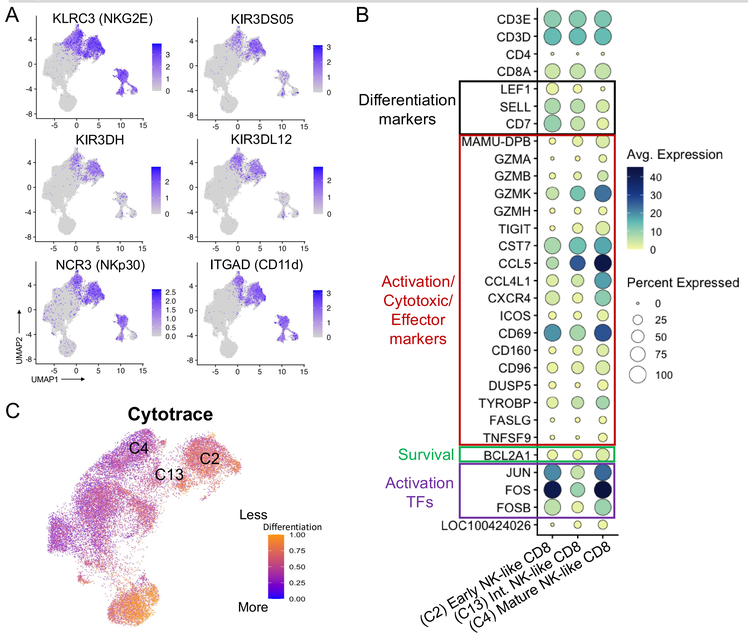


Single-cell analysis identifies distinct LN CD8 T cell and NK cell subsets. Clusters reflect lineage and differentiation state. Interestingly, a large proportion of memory (FAS/CD95+) CD8 T cells express NKG2A and NKG2C (C2, C4, C13).

Single-cell analysis identifies lymph node NK-like CD8 T cell and NK cell subsets with distinct effector transcriptional programs associated with post ATI outcomes.

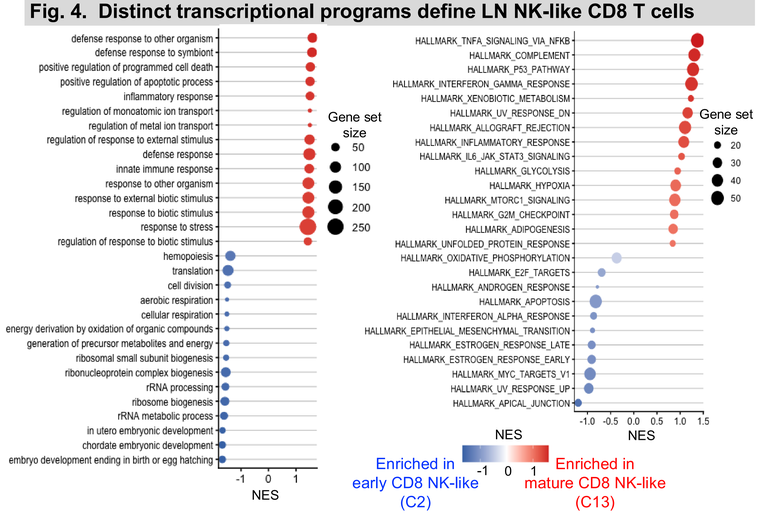
RESULTS

Fig. 3. Identification of LN NK-like CD8 T subsets in ART-treated, SIV$^+$ RMs



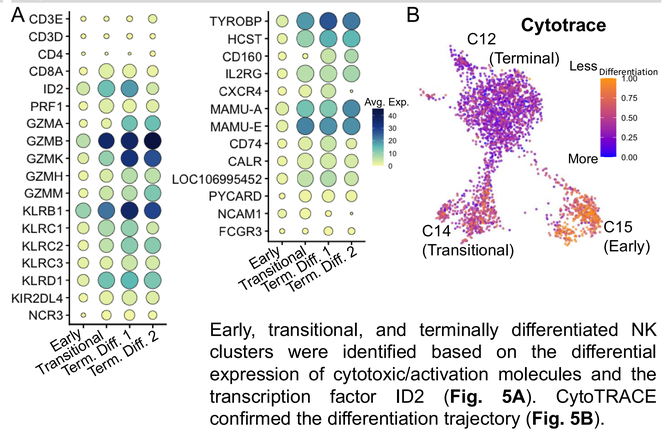
We identified NK-like CD8 T cells expressing NK markers (**Fig. 3A**) and classified them as early (C2), intermediate (C13), and mature (C4) based on differentiation and effector gene expression (**Fig. 3B**). CytoTRACE analysis confirmed the cell trajectory (**Fig. 3C**).

Fig. 4. Distinct transcriptional programs define LN NK-like CD8 T cells



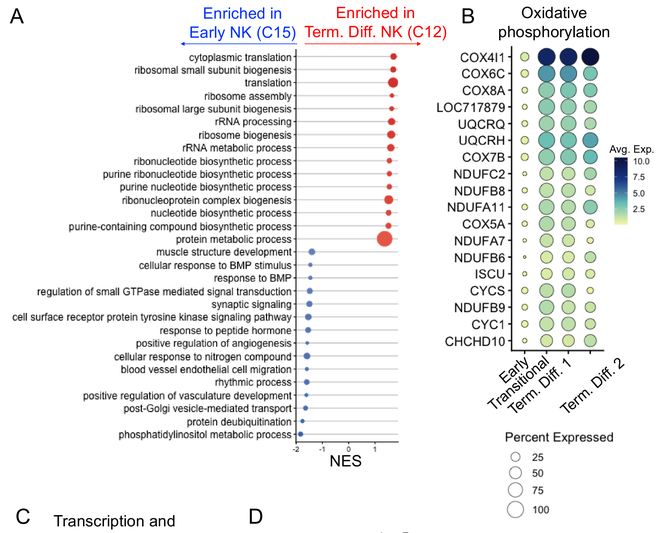
Pathway analysis reveals distinct transcriptional programs in NK-like CD8 T subsets. Mature subsets are enriched for immune activation pathways, whereas early subsets are enriched for metabolic and translational/biosynthetic pathways.

Fig. 5. Identification of LN NK cell subsets in ART-treated, SIV$^+$ RMs



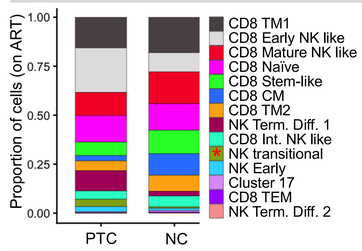
Early, transitional, and terminally differentiated NK clusters were identified based on the differential expression of cytotoxic/activation molecules and the transcription factor ID2 (**Fig. 5A**). CytoTRACE confirmed the differentiation trajectory (**Fig. 5B**).

Fig. 6. Distinct transcriptional programs define LN NK cell subsets



LN NK cell subsets display distinct transcriptional programs across differentiation states associated with metabolic and regulatory pathway shifts (**Fig. 6A**). Compared to early NK cells, terminally differentiated NK cells are enriched for ribosomal activity (translation) (**Fig. 6A**), oxidative phosphorylation (**Fig. 6B**), transcriptional and chromatin regulation (**Fig. 6C**) and inflammatory (**Fig. 6D**) signaling programs.

Fig. 7. Enrichment of transitional NK cells in PTC RMs



Transitional NK cells were the only population significantly enriched on ART in post-treatment controller (PTC) RMs, while all other clusters showed comparable proportions (propeller test).

Fig. 8. Distinct transcriptional programs in PTC and NC



PTCs displayed elevated interferon-stimulated associated genes in early NK-like CD8 T and transitional NK subsets. In contrast, non-controllers (NCs) showed enrichment of TGF-β signaling and lipid metabolism pathways in mature NK-like CD8 T cells, transitional NK cells, and terminally differentiated NK cells, along with JAK-STAT signaling in mature NK-like CD8 T cells and apoptotic signaling in transitional and terminally differentiated NK cells.

CONCLUSIONS

This study uncovers transcriptionally distinct NK-like CD8 T cell and NK cell subsets within lymphoid tissues, providing a high-resolution map of effector heterogeneity in ART-treated, SIV-infected RMs. Our findings suggest that distinct transcriptional programs, rather than subset frequencies, are associated with post-ATI outcomes. This work refines our understanding of immune mechanisms relevant to HIV persistence and control.

ACKNOWLEDGMENTS

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PLAIN LANGUAGE SUMMARY

Differences in how immune cells function in lymphoid tissues help explain why some animals control the virus when ART is interrupted.

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