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Decoding The Transcriptional Programs Governing Natural Killer Cell Memory Establishment

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DECODING THE TRANSCRIPTIONAL PROGRAMS GOVERNING NATURAL KILLER CELL MEMORY ESTABLISHMENT

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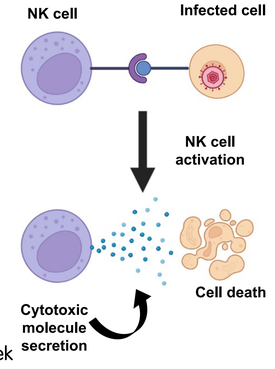
Abstract

Natural killer (NK) cell memory can be established in response to short-term stimulation with myeloid-derived cytokines, specifically, IL-12, IL-15, and IL-18. Memory NK cells have been shown to produce more interferon-gamma and display higher cytotoxic function upon re-challenge, compared to unstimulated NK cells, much like adaptive cytotoxic T-cells. Memory NK cells have been demonstrated to display increased killing of leukemic tumor cells and are currently in clinical trials for cancer cellular therapy. Other recent studies have also identified that memory NK cells expand in response to infections, such as tuberculosis and HIV-1. However, it remains unclear the precise transcriptional programs that govern the transition of NK cells to memory NK cells and how this this long-term cellular phenotype is preserved. Here, we utilized single-cell RNA-sequencing (scRNA-seq) to study the dynamics of the transcriptome of NK cells during memory establishment. We stimulated human peripheral blood NK cells from five distinct donors overnight with the myeloid cytokine cocktail, or a basal amount of IL-15 as a control, and performed scRNA-seq at two, three- and eight-days post-stimulation. We observed alterations in metabolic genomic signatures, including an upregulation of reduction-oxidation transcripts associated with oxidative stress mitigation in the primed samples at days two and eight, indicating key changes in metabolic pathways. Defining the NK cell transcriptome during memory establishment will reveal potential targets for exploitation during vaccine design against pathogens such as HIV-1, or to overcome barriers related to immunotherapy. Our findings define novel transcriptional trajectories for memory NK cell differentiation up to one week post exposure and suggest that resting memory NK cells readily express metabolic machinery associated with enhanced cytotoxic function and cytokine secretion.

Background

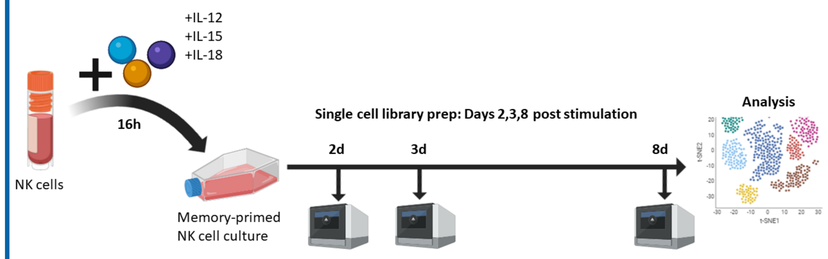
- Natural killer (NK) cells are cytotoxic lymphocytes that kill infected and cancer cells (1).
- NK cells have historically been considered as members of the innate immune system and lack the ability to establish immunological memory
- Recent reports have elucidated that memory-like states can be induced in human NK cells via stimulation with cytokines, or viral antigen (2,3,4).
- Memory NK cells are better at killing virus and cancer than naïve NK cells (2,5).
- The transcriptional mechanisms governing NK cell memory establishment are poorly understood
- **Memory NK cells could potentially be induced with a vaccine against infectious diseases such as HIV.**
- We performed single-cell RNA sequencing on NK cells up to 1 week following a memory-priming stimulation with cytokines *ex vivo*.



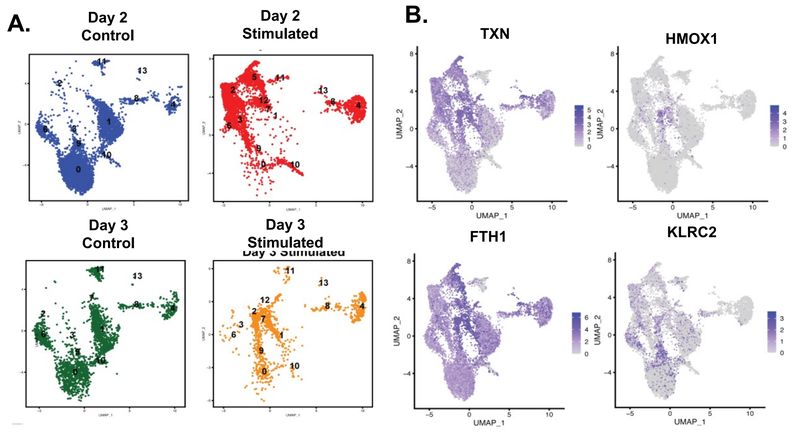
Purpose

To define the transcriptional programs regulating NK cell memory establishment.

Experimental Approach



Memory-primed NK cells upregulated transcripts associated with oxidative stress mitigation



A. UMAP projections from single cell RNA analysis of control (left) and stimulated (right) NK cells at Days 2 and 3 post-stimulation. **Clusters 7 and 12 expanded in memory-primed NK cells** vs control.

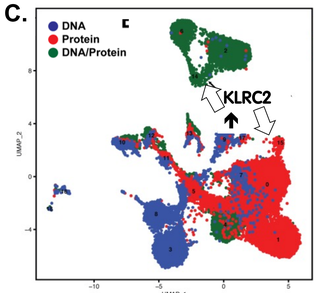
B. Composite image of Figure A. The **redox-associated transcripts** thioredoxin (TXN), heme oxygenase 1 (HMOX1), and Ferritin Heavy Chain 1 (FTH1) were **upregulated in memory-primed NK** cell subsets. Killer Cell Lectin Like Receptor C2 (KLRC2), a **marker of memory** in NK cells, was also **upregulated in memory-primed NK** cell subsets.

Specific HIV vaccine platforms induced expression of NK cell memory marker

	Vaccine administration timpoints (months)			
	0	1	3	6
Cohort 1 (n=26)	Protein	Protein	DNA	DNA
Cohort 2 (n=26)	DNA	DNA	Protein	Protein
Cohort 3 (n=26)	DNA	DNA	Protein + DNA	Protein + DNA
Cohort 4 (n=26)	Protein + DNA	Protein + DNA	Protein + DNA	Protein + DNA
PBMC collection	X	n=104	X	n=104

HVTN-105 cohorts and PBMC samples. 4 distinct cohorts received combinations of either DNA, Protein, or both, at 0,1,3, and 6 months. PBMCs were collected 2 weeks post the second and fourth timepoint, n=208 samples.

C.



C. UMAP of scRNA analysis of **NK cells** derived from the blood of individuals in the HVTN-105 **HIV vaccine trial**.

KLRC2 partially **defined memory-like clusters** prominent in individuals given a combination DNA and protein vaccines (green), or only Protein vaccines (red).

Conclusions

- **Stimulated NK cells display a unique, memory-primed transcriptional profile**
- **Redox enzymes associated with oxidative stress mitigation are upregulated in memory-primed NK cells**
- **DNA/Protein and Protein vaccine platforms elicit NK cell memory marker expression in humans**

Future directions

Future studies will mechanistically target TXN, HMOX1, and FTH1 to determine **if oxidative stress mitigation is critical for NK memory formation** induced both *ex-vivo* with cytokines, or *in-vivo* via vaccination.

Acknowledgements and References

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