Innate NK Cell Recognition Drives Killing of HIV-Expressing Authentic Reservoir Clones

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INTRODUCTION

- HIV-1 persists by integrating into mCD4⁺ T cells that **clonally expand to become the viral reservoir**
- Reservoir cells are incredibly **rare in circulation**, representing approximately 10-100 per million CD4⁺ T cells
- **NK cells are essential for long-term viremic control**
- Known mechanisms by which NK cells identify target, HIV-infected cells include **recognition of stress ligands via NKG2D** and natural cytotoxicity receptors, disinhibition by decrease of regulatory surface HLA proteins (**"missing self"**), and antibody-dependent cellular cytotoxicity (**ADCC**)
- Assessments of the interactions between the immune system and cells harboring intact (or defective) integrations are largely **associative in nature**
- The rarity of such targets and historic inability to isolate them for long term culture has limited the field's ability to functionally assess their susceptibility to immune-mediated elimination
- We have developed a method to **isolate, clone, and expand Authentic Reservoir Cells (ARCs)** from PLWH to functionally assess mechanisms of resistance to NK cell-mediated elimination

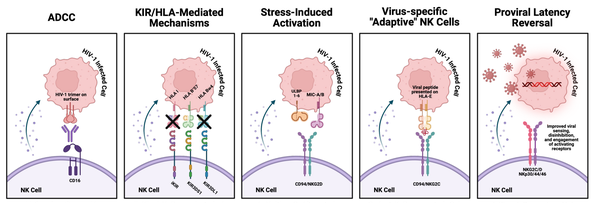


Figure 1. Mechanisms by which NK cells identify and eliminate HIV-infected cells. Availability of ARCs allows for these mechanisms to be assessed *in vitro* between reservoir cells and autologous NK cells.

METHODS

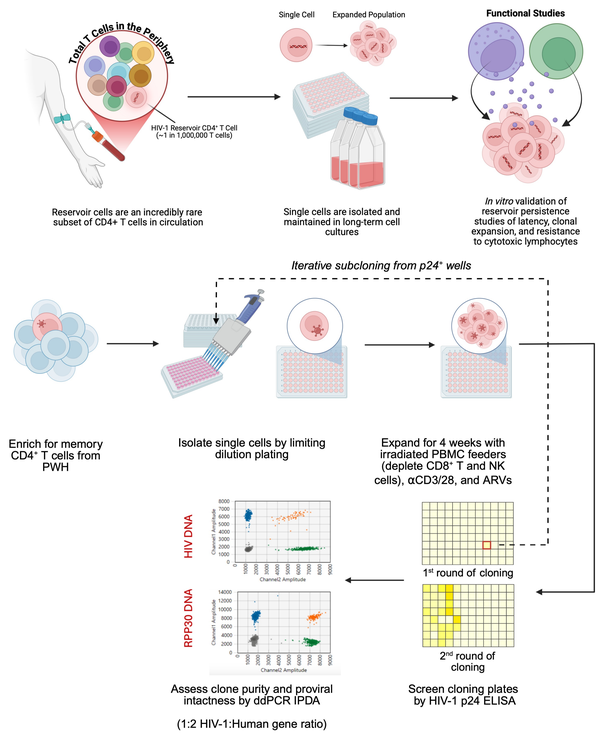


Figure 2. Schematic for the isolation, cloning, and characterization of HIV-1-infected mCD4+ T cell clones from the viral reservoirs of PLWH.

RESULTS

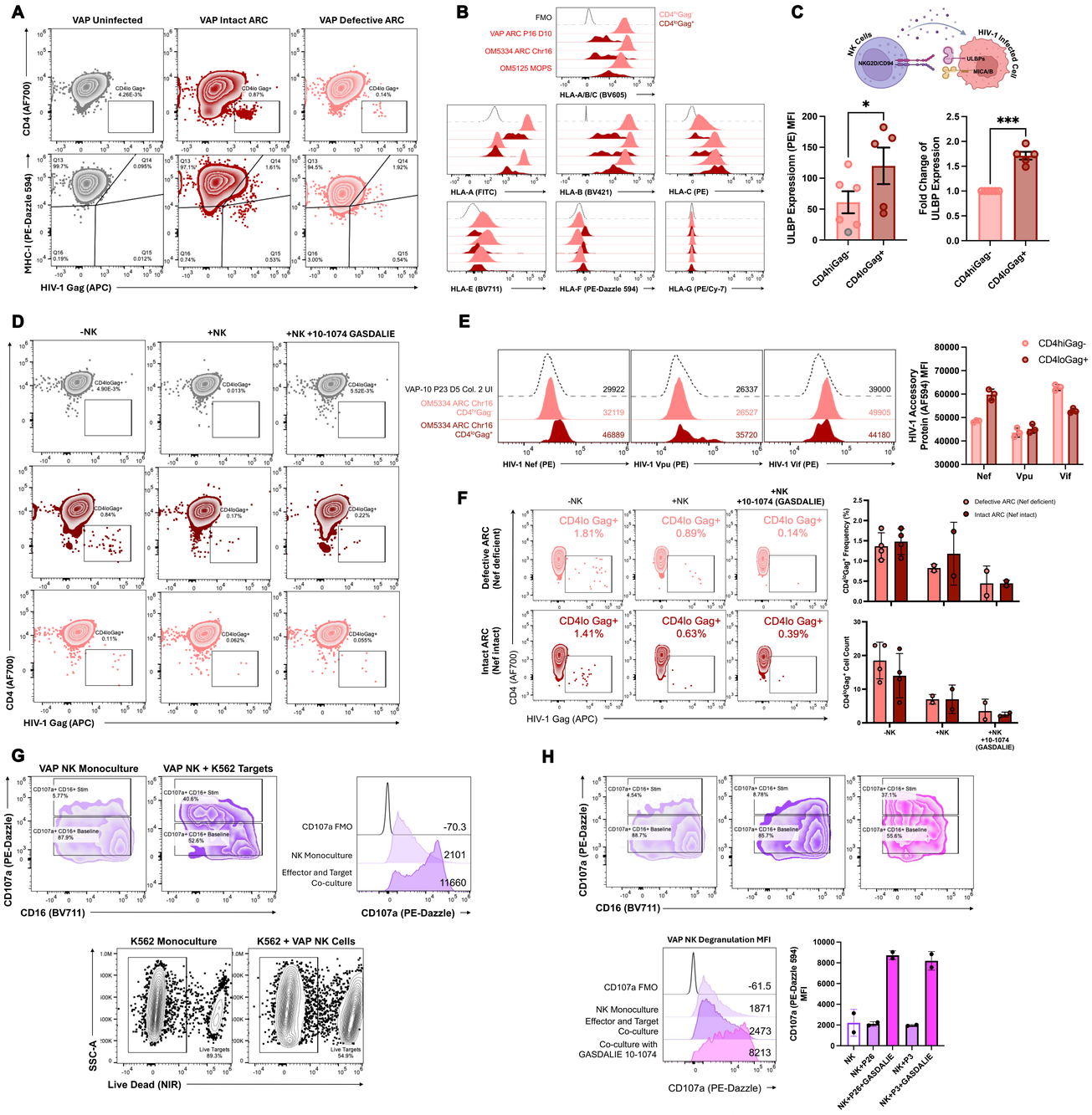


Figure 3. Characterization of ARCs uncovers potential vulnerability to NK cell immunity with innate NK cell recognition driving elimination of HIV-expressing authentic reservoir clones.
(A and B) ARCs maintained in culture exhibit downregulation of surface CD4 and MHC I similar to lab-derived infection models of HIV-1, representing potential vulnerabilities that make them resistant to CD8⁺ T cell but vulnerable to innate recognition by NK cells.
(C) Gag+ ARCs demonstrate increased surface expression of NKG2D ligands, including pooled ULBPs. Data points represent averaged triplicate values from five unique ARCs across three different donors.
(D) In co-culture with autologous NK cells, HIV-1 Gag⁺ ARCs are readily eliminated after 24 hours. This elimination is nominally improved with the addition of 10-1074 GASDALIE.
(E and F) Intact and defective ARCs may differentially express HIV accessory proteins. A Nef-deficient ARC with known resistance to autologous CTLs was susceptible to NK-mediated elimination.
(G) ARC-autologous NK cells isolated from PLWH on long-term ART retain their robust cytotoxic capacity and potently eliminate the MHC I-negative K562 target cell line.
(H) Despite efficient elimination of HIV-1 Gag⁺ ARCs, autologous NK cells degranulate nominally above base line, potentially implying other mechanisms of target elimination such as through the activation of death receptors. Additionally, while the 10-1074 GASDALIE antibody primes NK cells to degranulate more readily, this is not reflected by improved target elimination.

CONCLUSIONS

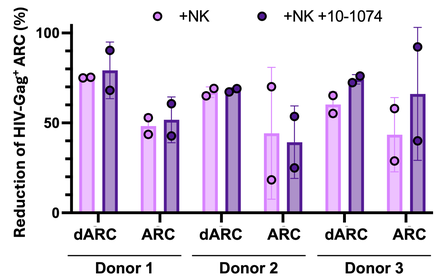


Figure 4. Summary graph of ARC cell reduction by NK cells. Gag⁺ ARC cell reduction of unique, participant-derived pure populations of intact ("ARC") and defective ("dARC") Authentic Reservoir Clones from three different donors as assessed by flow cytometry following co-culture with or without autologous NK cells in the presence or absence of 10-1074 GASDALIE. Frequency changes are assessed on cells gated as follows: Lymphocytes/Single Cells/Live/CD3⁺CTV⁺/CD8⁻/CD4ˡᵒGag⁺.

- Authentic Reservoir Clones retain phenotypic similarities to lab-derived infection models of HIV-1-infected mCD4⁺ T cells
- ARCs **downregulate classic MHC I and upregulate expression of NKG2D ligands**, uncovering vulnerability to NK cell-mediated elimination
- Autologous **NK cells readily eliminate HIV-1 Gag⁺ ARCs**, but fail to eliminate Gag⁻ cells, demonstrative of reservoir persistence when cells with integrated provirus do not express viral protein
- Despite effective cytotoxic priming by 10-1074 GASDALIE bnAb, NK cell-mediated elimination is only nominally improved, which may **imply escape by decreased HIV-1 Env expression in ARCs**

FUTURE DIRECTIONS

- Optimize methods for a prolonged co-culture between ARCs and NK cells over a course of 7-10 days to assess spontaneous production of HIV-1 Gag by ARCs and resulting elimination
- Assess recognition capacity of ARCs by autologous NK cells by measuring intracellular production of IFNγ and TNFα
- Comprehensively phenotype extracellular NK cell ligands on uninfected clones and ARCs
- Sequentially block NK receptors in elimination assays to assess their importance in mediating NK cell recognition
- Mine single and bulk-cell multiomics data related to NK susceptibility

PLAIN LANGUAGE SUMMARY

Even when HIV is well controlled by treatment, some infected cells persist in the body as a hidden reservoir. We found that certain reservoir cells can be detected and killed by natural killer (NK) cells because they show signs of stress and lose protective surface signals. Our work suggests NK cells may already play an underappreciated role in controlling HIV — and could potentially be harnessed to shrink the reservoir further.

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