Natural Killer cells preferentially kill HIV-infected CD4 T cells using death receptors

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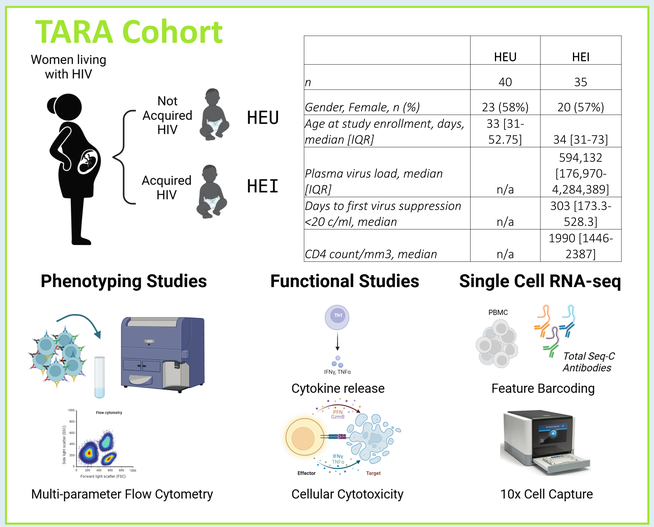
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BACKGROUND

Early infant diagnosis of perinatal HIV enables rapid ART initiation, improving virologic control and limiting HIV reservoirs. Infant HIV reservoirs may differ from adults due to the developing immune system, yet data on immune responses in perinatal HIV remain limited. Infant immune systems feature high CD4 T cell frequencies, Th2 bias, and reduced cytotoxic activity in T and NK cells. NK subsets, particularly CD56dimCD16+ cells, are linked to HIV control. Emerging evidence suggests NK cells play a role in early HIV suppression. This study examines NK cell phenotype and function in HIV-exposed infants from Mozambique at pre-ART and longitudinally post-ART, to investigate impacts of HIV acquisition and viral suppression on their role in perinatal HIV.

METHODS



	HEU	HEI
n	40	35
Gender, Female, n (%)	23 (58%)	20 (57%)
Age at study enrollment, days, median [IQR]	33 [31-52.75]	34 [31-73]
Plasma virus load, median [IQR]	n/a	594,132 [176,970-4,284,389]
Days to first virus suppression <20 c/ml, median	n/a	303 [173.3-528.3]
CD4 count/mm3, median	n/a	1990 [1446-2387]

Single Cell RNA Seq: Multiomic single cell sequencing was performed combining the transcriptome, proteome and V(D)J sequencing via 10x genomics ECCITEseq (Expanded Crisper Compatible Cellular Indexing of Transcriptome and Epitopes) protocol. PBMC were thawed and rested overnight prior to labeling with TotalSeq-C Universal Cocktail (Biolegend) for feature barcoding and cell capture.

NK effector function Assay: NK cells were purified from PBMC at Entry (1-2 months of age) and 12 months were co-cultured with target cells at 4:1 ratio. 3 cell lines were tested: CEM.NKR; NK resistant, K562; MHCI deficient/NK sensitive, HUT78/SF2; HIV-infected/NK sensitive with and without IL-15. To deal with the paired nature of the data, at different timepoints, a mixed effects model was used to analyze the data, correcting for batch effect.

Functional NK cells that can target HIV-infected cells are present at age 1 month (pre-ART) in infants with perinatally acquired HIV. At age 12m, the magnitude of NK cytotoxicity against HIV-infected target cells is negatively associated with virus load. Killing of HIV infected target cells was associated primarily with the Fas-FasL pathway rather than CD107a degranulation.

RESULTS

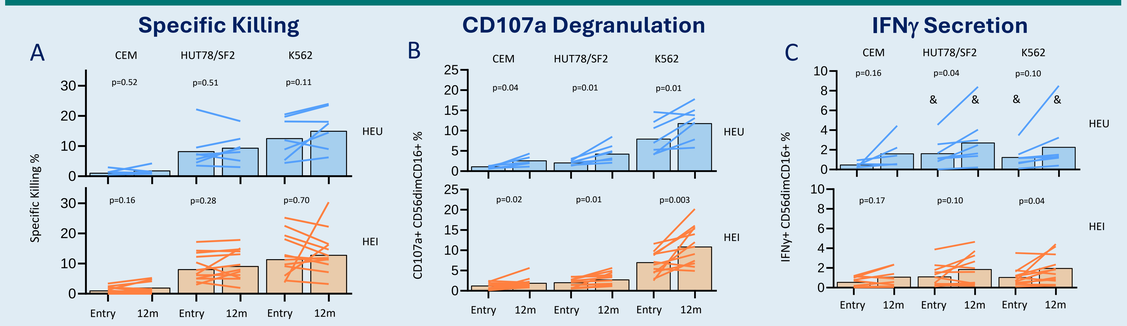


Figure 1. NK Cytotoxic Functions are Equivalent in Infants with and without Perinatal HIV in Response to Target Cells . A) % specific killing was calculated for each sample by subtracting the frequency of Dead cells in the Target only condition from the NK+Target condition. Frequencies of CD107a (B) and IFNγ (C) expressing CD56dimCD16+ cells are shown for HEU and HEI. Data represents 6 independent experiments, each containing the paired samples from HEI and HEU. Paired t test was used to assess significant differences between timepoints, p values shown. (&) indicate non-significant comparisons only for K562 or HUT78/SF2 compared to CEM. No significance observed when comparing HEI and HEU within a timepoint.

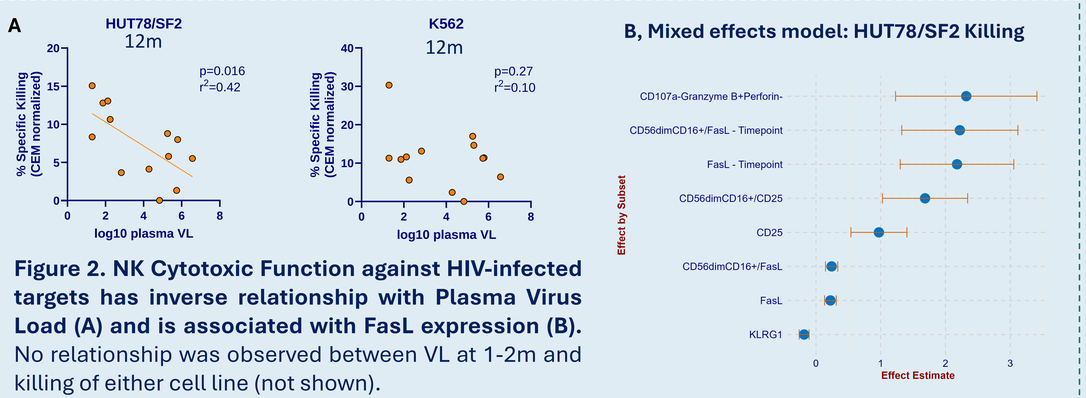


Figure 2. NK Cytotoxic Function against HIV-infected targets has inverse relationship with Plasma Virus Load (A) and is associated with FasL expression (B). No relationship was observed between VL at 1-2m and killing of either cell line (not shown).

RESULTS

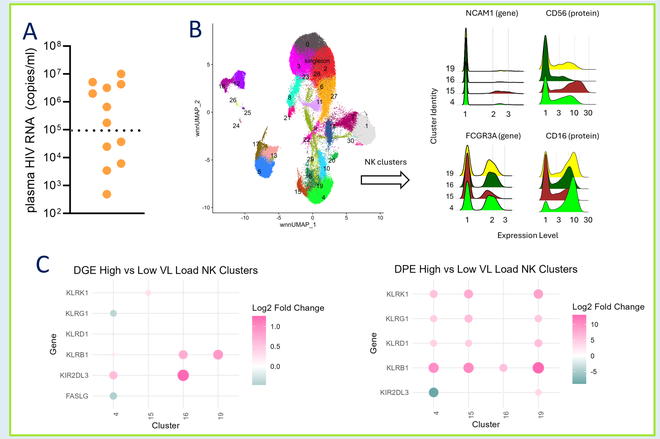


Figure 3. Inhibitory NK Receptor Expression is Associated with high pre-ART Virus Load. A) Infants were split into "high" and "low" VL groups for comparative analysis. B) Multi-omic scRNA seq clustering of NK cells using weighted nearest neighbors. C) Cluster specific differential gene and protein expression of NK receptors in High vs. Low viremia infants showing higher NKR expression and reduced *FasL*.

SUMMARY/CONCLUSIONS

1. In response to HIV-infected targets, NK from infants with perinatal HIV have comparable functionality as HIV exposed uninfected controls at pre-ART and post-ART (Fig 1).
2. High viremia in infants (12m) is associated with impaired NK-mediated killing of HIV-infected cell line (HUT78/SF2) but not K562 (Fig 2).
3. scRNAseq and mixed effects modeling reveal reduced NK function is associated with reduced FasL and greater inhibitory NKR expression with high viremia (Figs 2 and 3).
4. Results point to Fas/FasL as an alternate mechanism for killing of HIV-infected cells, complementary to CD107a degranulation, that could be targeted for novel immunotherapeutic approaches for HIV remission.

ACKNOWLEDGEMENTS

Funding support was provided from NIH through R01 AI127347 grant to Savita Pahwa, PAVE MDC Collaboratory 5UM1AI164566-02, and PENTA foundation (EPIICAL). We used facilities in the Immunology Core of the Miami CFAR (supported by NIH program grant P30 AI073961) and OncoGenomics Core of the Sylvester Cancer Center. We thank caregivers who participated in the study, the staff at the Matola Provincial Hospital who took care of infants and caregivers; the director of Matola Provincial Hospital and the Provincial Health Directorate of MOH for the support in implementing cohort enrollment in Mozambique.