KIR+RA+CD8 T CELLS EXHIBIT ANTIGEN INDEPENDENCE AND CORRELATE WITH HIV RESERVOIR SIZE

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BACKGROUND

CD45RA+CD8+ T cells that express killer cell immunoglobulin-like receptors (KIRs) (KIR+RA+) are active during autoimmunity and infectious disease, contain restricted TCR repertoires, and elicit IL-15-dependent protective immunity. We sought to reveal these features in ART-treated HIV+ individuals and further hypothesized KIR+RA+ harbor antigen-independent activity and would negatively correlate with reservoir size.

METHODS

CD8+ T cells from HIV+ individuals were sorted into distinct CD3+CD8+ subsets: TN (CD45RA+panKIR-CD27+), KIR+RA+ (CD45RA+panKIR+), TEMRA (CD45RA+ panKIR-CD27-), TCM (CD45RA-CD27), and TEM (CD45RA-CD27-); RNA was sequenced (NovaSeq6000; n=3). In PBMCs from HIV+ individuals (n=15), RNA profiles were validated via flow cytometry, and subsets were correlated to reservoir size using the intact proviral DNA assay (IPDA). We developed an antigen-independent Viral Inhibition Assay (VIA) which abrogated surface expression of MHC I on CD4+ T cells by CRISPR/Cas9 (n=7). Activated cells were infected with HIV and cocultured for 7 days with CD8+ T cell subsets then assessed for HIV Gag via flow cytometry. The impact of IL-15 superagonist (N803) on KIR+RA+ was also assessed (n=5).

RESULTS

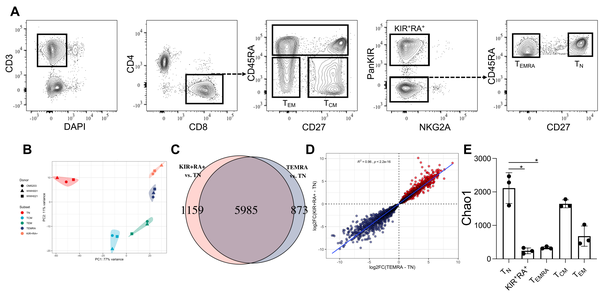


Fig. 1. KIR+RA+ cells have less diverse TCR sequences compared to T_N and are more closely related to T_{EMRA} cells. Fluorescence activated cell sorting (FACS) was used to sort various CD8+ T cell subsets, using a gating strategy (**A**). RNA was extracted from cells and sequenced on the NovaSeq6000 platform. Donor-adjusted principal component analysis was calculated for the top 500 variable genes (**B**). Overlapping differentially expressed genes (DEGs) between KIR+RA+ and T_N (**C**) were displayed as a scatterplot correlation analysis of log2 fold-changes between genes that change in both KIR+RA+ and T_N (**K**). Chao estimates were calculated to measure TCR diversity (in CDR3 amino acid sequences) in each subset.

CD45RA+CD8+ T cells that express inhibitory KIRs impede HIV replication in an antigen-independent manner

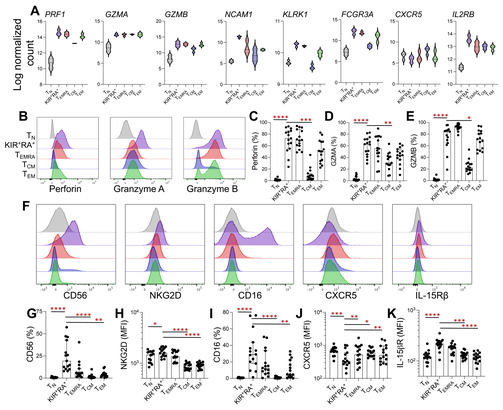


Fig. 2. KIR+RA+ cells have high cytotoxic profiles and express markers of innate immunity. Log normalized gene expression counts from RNAseq data were displayed as violin plots (**A**) and flow cytometry was used to measure protein expression and validate the RNAseq data (**B-K**).

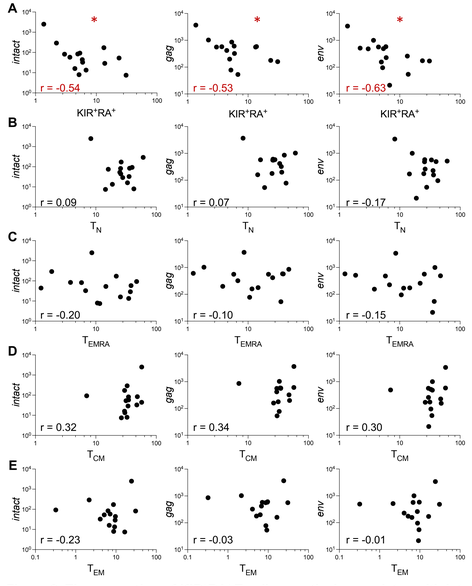
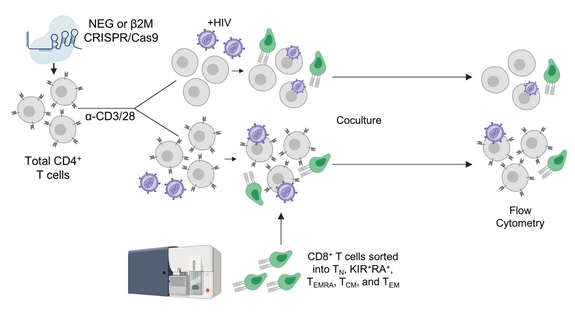


Figure 3. The proportion of KIR+RA+ T cells negatively correlates with intact HIV DNA. Flow cytometry was used to determine CD8+ T cell subsets and IPDA was performed on purified CD4+ T cells; correlations between *intact*, *gag*, or *env* DNA and the proportion of each CD8+ T cell subset were calculated for KIR+RA+ (**A**), T_N (**B**), T_{EMRA} (**C**), T_{CM} (**D**), and T_{EM} (**E**) cells. Spearman r was calculated and recorded in each plot, and significant p values (p<0.05) were listed in red.

Fig. 4. Schematic of the Viral Inhibition Assay



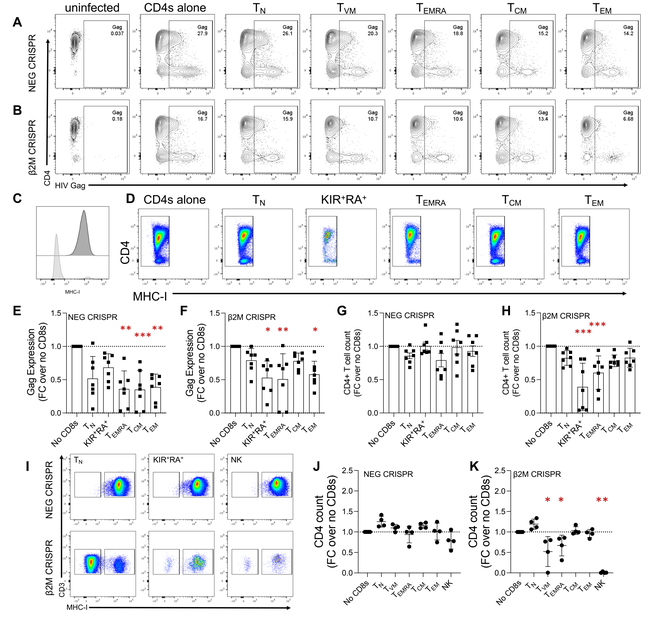


Fig. 5. KIR+RA+ cells significantly reduce HIV infection in MHC-I negative cells through a viral inhibition assay (VIA), and significantly deplete populations of uninfected MHC-I negative CD4+ T cells. Representative examples of the VIA demonstrate the impact of CD8+ T cell exposure on Gag expression in CD4+ T cells that are MHC-I positive (**A**) vs. MHC-I negative (**B**), including histograms of MHC-I expression following NEG CRISPR (dark grey) vs β2M CRISPR (light grey) (**C**); Gag expression is displayed as fold change (FC) over CD4+ T cells cultured alone for MHC-I positive (**E**) vs. MHC-I negative (**F**) cells. Representative example of death of MHC-I negative CD4+ T cells (**D**), and cell numbers between MHC-I positive (**G**) and MHC-I negative (**H**) A representative example show the number of MHC-I positive and MHC-I negative CD4+ T cells (HIVneg donor) remaining when exposed to autologous T_N, KIR+RA+, or NK cells (**I**), and exposure to all CD8+ T cell subsets (**J, K**).

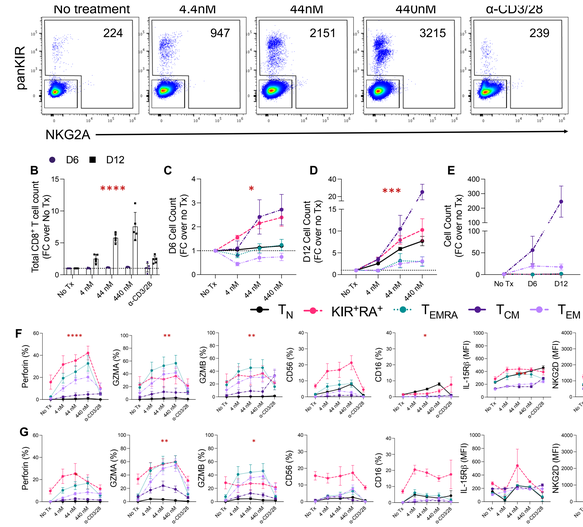


Figure 6. The IL-15 superagonist N803 increases KIR+RA+ numbers and enhances cytotoxic profiles of naïve CD8+ T cells. Naive CD8+ T cells were treated with 4-, 44-, or 440-nM of N803, α-CD3/28, or left unstimulated; a representative example shows the impact of various treatments on CD8+ T cell subsets at D12 (**A**) including a graph of total CD8+ T cell counts as FC over untreated cells for D6 and D12 (**B**) and changes to subset numbers at D6 (**C**) and D12 (**D**). Changes to cytotoxic profiles and the expression of NK markers on each CD8+ T cell subset were tracked following treatment on D6 (**F**) and Day 12 (**G**).

CONCLUSIONS

Here, we elucidate important phenotypic and functional characteristics of CD45RA+KIR+CD8+T cells. We demonstrate they are most closely related to T_{EMRA} cells, with highly cytotoxic profiles and restricted TCR sequences. Compared to the other subsets studied, KIR+RA+ cells are the only subset to correlate with intact proviral DNA, where higher proportions of KIR+RA+ cells associate with smaller viral reservoirs. KIR+RA+ cells expand in response to N803 stimulation. We show that KIR+RA+ cells can reduce viral replication in an antigen-independent manner during a viral inhibition assay, and importantly, KIR+RA+ cells can generally diminish populations of CD4+ T cells that do not express MHC I, independent of infection.

ADDITIONAL INFORMATION

Contact Talia Mota, PhD, MPH tam2036@med.cornell.edu

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