Investigating Antigen-independent CD8⁺ T cell enforcement of HIV Latency

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Background

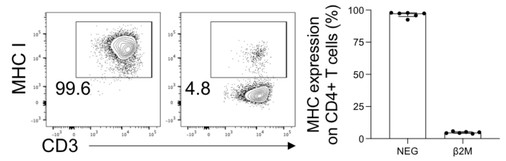
Whereas a number of lines of evidence have firmly established a critical role for HIV-specific CD8⁺ T cells acting in a canonical antigen-dependent manner, research in this area has also provided evidence in support of additional non-TCR mediated antiviral activity. Recent research supports the possibility that non-canonical behaviors of CD8⁺ T cells may exert distinct mechanisms of noncytolytic control to reduce viral gene expression, as observed in HIV and other pathogenic infections.

Hypothesis: Primary CD8⁺ T cells from HIV⁺ individuals may exhibit distinct features of nonclassical control of HIV infection. These features include antigen-independent antiviral suppression of viral transcription without cytotoxicity, thus maintaining latency.

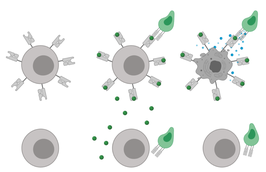
Methods

We established a system to abrogate surface expression of MHC I (HLA-A/B/C/E) using CRISPR/Cas9 and two guide RNAs against beta-2-microglobulin (β2M). MHC I KO was performed in HIV-JRCSF-infected CD4⁺ T-cells from people with HIV and exposed overnight to autologous primary unstimulated or TCR-stimulated CD8⁺ T-cells (n=6), including CD8⁺ T-cells with CRISPR KO of cytotoxic granules including Perforin, Granzyme A and B, the TCR, and death receptor ligands including FasL, TRAIL, and NKG2D (n=2).

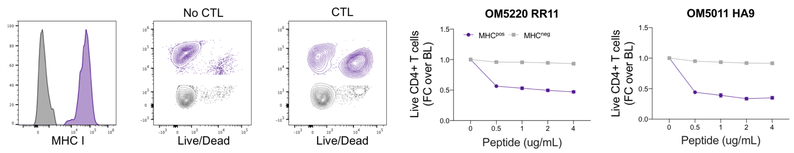
Results – Development of MHC I knockout system



Using the CRISPR/Cas9 system and two guide RNAs designed against β2M, surface expression of MHC I (HLA-A/B/C/E) was abrogated within 48hrs



This knockout protected peptide-pulsed target cells from killing by autologous HIV-specific CTL clones (grey plots below, purple remain MHC I⁺), consistently in 2 donors with different CTL specificities and across multiple peptide concentrations



These results established a platform to study the antigen-independent antiviral activity of CD8⁺ T cells from donors living with HIV

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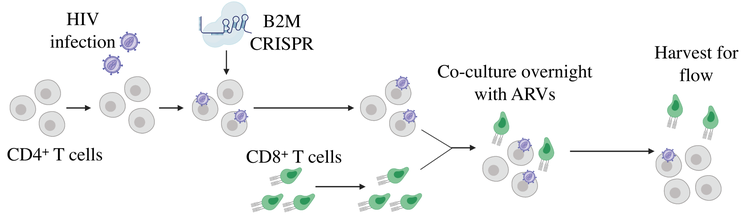
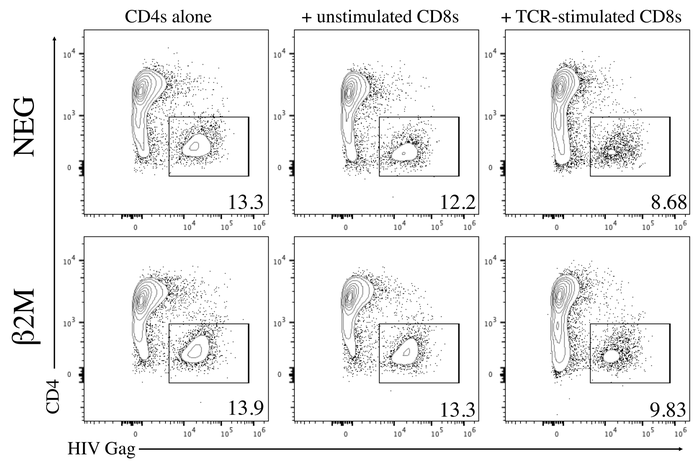
TCR-stimulated primary autologous CD8⁺ T cells can reduce HIV Gag expression overnight in infected CD4⁺ T cells in an **antigen-independent, noncytolytic** manner, driving cells into viral latency

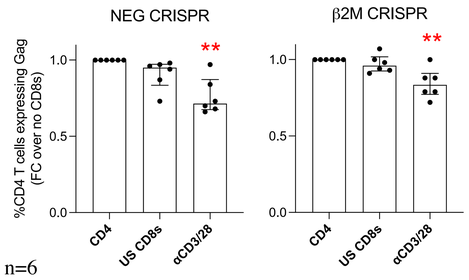
Results – TCR-stimulated CD8⁺ T cells reduce HIV Gag expression overnight in an antigen-independent manner



Schematic of antigen-independent assay

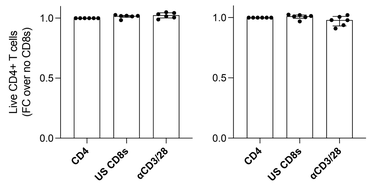


Representative example. Anti-CD3/28-stimulated CD8⁺ T cells could reduce HIV Gag expression overnight, even in the absence of MHC I expression, whereas unstimulated CD8⁺ T cells did not have this affect



n=6

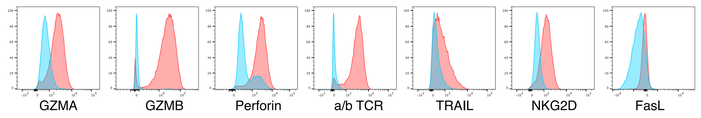
Anti-CD3/28-stimulated CD8⁺ T cells significantly reduced HIV Gag expression overnight, in the presence or absence of MHC I surface expression



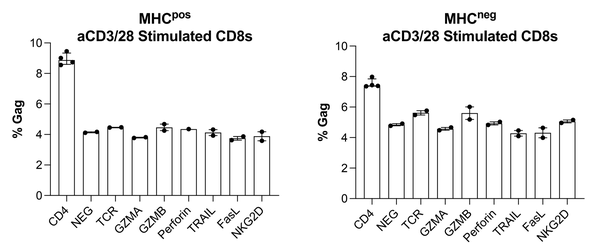
This reduction in HIV Gag occurred in the absence of cell death, indicating a noncytolytic reduction in HIV expression

These results indicate the presence of an antigen-independent noncytolytic activity of TCR-stimulated primary CD8⁺ T cells on driving HIV-infected CD4⁺ T cells into a latent state

Results – Canonical Cytotoxic and death receptor pathways are not involved in the reduction of HIV Gag



CRISPR/Cas9 was used to knock out canonical cytotoxic and death receptor pathways to rule out their impact in reducing HIV Gag expression from TCR-stimulated CD8⁺ T cells. Above demonstrates CRISPR guide RNAs to successfully knock out Granzyme A and B, Perforin, the TCR, TRAIL, NKG2D, and FasL.



Knocking out these pathways did not rescue CD4⁺ T cells from the impact of TCR-stimulated CD8⁺ T cells in reducing Gag expression overnight. These are ongoing studies (n=2), but so far suggest these pathways are not involved in the observed antigen-independent behavior, further supporting that this activity is also noncytolytic.

Conclusion/Discussion

These results add to the evidence suggesting that an antigen-independent noncytolytic activity contributes to CD8⁺ T cell-mediated suppression of HIV. This provides further impetus to study underlying mechanisms, which may reveal potential therapeutic targets to either augment viral suppression or enhance latency reversal.

Acknowledgments

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