HIV-Infection Does Not Generally Confer Higher Resistance to CTL-mediated Lysis

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BACKGROUND

While current antiretroviral treatment regimens can reliably prevent new infection events in people living with HIV (PLWH), they cannot actively eliminate the persistent population of CD4⁺ T cells harboring intact provirus that can give rise to viral rebound if treatment is interrupted. Various approaches to HIV cure rely on the elimination of this reservoir through cytotoxic T lymphocytes (CTLs). However, some studies have suggested that HIV-infection might confer some level of intrinsic resistance to CTL-mediated lysis through various mechanisms such as the upregulation of anti-apoptotic factors. Here, we show that the rate of lysis did not differ between primary CD4⁺ T cells that are uninfected, harboring intact HIV provirus, or actively expressing an HIV reporter construct.

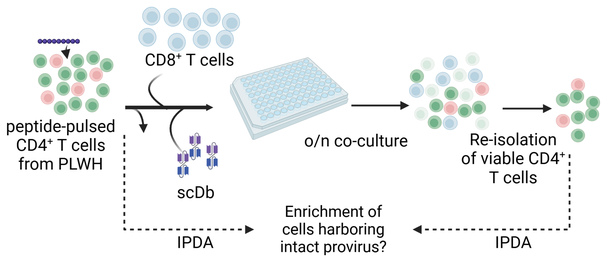


Fig. 1. Assay overview. PLWH-derived CD4⁺ T cells are pulsed with an exogenous peptide and cultured with autologous CD8⁺ T cells and specific diabody. Frequencies of intact provirus in the viable CD4⁺ population before and after culture are determined by IPDA.

METHODS

We performed co-culture killing assays using either primary CD4⁺ T cells of 10 aviremic PLWH or healthy donor CD4⁺ T cells that were infected with different NL4-3 HIV-1 eGFP-reporter constructs, with autologous CD8⁺ T cells and CTL-engaging single-chain diabodies specific for exogenous peptides in the context of HLA-A2 or HLA-E. CD4⁺ T cells were converted into target cells by pulsing them with saturating concentrations of the respective peptides. Following the co-culture, killing was assessed via flow cytometry. The frequency of HIV-infected cells before and after the co-culture was determined using the intact proviral DNA assay (IPDA) in the case of primary cells from PLWH, or by quantifying the frequency of GFP+ reporter cells for *in vitro* infected CD4⁺ T cells.

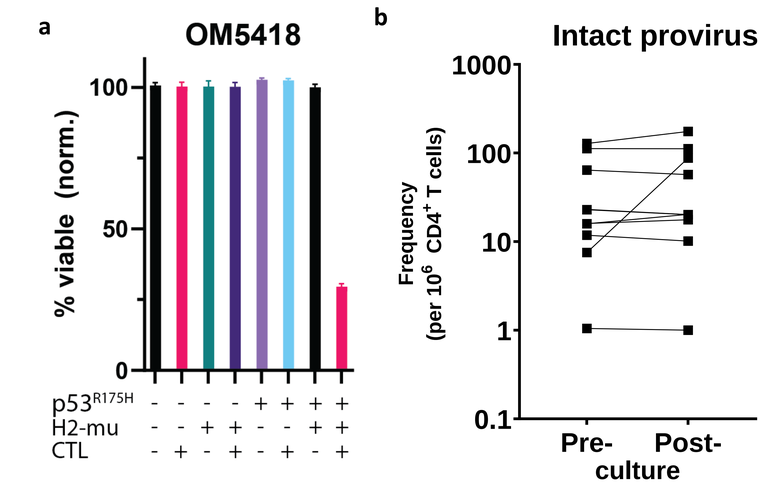


Fig. 2. Observed cytotoxicity is antigen- and diabody-dependent and does not generally lead to enrichment of cells harboring intact provirus. **a** Viability of CD3⁺CD8⁻ cell population in co-culture setup of one representative study participant OM5418. **b** Frequency of intact provirus in copies per 10^6 CD4⁺ T cells before and after the co-culture. Each data point represents the average of two independent replicates for each study participant.

RESULTS

In 9 of the 10 study participants, we did not see any enrichment for cells harboring intact provirus over the course of the co-culture (Fig. 2). When testing healthy donor cells expressing NL4-3 reporter virus using an HLA-A2 antigen, we saw nef-dependent enrichment of actively infected cells. Surface MHC staining showed that in cells infected with nef-sufficient virus, HLA-A2, but not HLA-E, is downregulated. When we repeated the assay targeting an HLA-E antigen, GFP+ and GFP- cells were eliminated at the same rate for all three viral reporter constructs (Fig. 3).

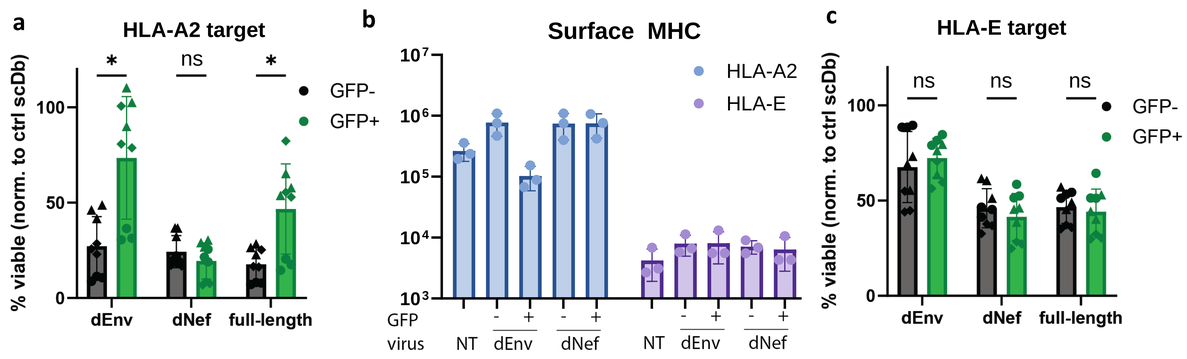


Fig. 3. Nef-dependent enrichment for HIV-expressing cells observed only when targeting a classical MHC I antigen. **a** Viability of uninfected (GFP-) and virus-expressing (GFP+) target cells after overnight co-culture. Healthy donor CD4⁺ T cells were infected with NL4-3-based eGFP-reporter constructs lacking env (Δenv), nef (Δnef) or neither (full-length), and pulsed with the exogenous $p53^{R175H}$ peptide. Cells were then co-cultured with autologous CD8⁺ T cells and HLA-A2:$p53^{R175H}$-specific diabody H2-mu or irrelevant control diabody. Data shown was obtained from independent experiments across three different healthy donors. **b** Mean Fluorescence Intensity (MFI) of primary CD4⁺ T cells that were untreated (NT) or PHA-activated and infected with indicated NL4-3-based eGFP-reporter constructs, and surface-stained for HLA-A2 or HLA-E. **c** Viability of uninfected (GFP-) and virus-expressing (GFP+) target cells after overnight co-culture. Cells were prepared as in **a**, but pulsed with Mtb44 peptide and co-cultured with HLA-E:Mtb44-specific diabody RLP13 or irrelevant control diabody.

Under identical CTL pressure, latently infected cells from PLWH and cells actively expressing HIV-1 reporter virus were both killed at similar rates as uninfected cells.

CONCLUSIONS

Our findings indicate that HIV-infection does not generally render CD4⁺ T cells more resistant to CTL-mediated lysis. In our co-cultures, the population of PLWH-derived cells harboring intact provirus was killed at similar rates as uninfected cells. In the setting of active viral expression, a nef-dependent survival advantage was observed only when targeting an HLA-A2 antigen, which could be explained by the reduced surface levels of classical MHC I in the context of HIV nef expression. Since this enrichment could not be reproduced when targeting HLA-E, which is not downregulated, the overall susceptibility to CTL killing mechanisms is likely not decreased.

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