Targeting HIV-Infected Cells for Immunotherapy Through HLA-E

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BACKGROUND

HIV has evolved multiple ways to evade cell-mediated immunity, one of which is the downregulation of MHC Class I. HLA-E, a non-classical class I molecule, however, is upregulated during infection, potentially presenting virally derived peptides. In addition, the virtually monomorphic nature of HLA-E makes it an attractive immunotherapeutic target. Using Mtb44, an M. tuberculosis-derived HLA-E binding peptide, we show that epitopes transcribed from the HIV provirus can be presented in the context of HLA-E and recognized by single-chain diabodies (scDb) to induce CTL-mediated specific lysis of infected cells.

METHODS

In order to assess specificity and efficiency of killing, we co-culture target cells (peptide-pulsed PBMCs or Jurkat E6.1 cells that were infected with Mtb44-lacking or Mtb44-expressing HIV pNL4.3 dEnv-eGFP reporter virus) with primary CD8+ T cells and RLP13, an in-house generated scDb that recognizes HLA-E:Mtb44 and binds CD3 to induce CTL activation. Following overnight incubation, cells are stained for viability and activation markers and analyzed by flow cytometry.

HLA-E can present epitopes encoded in the HIV provirus and can be targeted for specific CTL-mediated lysis of infected cells.

RESULTS

PBMCs from healthy donors homozygous for HLA-E*0101 or HLA-E*0103 were pulsed with varying amounts of Mtb44 or control peptide and co-cultured with RLP13 scDb and autologous CD8+ T cells. RLP13 induced lysis of cognate target cells at similar efficiency regardless of donor HLA-type (Fig. 2a). When co-culturing Jurkat cells infected with reporter virus that lacks (NL4.3 dEnv) or includes (NL4.3 dEnv-Mtb44) the sequence encoding for Mtb44 (Fig. 2b), we observed RLP13-dose-dependent reduction of actively infected cells only if they expressed Mtb44, but not in the uninfected or NL4.3 dEnv-infected population (Fic. 2c)

CONCLUSIONS

- Virally encoded peptides can be presented by HLA-E on the cell surface and recognized by scFvs
- ScDbs specific for HLA-E antigens can induce specific lysis of cells presenting cognate pMHC
- Unlike with most classical MHC class I, peptide:HLA-E complexes can be targeted independent of HLA type.

Our observations using Mtb44 as model epitope for HLA-E antigens indicate that this non-classical MHC may be an attractive target for HIV vaccine and immunotherapy approaches. We show that epitopes transcribed from the integrated HIV-provirus can be processed and presented by HLA-E on the cell surface. The high level of homology between the two alleles allows for universally applicable reagents that show similar efficiency for either HLA-E type, and unlike classical class I molecules, it is not downregulated during HIV infection. Studies with HIV-derived HLA-E binding peptides are ongoing and could unlock a new set of immunotherapeutic targets.

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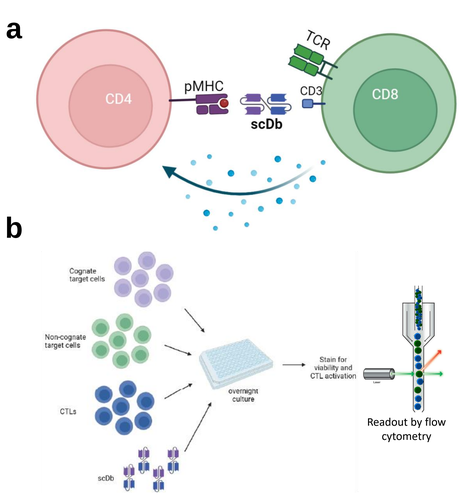


Fig. 1 Overview of scDb mechanism of action and co-culture killing assay. **a** scDb recognizes cognate pMHC on infected cell and activates CD8 T lymphocyte, inducing localized cytotoxic effector molecule secretion. **b** *In vitro* killing assay setup.

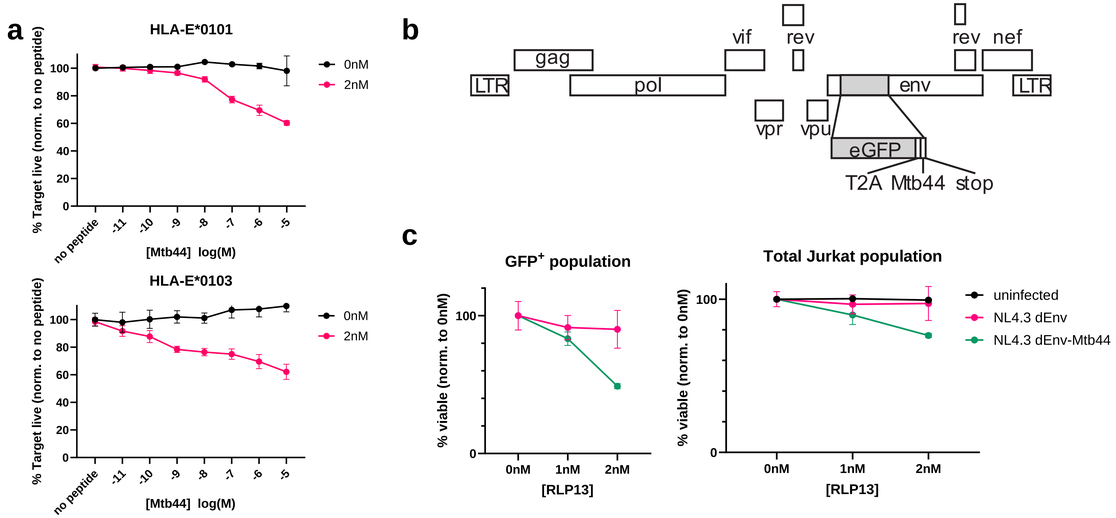


Fig. 2 Targeting of cognate cells through scDb. **a** Viability of PBMCs from healthy donors homozygous for either HLA-E allele pulsed with indicated amounts of Mtb44 peptide upon overnight co-culture with autologous CD8+ T cells and with or without HLA-E:Mtb44-specific RLP13 scDb. **b** Map of viral construct NL4.3 dEnv-Mtb44. pNL4.3 dEnv-eGFP was used as foundation, and a non-coding T2A sequence followed by the DNA sequence encoding for the Mtb44 peptide were inserted before the STOP-codon at the 3' end of the eGFP cassette. **c** Viability of Jurkat cells infected with HIV reporter construct pNL4.3 dEnv-eGFP without (red) or with (green, described in b) the sequence encoding for Mtb44. Frequency of GFP+ cells was comparable for both conditions (NL4.3 dEnv: 23.0 ±. 0.8%, NL4.3 dEnv-Mtb44: 23.4 ± 0.5%). Left: Viability of GFP+ population for each condition. Right: Viability of total Jurkat populations.