Supplementary Materials for

HLA-E–restricted, Gag-specific CD8+ T cells can suppress HIV-1 infection, offering vaccine opportunities

Hongbing Yang, Margarida Rei, Simon Brackenridge, Elena Brenna, Hong Sun, Shaheed Abdulhaqq, Michael K. P. Liu, Weiwei Ma, Prathiba Kurupati, Xiaoning Xu, Vincenzo Cerundolo, Edward Jenkins, Simon J. Davis, Jonah B. Sacha, Klaus Früh, Louis J. Picker, Persephone Borrow, Geraldine M. Gillespie, Andrew J. McMichael*

*Corresponding author. Email: andrew.mcmichael@ndm.ox.ac.uk

Published 25 March 2021, Sci. Immunol. 6, eabg1703 (2021)
DOI: 10.1126/sciimmunol.abg1703

The PDF file includes:

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Fig. S4. Activation of primary CD8+ T cells transduced with RL9-responsive TCRs by HLA-E RL9 stimulation.
Table S1. CRISPR guides for TCR and CD4 deletion in Jurkat cells.

Other Supplementary Material for this manuscript includes the following:

(available at immunology.scientificmag.org/cgi/content/full/6/57/eabg1703/DC1)

Table S2. Raw data (in Excel spreadsheet).
Fig. S1. Enhanced stability of disulfide-linked RL9 HLA-E complexes versus conventionally refolded RL9-HLA-E material, illustrated by thermal melt analysis.

Thermal melt analysis of (A) conventional and (B) disulfide-linked RL9-HLA-E complexes as measured by fluorescent (ROX) dye incorporation using the Applied Biosystems Thermal Shift Assay. The raw melt curve data is illustrated, with the Y-axis denoting fluorescence intensity (arbitrary units) and the X-axis depicting time (minutes). The inflection point of each melt curve, defined as the derivative melting temperature (TmD), was calculated automatically using Protein Thermal Shift Software v1.3. Two technical replicates were tested per run, and two biological replicates were performed for each tetramer. Representative data from a single assay illustrating Tm Derivative (TmD) values (denoted as black vertical lines on curves) +/- standard deviations are reported.
Fig. S2. Representative FACs plot of a RL9 clone stained with HLA-E-RL9 disulfide-linked tetramer and fixed without wash. 1 million RL9 clone cells were stained with 0.2μg HLA-E-RL9 disulfide- linked tetramer for 40 minutes at RT prior to fixation with 2% paraformaldehyde for 15 minutes in the absence of washing step before acquisition on a LSR Fortessa. The tetramer failed to bind a B*08:01 restricted EBV-specific CD8+ T cell control clone generated from the same donor.
Fig. S3. Creation of the CD8+ Jurkat (J8) T cell line. Jurkat (leukemic CD4+) T-cells were transduced simultaneously with lentiviruses expressing Cas9 and gRNA specific for the endogenous TCRα and TCRβ genes, using LentiCRISPRv2. Simultaneous knockout was possible owing to the efficiency of the individual guides (>90%). Cells were then sorted in bulk to remove residual CD3+ cells, with stable insertion of both guides further selected for using puromycin selection for one week. The CD8α/β chains were then introduced into the cell-line and CD8α/β expression matched by cell sorting to human-PBMC derived CD8+ T cell levels. Lastly, human CD4 expression was ablated using two gRNA-Cas9 containing lentiviruses, with cells sorted in bulk on negative CD4 expression without the need for further puromycin treatment, giving the J8 cell-line.
Fig. S4. Activation of primary CD8+ T cells transduced with RL9-responsive TCRs by HLA-E RL9 stimulation. (A) CD8+ transductants were activated by exposure to autologous B cells pulsed with RL9 peptide or (B) HIVNL4.3-infected CD4.221 cells, indicated by TNFα secretion and up-regulation of CD137. Responses were partially blocked by competitive inhibition with the signal peptide VL9. Gag p24+ cells were gated on CD3+CD8- T cells. Horizontal line of the bar indicated means. Error bars indicated SD. Data shown is representative of three independent experiments.
<table>
<thead>
<tr>
<th>Gene</th>
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<tr>
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Table S1. CRISPR guides for TCR and CD4 deletion in Jurkat cells.