Pathogen-derived HLA-E bound epitopes reveal broad primary anchor pocket tolerability and conformationally malleable peptide binding

Lucy C. Walters¹, Karl Harlos², Simon Brackenridge¹, Daniel Rozbesky², Jordan R. Barrett¹, Vitul Jain², Thomas S. Walter², Chris A. O’Callaghan³, Persephone Borrow¹, Mireille Toebes⁴, Scott G. Hansen⁵, Jonah B Sacha⁵, Shaheed Abdulhaqq⁵, Justin M. Greene⁵, Klaus Früh⁵, Emily Marshall⁵, Louis J. Picker⁵, E. Yvonne Jones², Andrew J. McMichael¹ & Geraldine M. Gillespie¹

Through major histocompatibility complex class Ia leader sequence-derived (VL9) peptide binding and CD94/NKG2 receptor engagement, human leucocyte antigen E (HLA-E) reports cellular health to NK cells. Previous studies demonstrated a strong bias for VL9 binding by HLA-E, a preference subsequently supported by structural analyses. However, Mycobacteria tuberculosis (Mtb) infection and Rhesus cytomegalovirus-vectored SIV vaccinations revealed contexts where HLA-E and the rhesus homologue, Mamu-E, presented diverse pathogen-derived peptides to CD8⁺ T cells, respectively. Here we present crystal structures of HLA-E in complex with HIV and Mtb-derived peptides. We show that despite the presence of preferred primary anchor residues, HLA-E-bound peptides can adopt alternative conformations within the peptide binding groove. Furthermore, combined structural and mutagenesis analyses illustrate a greater tolerance for hydrophobic and polar residues in the primary pockets than previously appreciated. Finally, biochemical studies reveal HLA-E peptide binding and exchange characteristics with potential relevance to its alternative antigen presenting function in vivo.

¹Nuffield Department of Medicine Research Building, Roosevelt Drive, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK. ²Division of Structural Biology, Wellcome Centre for Human Genetics, Roosevelt Drive, University of Oxford, Oxford OX3 7BN, UK. ³Henry Wellcome Building for Molecular Physiology, University of Oxford, Oxford OX3 7BN, UK. ⁴Department Molecular Oncology and Immunology, B6 Plesmanlaan 121, Amsterdam 1066CX, The Netherlands. ⁵Vaccine and Gene Therapy Institute and Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006, USA. These authors contributed equally: Andrew J. McMichael, Geraldine M. Gillespie. Correspondence and requests for materials should be addressed to A.J.M. (email: andrew.mcmichael@ndm.ox.ac.uk) or to G.M.G. (email: geraldine.gillespie@ndm.ox.ac.uk)
H
Human leucocyte antigen E (HLA-E) is a non-classical MHC class Ib molecule homologous to H-2 Qa-1 in mice and Mamu-E in rhesus macaques (RM)1-4. The two common human allotypes, HLA-E*01:01 and HLA-E*01:03, are essentially monomorphic, differing by a single amino acid substitution (Arg or Gly) at position 107, situated on a loop outside the peptide binding groove (PBG). Although relative expression is higher for HLA-E*01:03, both subtypes are present on the cell surface at lower levels than classical HLA-A or HLA-B molecules3,4. HLA-E exhibits preferential binding to a highly conserved set of nonameric signal (VL9) peptides derived from the leader sequence of HLA-A, B, C or G molecules5-7. Primary anchor residues are largely conserved among VL9 peptides: the canonical position 2 Met and position 9 Leu are accommodated by the primary B and F pockets, respectively. However, a position 2 Thr, present in a subset of HLA-B molecules, results in lower binding affinity to HLA-E and consequently, reduced surface expression7-9. VL9 peptide-bound HLA-E complexes engage the natural killer (NK) cell inhibitory receptor, CD94-NKG2A, thereby protecting healthy cells from NK cell-mediated lysis6,9. HLA-E also binds the NK cell activating receptor, CD94-NKG2C, although such interactions are of lower affinity10. Whilst VL9 peptide binding and NK cell regulation are ostensibly its primary function, HLA-E, and its rhesus and murine homologues, can present peptides from microbial and autologous sources to CD8+ T cells8,11-21. In Mycobacterium tuberculosis (Mtbt) infection, multiple mycobacterial peptides have been shown to stimulate HLA-E restricted CD8+ T cells15. Similarly, Mamu-E restricted CD8+ T cell responses are elicited in RM by an experimental rhesus cytomegalovirus (RhCMV68-1) vaccine, recombinant for simian immunodeficiency virus (SIV) genes13. In both cases, the antigen presentation pathways are atypical, arising from Mtbt-infected phagolysosomes in macrophage22 or vector-mediated disruption in RhCMV68-1-vaccinated macaques13. Remarkably, RhCMV68-1-stimulated SIV epitopes are particularly diverse with no simple sequence motif13, implying unexpectedly permissive peptide binding by Mamu-E22. These broad, Mamu-E restricted CD8+ T cell responses have been implicated as immune correlates of protection in RhCMV68-1 SIV vaccine studies13.

Here we explore pathogen-derived peptide binding to HLA-E from a structural and biochemical perspective. Through combined sandwich enzyme-linked immunosorbent assay (ELISA)-based and single-chain trimer approaches we confirm that the HLA-E peptide repertoire is broad. Via the mutagenesis and structural analysis of pathogen-derived peptides we also demonstrate an increased binding capacity of the primary pockets, with a greater breadth of tolerated anchor residues than originally reported for HLA-E-binding peptides5,23-26. This diversity also extends to the conformation of HLA-E-bound peptide which we show can differ dramatically from canonically orientated VL9, even in the presence of preferred primary anchor residues. Finally, we demonstrate that HLA-E is relatively stable without added peptide, favouring both low-affinity peptide binding and peptide exchange. These characteristics likely favour promiscuous peptide sampling in vivo, especially when the peptide loading complex (PLC) is disrupted, or absent in a peripheral intracellular compartment.

Results
Pathogen-derived peptide binding to HLA-E*01:03. A micro-refolding and sandwich ELISA-based approach enabled relative quantification of peptide binding affinity for HLA-E*01:03. Two nonameric peptide panels were tested: the first included the HIVGag-derived, RMYSPTSL (RL9HIV), a NetMHC predicted epitope27 homologous to the SIVGag-derived RMYNPTNIL peptide (RL9SIV), which constituted one of the two supertopes recognised by 100% of protected macaques in RhCMV68-1 vaccine trials13. Furthermore, since RL9HIV ranked as a strong binder relative to previously reported HLA-E restricted microbial peptides in the micro-refolding ELISA23,28 (Fig. 1a) and elicited Mamu-E restricted CD8+ T cell responses in RM vaccinated with an HIVGag-insert RhCMV68-1 vector (Fig. 1b), it was selected for crystallographic analysis.

A selection of published Mtbt-derived peptides15 constituted the second panel, of which four facilitated HLA-E*01:03 complex formation in the micro-refolding ELISA (Fig. 1c). One peptide, RLPAKAPLL (Mtbt44), exhibited comparable binding to that of the MHC class Ia-derived VL9 positive control peptide. As Mtbt44 also elicited Mamu-E-restricted CD8+ T cell responses in Bacillus Calmette–Guérin (BCG) vaccinated RM (Fig. 1d), it was pursued in crystallographic studies.

HLA-E*01:03—Mtbt44 structure. Previous crystal structures of HLA-E have been determined in complex with MHC class Ia leader sequence-derived VL9 peptides or HCMV UL40 protein-derived VL9 mimics8,10,23. Here we present the structure of HLA-E*01:03 bound to the Mtbt-derived peptide, Mtbt44 (RLPAKAPLL) (Fig. 2a). The complex packed in the P1 space group, diffraction to 2.1 Å and was assigned the PDB ID, 6GH1 (Supplementary Table 1). Clear electron density was visible in the PBG into which the Mtbt44 peptide was modelled (Fig. 2a). Despite sequence disparity, superposition of Mtbt44 and canonical VL9 revealed strong conformational similarity in peptide positioning (Fig. 2b). Both peptides adopt the classical kinked orientation in which the backbone arches away from the groove floor projecting residues four and five (P4-P5) towards the solvent. Furthermore, eight of the nine canonical hydrogen (H) bonds connecting peptide and heavy chain (HC) are conserved with 1MHE. Formation of the Mtbt44 P9-Leu [OXT] HLA-E Lys-146 [NZ] H-bond may counterbalance any loss of complex stability arising from the absent P5 H-bond, which connects VL9 to the α2 helix in 1MHE. Consistent with these structural observations and peptide binding assay data (Fig. 1 and Supplementary Table 2), Mtbt44 complex stability is also underscored by thermal melt (Tm) analysis: Mtbt44 shows a modest Tm increase (Tm = 50.2 °C ± 0.3) compared to the HLA-A2 leader sequence-derived VL9 peptide Tm documented here (Tm = 47.5 °C ± 0.4), and values previously reported for other MHC class Ia-derived leader sequence peptides8,10.

Analogous to previous VL9-bound structures, side chains of primary and secondary anchor residues in the Mtbt44 complex project into their corresponding pockets towards the groove floor. Minimal repositioning of HLA-E-derived B pocket-lining residues suggests that P2 primary anchor Met to Leu substitution is well tolerated. Furthermore, the small side chains of P3-Pro and P6-Ala occupy the shallow D and C pockets, respectively.

HLA-E*01:03—RL9HIV structure. Diffraction data from multiple isomorphous HLA-E*01:03—RL9HIV peptide crystals were merged in Xia2 yielding a 100% complete dataset to 2.6 Å resolution (Supplementary Table 1). The structure (PDB ID: 6GL1) was determined in the C2 space group with clear electron density visible in the PBG into which the RL9HIV peptide was modelled (Fig. 2d). RL9HIV’ positioning shows marked differences to the canonically orientated VL9 and Mtbt44 peptides, in which P4 and 5 backbone arching ensures optimally positioned secondary anchor residues at P3, 6 and 7 for secondary D, C and E pocket binding, respectively (Fig. 2e). RL9HIV exhibits an alternative, C-terminally shifted, kinked motif, wherein P6 and P7 arch away
from the base of the groove, disrupting C and E pocket occupancy. The P6 RL9HIV and VL9 Ca atoms are separated by 4.2 Å and the P7 Ca atoms by 3.4 Å (Fig. 2h (i)), disrupting the ability of the RL9HIV P6-Thr and P7-Ser side chains to occupy their respective C and E pockets (Fig. 2f (i) and (ii)), and concomitantly rendering this region of the peptide more solvent exposed (Fig. 2h (ii)). However, the extended conformation between P1 and 5 of the RL9HIV peptide, arising from C-terminally shifted backbone arching, positions the P5-Pro Ca atom only 1.8 Å from the VL9 P6-Thr Ca atom, with the two side chains deviating by as little as 1.2 Å, potentially permitting a degree of compensatory C pocket occupancy. Despite minimal Ca

![Graphs and images showing experimental data and results related to the effects of peptides on immune response and Mtb protein expression.](image-url)
deviation facilitating optimal secondary anchor residue-pocket alignment, the P3-Tyr side chain is prohibitively large for shallow D pocket binding (Fig. 2f (iii)), instead projecting toward the α2 helix in the C-terminal direction of the peptide. This in turn triggers the D pocket lining residue, His-99, to adopt an alternative rotamer. An extensive, yet distinct, hydrogen-bonding network secures RL9HIV in the PBG (Fig. 2i). Six of the nine H-bonds connecting VL9 and HC in 1MHE are conserved in the RL9HIV complex. However, four novel bonds are formed, one of which (P9-Leu — OXT—Lys-146 [NZ]) is also present in the Mtb44 complex. Glu-152, situated on the α2 helix, forms novel bonds with the P3-Tyr side chain and P7 backbone, the latter of which canonically bonds with Asn-77 on the α1 helix in 1MHE. Thus, P6 and 7 are stabilized closer to the α2 helix in RL9HIV than in other HLA-E-peptide complex structures.

Analysis of Mtb44 primary anchor residue variants. Peptides previously shown to bind HLA-E predominantly have Met at P2 for optimal B pocket binding, in addition to a strong F pocket binding preference for Leu at P9. We investigated these specificities using a single-chain peptide-b2m-HC construct, where P2 variants of the Mtb44 and VL9 peptides were tested for cell-surface expression in transfected 293T cells. As illustrated in Fig. 3a, the B pocket tolerated all hydrophobic side chains at P2, in addition to polar residues such as Gln, Ser and Thr. Despite Mtb44 and VL9 producing slightly varying hierarchies of binding for P2 substituted residues, cell surface expression was not supported by the charged residues Gln, Asp, or Arg in either peptide. We then sought to analyse the ability of the B pocket to accommodate a selection of these hydrophobic and polar residues, some of which are present in HLA-E restricted microbial peptides, by crystal structure determination of Mtb44 P2 variant peptide complexes. Diffraction quality crystals could be grown for HLA-E*01:03 in complex with Mtb44 P2-Gln and Phe variants, termed Mtb44*P2-Gln (PDB ID: 6GH4) and Mtb44*P2-Phe (PDB ID: 6GGM), respectively. HLA-E*01:03 was also crystallised bound to an Mtb44 peptide in which the aliphatic hydrophobic P9 primary anchor, Leu, was substituted by the aromatic hydrophobic residue, Phe, termed Mtb44*P9-Phe (PDB ID: 6GHN).

Reflections from multiple isomorphous Mtb44*P2-Phe crystals were merged and integrated by Xia2, producing a dataset to 2.7 Å in the P1 space group. Similarly, Mtb44*P2-Gln crystallised in P1, however, diffraction data were collected to a higher resolution of 2.1 Å (Supplementary Table 1). Electron density was present for both peptides including the substituted P2 side chains (Fig. 3b). Superposition of Mtb44*P2-Gln and Mtb44*P2-Phe with the original Mtb44 complex structure revealed similarly orientated peptides in the PBG (Fig. 3c). Perhaps unsurprisingly, considering its depth and hydrophobicity, P2 Leu to Gln or Phe substitutions were tolerated by the B pocket with minimal repositioning of the pocket-forming residues (Fig. 3d).

However, the P2 Mtb44*P2-Phe Ca atom is elevated 0.7 Å away from the groove floor in the direction of the α1 helix, relative to Mtb44, permitting accommodation of the larger aromatic side chain (Fig. 3c). Additionally, P6 and 7 of the Mtb44P2-Phe variant sit 0.9 and 0.8 Å deeper in their respective C and E pockets, in turn reducing the height of the P3 kink and increasing the P5 buried area 40% relative to Mtb44 and Mtb44*P2-Gln (Fig. 3e). Furthermore, Mtb44*P2-Phe forms three additional H-bonds securing P1 and 2 in the groove. Such features align with single-chain trimer-based transfected data demonstrating that Mtb44 P2-Phe drives the highest relative levels of HLA-E surface expression. However, these minor readjustments in peptide positioning due to P2 substitution do not disrupt immune recognition: CD8+ T cells isolated from the spleens of BCG-vaccinated RM mounted responses of similar magnitude when stimulated with Mtb44*P2-Phe, Mtb44*P2-Gln or the index Mtb44 epitope, emphasising the similarity in positioning of solvent exposed side chains and thus their antigenicity in vivo (Fig. 3f).

Reflections from multiple isomorphous Mtb44*P9-Phe complex crystals were merged in Xia2, yielding a dataset in the P1 space group to 2.5 Å. Clear electron density was present for the peptide including the substituted P9-Phe side chain (Fig. 4a). Similarly to the Mtb44 P2 variants, Mtb44*P9-Phe adopts the classical kinked conformation in the PBG and exhibits minor repositioning relative to the original Mtb44 peptide (Fig. 4b–d (i) and (ii)). Superposition revealed a slight elevation in the P9 Ca atom of Mtb44*P9-Phe compared to Mtb44 and an alternative rotamer for the F pocket-lining Phe-116 side chain, which tilts more acutely towards the groove floor, increasing pocket volume to accommodate the larger aromatic side chain (Fig. 4e). Minor repositioning also impacts the hydrogen-bonding network: three of the nine H-bonds securing Mtb44 to the groove are lost in Mtb44*P9-Phe at positions 1, 2 and 9.
Fig. 2 Structural analysis of HIV- and Mtb-derived peptide-bound HLA-E*01:03 complexes. a Mtb-derived Mtb44 peptide (RLPAKAPPLL) visualised as sticks in purple-slate side-on and from above with electron density overlaid in grey mesh and HLA-E*01:03 HC + β2M omitted for clarity. (All electron density contoured at 1 sigma.) b Alignment of Mtb44 (purple-slate) and canonical VL9 (VMAPRTVLL) (violet) peptides depicted as sticks in the peptide binding groove displayed in grey cartoon with the omission of the α2 helix for clarity. c Intermolecular Mtb44 peptide-HC hydrogen-bonding network visualisation. Peptide and HC-derived bonded residues displayed as solid sticks and H-bonds depicted as dashed lines (lime green). Peptide binding groove displayed as grey cartoon with the β-sheet floor omitted for clarity. d HIV-derived epitope “RL9HIV” (RMYSPTSIL) (lime green) in grey mesh electron density visualised side-on and from above with peptide binding groove omitted for clarity. e Alignment of RL9HIV (lime green) and canonical VL9 (violet) peptides depicted as sticks in the peptide binding groove with the α1 helix and β-sheet floor displayed in grey cartoon and the α2 helix omitted for clarity. f (i) E, (ii) C and (iii) D pocket visualisation for RL9HIV (lime green) superposed to VL9 (violet) with pocket-forming residues derived from the heavy chain of the RL9HIV-HLA-E complex depicted as grey sticks. Distances between the superposed peptide Ca atoms shown as grey dashed lines. g Ca backbone alignment of Mtb44, RL9HIV and VL9 peptides visualised side-on (i) and from above (ii) with peptide binding groove α1 + 2 helices depicted in grey cartoon. h (i) Distance in Å between superposed Ca atoms of Mtb44 versus VL9 and RL9HIV versus VL9 with peptide residue position along the x axis and distance on the y axis. (ii) Buried residue area percentage for Mtb44, RL9HIV and VL9 peptides with residue position along the x axis and buried area % on the y axis. i Intermolecular RL9HIV peptide-HC hydrogen-bonding network visualisation. Peptide and HC-derived bonded residues displayed as solid sticks with H-bonds depicted as dashed lines (lime green). Peptide binding groove displayed as grey cartoon with the β-sheet floor omitted for clarity.
VL9- and Mtb44-refolded HLA-E-β2m material. The immediate incorporation of dye suggested issues relating to sample non-uniformity and stability, and indicated that heterogeneous protein species—either higher order aggregates or mixed protein forms—were probably present. To explore this, we performed blue-native polyacrylamide gel electrophoresis (BN-PAGE) analysis to compare freshly purified RL9HIV-, Mtb44- and VL9-refolded HLA-E-β2m complexes. Unusually, HLA-E and β2M readily formed dimers in the absence of exogenously added peptide, and this material was also included in these experiments. The results (Fig. 5a) illustrate distinct gel signatures for the various HLA-E-β2m samples. For HLA-E refolded in the presence of the higher affinity binding Mtb44 and VL9 peptides, singular, compactly formed bands (”compact form” (CF)) resolved downstream of the 66 kDa protein marker. The gel signatures of VL9- and Mtb44-refolded HLA-E complexes were similar in form, but positionally distinct, presumably due to the charge difference of solvent exposed peptide residues (two positively charged amino acids for Mtb44 and one for VL9). In contrast, HLA-E-RL9HIV complexes resolved heterogeneously as two main bands: a faint, CF that resolved comparably to the HLA-E-Mtb44 CF species, and a more dominant, diffuse form (DF). Finally, HLA-E-β2m refolded in the absence of peptide, resolved only as diffuse material similar to the DF observed for RL9HIV.

Fig. 3 Structural analyses of Mtb44 position 2 peptide variants: Mtb44*P2-Gln and Mtb44*P2-Phe. **a** Relative binding of Mtb44 (i) and VL9 (ii) position 2 peptide variants from single-chain peptide-β2M-HC DNA constructs transfected in 293T cells and tested for HLA-E surface expression by flow cytometry using the MHC-E-specific 3D12 antibody. Data scaled relative to the index position 2 residue of each peptide (Leu for Mtb44 and Met for VL9). Relative binding displayed on the y axis, with position 2 residue mutations on the x axis. The mean ± SEM of the MFI is reported (n = 4). **b**. (i) The Mtb44 position 2 Glutamine variant Mtb44*P2-Gln (green-cyan) and (ii) the Mtb44 position 2 phenylalanine variant Mtb44*P2-Phe (yellow) visualised side-on with electron density overlay in grey mesh and HC backbone alignment of Mtb44*P2-Gln (green-cyan), Mtb44*P2-Phe (yellow) and Mtb44 (purple-slate) peptides visualised side-on and from above, respectively, with peptide binding groove α1+2 helices depicted in grey cartoon. (ii) Distance in Å between superposed Cα atoms of Mtb44*P2-Gln versus Mtb44 and Mtb44*P2-Phe versus Mtb44 with peptide residue position along the x axis and distance (Å) on the y axis. (iii) Superposition of Mtb44*P2-Gln (green-cyan), Mtb44*P2-Phe (yellow) and Mtb44 (purple-slate) peptides with peptide binding groove α1 helix and β-sheet floor depicted in grey cartoon. **d** B pocket visualisation for (i) Mtb44*P2-Gln (ii) Mtb44*P2-Phe (iii) Mtb44 and (iv) Mtb44*P2-Gln/Mtb44*P2-Phe/Mtb44 superposition. HLA-E*01:03 HC-derived pocket-forming residues depicted as grey sticks with electron density overlay as grey mesh. Buried residue area percentage for Mtb44*P2-Phe, Mtb44*P2-Gln and Mtb44 peptides with residue position along the x axis and buried area % on the y axis. **f** BCG-elicited Mamu-E-restricted Mtb44-specific CD8+ T cell recognition determined and shown as described in Fig. 1b.
To determine if these gel signatures are comparable to classical MHC class I, the BN-PAGE gel profiles of HLA-B*57-β2m complexes refolded with epitope peptide were also explored. As depicted in Fig. 5b, Cf signatures exclusively represented all samples tested, confirming that the compact gel signature species most likely represents optimally folded, peptide-loaded MHC class I protein.

We next assessed whether the HLA-E Cf and Df species represented HLA-E-β2m co-complexes, as indicated by the presence of both the HC and β2m. This was particularly relevant for the Df species, as the native gel band size resolution error is high (~15%), and this smaller species could represent monomeric HC protein. To investigate this, Mtb44- and RL9HIV-refolded HLA-E-β2m complexes were probed in a second dimension by native/SDS-PAGE analysis. Individual Cf and Df bands resolved by BN-PAGE were individually excised and inserted in standard 12-well slots of NuPAGE 10% SDS-PAGE gels for subsequent separation. This approach verified the presence of HC and β2m in all folded forms (Fig. 5c).

**Fig. 4** Structural analyses of the Mtb44 position 9 peptide variant: Mtb44*P9-Phe. **a** The Mtb44 position 9 phenylalanine variant Mtb44*P9-Phe (blue-cyan) visualised side-on with electron density overlaid in grey mesh and HLA-E*01:03 HC + β2M omitted for clarity. **b** Superposition of Mtb44*P9-Phe (blue-cyan) and Mtb44 (purple-slate) peptides depicted as sticks with the binding groove α1 helix and β-sheet floor depicted in grey cartoon. **c** Ca backbone alignment of Mtb44*P9-Phe (blue-cyan) and Mtb44 (purple-slate) peptides visualised side-on (i) and from above (ii), with binding groove α1 + 2 helices depicted as grey cartoon. **d** (i) Distance in Å between superposed Ca atoms of Mtb44*P9-Phe versus Mtb44 with peptide residue position on the x axis and distance (Å) on the y axis. (ii) Buried residue area percentage for Mtb44*P9-Phe (blue-cyan) and Mtb44 (purple-slate) peptides with residue position along the x axis and buried area % on the y axis. **e** F pocket visualisation of (i) Mtb44*P9-Phe, (ii) Mtb44 and (iii) Mtb44*P9-Phe/Mtb44 superposed complexes. HLA-E*01:03 HC-derived F pocket-forming residues depicted as grey sticks with electron density overlaid as grey mesh.
We questioned whether pre-refolded HLA-E-β2m, with optimal tertiary structure and disulphide-bridge formation, was more receptive to RL9HIV peptide binding. To evaluate this, the method of UV-mediated peptide exchange was employed. HLA-E refolded with the UV-labile VL9-based 7MT2 epitope incorporating a light sensitive J moiety at position 5 along the peptide was photo-illuminated in the presence or absence of 100 M excess Mtb44 and RL9HIV peptide. As indicated by the transition of gel signatures from Df to Cf species (Fig. 5e(ii)), peptide-receptivity was also a feature of the presumed peptide “empty” HLA-E-β2m forms.

The ability of HLA-E-β2m peptide-loaded material to exchange peptide was also evaluated. As Mtb44 and VL9-refolded HLA-E-β2m complexes produced BN-PAGE Cf gel signatures that are position-distinct, this feature was used as a tool to gauge peptide exchange. Previously refolded and purified HLA-E-β2m-VL9 peptide complexes were incubated with 200 M excess of Mtb44 peptide and subsequently analysed by BN-PAGE. As evidenced by the VL9 to Mtb44 Cf gel signature transition, VL9-loaded HLA-E complexes were readily displaced by the Mtb44 peptide (Fig. 5e(ii)). Finally, we tested whether the pre-refolded HLA-E-β2m-VL9 peptide complexes were also susceptible to RL9HIV peptide exchange. In agreement with observations made for the higher affinity Mtb44 peptide, 200 M excess of RL9HIV also displaced VL9 from HLA-E-β2m complexes, as evidenced by the emergence of gel forms.
resembling the HLA-β2m-RL9HIV pre-refolded complex signature (Fig. 5e(ii)).

Discussion

Mamu-E restricted CD8+ T cell responses have been implicated as immune correlates of protection in RhCMV68-1 vectored SIV vaccination trials, triggering new interest in HLA-E as a potential driver of protective immunity against HIV-1. Although earlier work indicated that the HLA-E binding repertoire was restricted to MHC class Ia leader sequence-derived peptides for presentation to NK cells, it is increasingly apparent that HLA-E can also bind and present autologous and microbially derived peptides to CD8+ T cells. The breadth of epitopes identified in RhCMV68-1 SIV vaccine studies and mycobacterial infection indicates that both Mamu-E- and HLA-E-restricted peptide repertoires have the potential to be very diverse. In particular, the range of Mamu-E restricted RhCMV68-1 stimulated responses is consistent with increased diversity of HLA-E peptide binding compared to transport-associated antigen processing (TAP)-deficient cell lines. Despite sequence disparity between the human and rhesus homologues, the amino acids that comprise the five primary and secondary anchor residue-accommodating pockets are almost identical between HLA-E and 21 of the 22 Mamu-E alleles, with a single exception, 173T, in the C pocket (Supplementary Table 3). Furthermore, it has previously been shown that rhesus SIV-specific CD8+ T cells recognise peptides presented by both Mamu-E and HLA-E. Similarly, rhesus CD8+ T cell responses to RL9HIV, Mtbb44 or Mtbb44 P2 variant epitopes were of comparable magnitude when peptides were presented by either Mamu-E or HLA-E (Supplementary Figs. 2, 3), further supporting the functional similarity between the rhesus and human homologues. Finally, in a single-chain peptide-β2M-HC trimer-based comparison of VL9 P2 variant binding to HLA-E*01:03 versus Mamu-E*02:04, the human and rhesus homologues exhibited similar binding preferences (Supplementary Fig. 1A). Thus, although there are no published structures of Mamu-E, it is reasonable to extrapolate from these experimental data and the close relatedness of HLA-E that the two homologues behave similarly in folding and low-affinity peptide binding.

The breadth of the HLA-E peptidome was re-explored using a refolding-ELISA approach that quantified the relative capacity of peptides to stabilise HLA-E-β2M-peptide complex formation (Fig. 1a, c). Several HIV epitopes identified in RhCMV68-1 HIV-1 Gag-insert vaccine trials, including some that lacked canonical anchor residues, exhibited reproducible binding to HLA-E, albeit with considerably lower affinity than VL9 peptides (L. Picker, unpublished). Similarly, screens of previously reported HLA-E restricted microbial peptides, including an Mtb-derived panel, highlighted a selection that supported heterotrimeric complex formation including one, Mtb44, which exhibited comparable binding affinity to VL9 peptides. This assay also indicated that HLA-E and β2m-fold in the absence of added peptide, as evidenced by reproducible signals for peptide-free refolded samples. As previous structures of HLA-E were crystallised in complex with highly conserved, canonical VL9 peptides or HCMV UL40 protein-derived VL9 mimics, it was unclear how nonleader sequence epitopes could be accommodated in the PBG. To understand the structural basis of binding, two of the highest affinity pathogen-derived peptides identified in refolding-ELISAs, RL9HIV (HIV gag) and Mtbb44 (Mtb), were selected for crystallographic analysis (Figs. 1, 2). Irrespective of sequence disparity to VL9, including a positive charge at P1 and a primary anchor Met to Leu substitution at P2, Mtbb44 exhibited strong conformational similarity, adopting the classical kinked binding motif in the PBG. However, despite a similar backbone conformation and solvent exposure profile to VL9, Mtbb44 possesses sufficiently distinct exposed side chains to elicit specific, Mamu-E restricted CD8+ T cell responses in BCG-vaccinated macaques. In contrast, the RL9HIV peptide carries canonical anchors Met at P2 and Leu at P9, yet adopts an alternative C-terminally shifted kinked motif in the binding groove, resulting in a distinct solvent exposed signature with implications for immunogenicity and peptide-specific TCR interaction. This alternative backbone conformation, in turn, disrupts secondary C and E pocket occupancy with the P6 and 7 anchors projecting 4.2 and 3.4 Å further into the solvent, respectively, likely contributing to the lower overall stability of the complex. 

In line with diverse and non-canonical SIVGag epitopes defined by Hansen et al., we provide structural evidence illustrating that the B and F pockets of HLA-E can tolerate a wider range of side chains than previously predicted (Figs. 3, 4). HLA-E structures crystallised in complex with Mtbb44 variants encoding P2-Gln or Phe, or P9-Phe, demonstrated minimal conformational repositioning relative to Mtbb44 in the PBG. However, these primary anchor mutations did have minor ramifications on complex stability, via the gain (for Phe at position 2) or loss (for the Phe position 9 variant) of three H-bonds. Notably, Mamu-E restricted Mtbb44-specific CD8+ T cell responses in BCG-vaccinated macaques were preserved when the animals were challenged with mutant peptides harbouring Phe or Gln at P2. This supports the possibility of enhancing MHC-epitope complex stability via primary anchor optimisation of certain HLA-E-restricted epitopes in immunogen design without disrupting TCR recognition. A more comprehensive analysis of B pocket tolerability was conducted by examining surface expression of single-chain peptide-β2M-HC trimers encoding Mtbb44 P2 variants. The hierarchy of tolerated residues is consistent with our structural data, with P2-Phe up-regulating surface expression of HLA-E to the greatest degree. In fact, this assay indicates that the B pocket is capable of accommodating any hydrophobic residue, dramatically increasing the potential number of HLA-E restricted HIV-derived vaccine candidate epitopes. The single-chain-trimer data also indicate that tolerance of primary anchor substitution is to some extent dependent on the remaining amino acids that constitute the nonameric peptide, as when P2 substitutions in the Mtbb44 and VL9 peptides were compared, HLA-E surface expression was up-regulated in different ranking orders.

During the biochemical characterisation of HLA-E, we noted that, unusually, HLA-E HC and β2m assembled as heterogeneous dimers in the absence of added peptide (Fig. 5). These dimeric forms likely comprised transition-state intermediates as evidenced by their diffuse gel profiles and the multiple transition states observed during thermal melt analysis. It is possible that peptide fragments derived from partially degraded HLA-E HC or β2M are present in the binding groove of these refolded species and it has previously been shown that peptide fragments as short as two amino acids (“dipeptides”) are capable of stabilising the F pocket. However, it is not critical whether these refolded species are truly empty. More importantly, peptide-recognition is a characteristic of these “empty” HLA-E-β2m forms, a finding that also concurs with data generated using particular peptide-free MHC class Ia-β2m heterodimers. Intrinsically allotype-specific differences in the propensity of MHC folding intermediates to retain peptide-recognition exist, presumably reflecting the extent to which different allelic forms maintain these stable functional states. In relation to PBG integrity in the absence of peptide, recent Molecular Dynamic Simulation studies predict that the α1–α2 helices of HLA-E are rigid and remain open when devoid of peptide, which contrasts with classical molecules such as HLA-A2 whose malleable helices are projected to collapse without
This, in addition to the apparent stability of the HLA-E-β2m peptide-free heterogeneous form identified here, may provide insight into the features that contribute to the broad peptide receptivity of HLA-E and Mamu-E where classical TAP-dependent peptide loading is blocked, for example, by various CMV genes and when RhCMV68-1 tropism is restricted to as yet undefined cell types in vivo.

During our biochemical evaluation we also observed that HLA-E-bound VL9 leader peptide was readily displaced by exogenous challenge with Molar excess of the Mtb44 peptide. Additionally, an excess of lower affinity peptides appears to disrupt the integrity of VL9-refolded HLA-E complexes, as evidenced by the emergence of the “diffuse” dimer gel form upon challenge with the RL9HIV epitope. Remarkably, the highest affinity VL9 leader epitope imparts a relatively small increase (Tm = 10 °C) to the stability of the HLA-E-β2m complex versus no peptide refolded forms, which contrasts the much larger values that high affinity peptides contribute to MHC class I stability upon binding.10,41

Presumably this property of lower peptide affinity is driven from the perspective of NK cell recognition.45,46 However, in the context-specific setting generated by the RhCMV68-1 regimen, this feature might enhance peptide-exchange properties of refolded HLA-E-β2m complexes.

Based on the gel profiles of optimally loaded peptide complexes, it is highly likely that the homogeneous, CI of HLA-E-bound VL9HIV represented material that crystalised in vitro. In contrast, the diffuse, peptide-receptive VL9HIV-specific material may comprise suboptimally bound peptide forms. The apparent incomplete loading of VL9HIV and other vaccine-identified peptides presumably reflects their lower affinities, given that exogenous loading of the higher affinity VL9 and Mtb44 peptides facilitated complete recovery of peptide-loaded, compact forms. It was initially reported that the MHC PBG undergoes conformational readjustments upon peptide binding47, with more recent hypotheses purporting that this is characterised by a two-stage transition where the groove initially exists in an “open” partially hydralized form accommodating suboptimally bound peptide, that converts ultimately, to a dehydrated, “closed” form upon optimal peptide loading.48,49 A likely explanation is that the diffuse forms described here could include both “empty” heterodimer (open (o)) and weakly bound VL9HIV peptide (open-peptide (op)), of which the latter species transitions to the compact peptide bound form (Cp) at an equilibrium primarily influenced by the affinity of the epitope for HLA-E (Hpol Hpol Hpol). Thus, for the higher affinity VL9 and Mtb44 peptides, the balance is strongly skewed to the compact peptide-bound forms, whereas weaker epitopes such as RL9HIV, the equilibrium is shifted towards the open/open-peptide binding species. In Mtb and RhCMV68-1 infected cells we suggest that movement of peptide binding away from native presentation routes and the features of peptide recognition may comprise suboptimally bound peptide forms. The apparent incomplete loading of VL9HIV and other vaccine-identified peptides presumably reflects their lower affinities, given that exogenous loading of the higher affinity VL9 and Mtb44 peptides facilitated complete recovery of peptide-loaded, compact forms. It was initially reported that the MHC PBG undergoes conformational readjustments upon peptide binding, with more recent hypotheses purporting that this is characterised by a two-stage transition where the groove initially exists in an “open” partially hydralized form accommodating suboptimally bound peptide, that converts ultimately, to a dehydrated, “closed” form upon optimal peptide loading. A likely explanation is that the diffuse forms described here could include both “empty” heterodimer (open (o)) and weakly bound VL9HIV peptide (open-peptide (op)), of which the latter species transitions to the compact peptide bound form (Cp) at an equilibrium primarily influenced by the affinity of the epitope for HLA-E (Hpol Hpol Hpol). Thus, for the higher affinity VL9 and Mtb44 peptides, the balance is strongly skewed to the compact peptide-bound forms, whereas weaker epitopes such as RL9HIV, the equilibrium is shifted towards the open/open-peptide binding species. In Mtb and RhCMV68-1 infected cells we suggest that movement of peptide binding away from the quality control environment of the ER-based TAP-Tapasin-associated PLC to an alternative loading pathway potentially shifts the balance allowing low-affinity peptides with suboptimal sequence motifs (op forms) to bind HLA-E or Mamu-E. Whether MHC peptide editors such as TAP-binding protein related (TAPBPR) protein, further influence peptide selection in this alternative pathway is currently unknown.

The work of Hansen et al.13 suggests that RhCMV68-1 vaccinated macaques prime Mamu-E-restricted CD8+ T cells and subsequently recognise peptide targets on SIV infected cells following viral challenge. The mechanism underlying how these low-affinity peptide epitopes elicit CD8+ T cell responses remains unclear. It is also unknown whether unusual forms of Mamu-E resembling the HLA-E ‘open’ material described here are generated in RhCMV68-1 vaccinated macaques, and whether CD8+ T cells recognise these forms. Both the mechanisms underlying alternative presentation routes and the features of peptide recognition by Mamu-E restricted CD8+ T cells are the focus of ongoing investigations.

**Methods**

**Peptide synthesis.** Synthetic nonameric peptide was generated by Fmoc (9-fluorenylethoxycarbonyl) chemistry on a purity of >95% by GL Biochem USA. All peptides were provided as lyophilised power, reconstituted in DMSO to a concentration of 200 mM, and stored at –80 °C. A UV photolable version of the HLA-B leader sequence peptide, VMApRTLVL, incorporating a UV-sensitive 3-amino-3-(2-nitrophenyl)-propionic acid residue (I residue) substitution at position 5 (termed 5m)72, was synthesised by Dria Elatmioui at UMC. The N7M2 peptide was stored as lyophilised power, and dispensed/reconstituted as required.

**RM and vaccines.** A total of 9 purpose-bred male RM (Macaca mulatta) of Indian genetic background (3–7 years of age) were used in the animal experiments reported here. All animals were used with the approval of the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee, under the standards of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (IACUC). The ONPRC is accredited as a Category 1 facility by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and has an approved Assurance (#A3304-01) for the care and use of animals on file with the Office for Protection from Research Risks at NIH. The IACUC adheres to national guidelines outlined in the Animal Welfare Act (7 U.S.C. Sections 2131–2139) and the Guide for the Care and Use of Laboratory Animals57 as mandated by the US Public Health Service Policy. The ONPRC IACUC approved care of RM, in addition to all experimental protocols and procedures. All RM were housed at the ONPRC in animal facility level (AFL) 2 with autonomously controlled lighting, temperature and humidity. They were fed with commercially prepared primate chow (Purina Lab Diet: Fibre-Balanced Monkey Jumbo, 5000; High Protein Monkey Diet, 5045) twice daily and received daily supplemental fresh fruit or vegetables. Fresh, potable water was provided via automatic water systems. Physiological, blood work and complete blood counts, were performed at all protocol time points. A number of criteria were used to normalise the animals within vaccine groups, including MHC haplotype, age and sex. Once the groups were set, compatible animals within the same vaccine group were pair housed for the duration of the immunisation phase. Animals for which no compatible pair mate was identified, but otherwise met the pairing criteria, were single cage-housed for the duration of the immunisation phase or until a suitable compatible pair mate were identified. RM used in these experiments were free of cercopithicine herpesvirus 1, D-type simian retrovirus, and simian T-lymphotrophic virus type 1. Six RM (3–4 years of age) were subcutaneously vac-cinated with the Rhesus cytomegalovirus 68-1 strain (RhCMV68-1)13 expressing HIV-M-Gag-Nef fusion and HIV-M-Pol from the Episensus1 (RL9 RMYSPTSIL) or Episensus2 and Episensus2 (IL9 RMYSPTSTI)33. Three RM were immunised intraovely with 1.25 × 10^6 HIV M1 1°C T cells are the focus of ongoing investigations.
CD4+/CD8+ T cell subsets following gating on the light scatter signature of small lymphocytes. Antigen specific CD8+ T cell response frequencies were determined from intracellular expression of IFN-γ 5 ng/1e6 cells (that also included an additional change creating a BamHI R restriction site), 5′-GACCTGGGGGGTATCCACCTTCTTCAAAATTGGTTCC-3′, and the reverse primer 5′-ggttaccaagccgttgatgacatctgacacc-3′ [GACCC]. This construct was inserted into pEGFP-N1 downstream of a HindIII-BamHI I cassette that contains the signal sequence of HLA-E*01:03, the central core region of the mature HLA-E transmembrane mature peptide, M(*)[GGGGS]* linker. Wild type and position 2 (p) mutants of the V19 and Mtb44 peptide sequences, followed by a flexible [GGGGS]* linker, were introduced in between the HLA-E signal sequence and the start of the β2-microglobulin sequence by overlap extension PCR.

Peptide β2m-HLA-E transient transfection of 293 T cells. HEK 293T cells were maintained in 5% CO2 in DMEM (Life Technologies) supplemented with 10% Foetal Bovine Serum (SeraLabs), and Penicillin/Streptomycin (50 and 50 µg/ml, respectively, Life Technologies). Transfections were carried out at 70% confluency in six well plates using GeneJuice (Millipore) according to the manufacturer’s instructions. Following 24 h, cells were harvested. 1 million 293T cells were stained with 1 µL of the anti-HLA-E monoclonal antibody, 3D12 (Biogen, 310941). Alexafluor 488-labeled anti-CD8a (SK1: PerCP-eFluor 710; 7.5 ng/1e6 cells; eBioscience, 46-0087-42), and PE/Dazzle 594; 60 ng/1e6 cells BioLegend, 310941).

Protein production HLA-E*01:03 HC residues 1–274 and β2-microglobulin (β2m) were cloned into pET22b prokaryotic vectors and expressed in BL21 competent E.coli. Inclusion bodies were purified via sonication and homogenisation in a Triton-based buffer prior to final re-solubilisation in 8 M urea, 50 mM MES pH 6.5, 0.1 mM EDTA and 0.1 mM DTT23.

Peptide binding affinity assays. A peptide binding affinity assay was adapted from published micro-scale refold-ELISA-based methods38,39. In brief, 1 µM HC and 1.5 µM pre-refolded β2m were refolded in 0.33 mM Tris-Maleate and 0.5% Lutrol-F68 in the presence of 70 µM peptide, pre-diluted to 2 mM working stocks in 100 mM Tris-HCl pH 8. Micro-refolds were incubated at room temperature for 48 h before the relative capacity of each peptide to support stable HLA-E-β2m- peptide complex formation was quantified by sandwich ELISA. Correctly refolded heterotrimetric complexes diluted 1:100 in 2% IgG-free bovine serum albumin (BSA) were loaded onto HLA-E*01:03 HC pre-captured by the anti-human HLA-E monoclonal, 3D12 (10 µg/ml) in ELISA wells previously blocked and washed, respectively, with 2% BSA and 0.05% Tween-based wash buffer. 0.2 µg/ml polyclonal detection IgG raised in rabbits, specific for human β2m and enhancement antibodies specific for rabbit IgG, diluted 1:15 in 2% BSA, both conjugated to horseradish peroxidase, were sequentially added to ELISA wells to ensure detection of β2m-associated forms of HLA-E only. Tetramethyl benzidine substrate and STOP solution were used to develop and terminate reactions, respectively, before obtaining absorbance readings at 450 nm on a FLUOstar OMEGA plate reader.

Protein refolding and purification. β2m (at a final concentration of 2 µM) was refolded in 100 mM Tris pH8.0, 400 mM 1-arginine monohydrochloride, 2 mM ethylenediaminetetraacetic acid, 5 mM reduced glutathione and 0.5 mM oxidised Glutathione at 4 °C for 30 min before the addition of 20–50 µM peptide. HLA-E*01:03 HC was pulsed into the refolding buffer until a final concentration of 1 µM was reached. Following incubation for 72 h at 4 °C, HLA-E refolds were filtered through 1.0 µm cellular nitrate membranes to remove aggregates prior to concentration by centrifugation at 10000× g for 48 h at 4 °C in a Amicon Centricon Plus-70 and Ultra-15 10-kDa cut-off centrifugal filter devices. Samples were concentrated according to size into 20 mM Tris pH8, 100 mM NaCl by fast protein liquid chromatography on a Superdex 75 16/60 column. Elution profiles were visualised by UV absorbance at 280µAU, enabling differentiation of correctly refolded HLA-E-β2m complexes from smaller unassembled β2m and larger refolded aggregates. Proteins were concentrated to 10 mg/ml for crystallisation and aliquots further analysed by SDS-PAGE electrophoresis to confirm presence of non-aggregated HLA-E HC and β2m.

Crystallisation screening. A total of 100 mL protein, at 10 mg/ml, and 100 mL reservoir buffer were mixed in crystallisation wells and equilibrated by sitting drop vapour-diffusion at 20 °C. Commercial sparse matrix grid screens were used to identify optimal crystallisation conditions, around which ammonium sulphate pH 4.0 and Dulbecco’s PBS (D-PBS) were merged in Xia2 to increase completeness of the dataset58,59. Diffraction data were collected at Diamond Light Source Beamlines I04 and I24. Data collection statistics were merged in Xia2 to increase completeness of the dataset58,59. Diffraction data were collected at Diamond Light Source Beamlines I04 and I24. Data collection statistics were merged in Xia2 to increase completeness of the dataset58,59. Diffraction data were collected at Diamond Light Source Beamlines I04 and I24.

Crystallographic analysis. Diffraction images from multiple isomorphous crystals were merged in X2iA to increase completeness of the dataset60,61. Diffraction data were auto-indexed by Xia2 DIALS using the default parameters since 2015: 1 sig(I) > 0.25, merged I sig(I) > 1 and CC ½ > 0.58–60. Initial phasing was carried out using the coordinates of the VL9-bound HLA-E*01:01 structure (PDB code 1MHE), stripped of peptide, hydrogens and waters as the search model in MolRep of CCP4™62. Molecular replacement for RUSHV and Mtb44 variant datasets was subsequently carried out in Phoenix63 using the refined Mtb44 structure coordinates as the phasing model. Rigid body, restrained and TLS refinement were computed by CCP4™ REFMAC561 or Phenix.refine64 applying non-crystallographic symmetry restraints between iterative cycles of manual model building in Coot65. Models were validated using MolProbity66, visualised using the PyMOL Molecular Graphics System, version 2.0 (Schrodinger, LLC) and further investigated by PDBePISA67 and PDBeFOLD68.
Peptide exchange into pre-refolded HLA-E-β2M-VL9 complexes and no peptide refolded complexes. A total of 20 μg of purified HLA-E-β2m complexes, previously refolded without peptide or in the presence of VL9, were pulsed, respectively, with 50 or 200 M excess of Mtb4 or RLV/HIV peptides for 2 h at room temperature, in a final volume of 20 μl. Then, 10 μl of each sample was loaded onto a BN-PAGE gel and their signature profiles were subsequently analysed.

Data availability. Structural factors and atomic coordinates have been deposited within the Protein Data Bank with accession codes: 6G1H, 6G4H, 6GGM, 6GHN and 6GL1.

All relevant data outlined in this study are available from the authors.

Received: 23 January 2018 Accepted: 4 July 2018
Published online: 07 August 2018

References

1. Boyson, J. E. et al. The MHC-E locus in macaques is polymorphic and is conserved between macaques and humans. Immunogenetics 41, 59–68 (1995).
2. Knapp, I. A., Cadavid, L. F. & Watkins, D. I. The MHC-E locus is the most well conserved of all known primate class I histocompatibility genes. J. Immunol. 160, 189–196 (1998).
3. Pietra, G., Romagnani, C., Manzini, C., Moretta, L. & Minigari, M. C. The emerging role of HLA-E-restricted CD8+ T lymphocytes in the adaptive immune response to pathogens and tumors. J. Biomed. Biotechnol. 2010, 907092 (2010).
4. Sullivan, L. C., Clements, C. S., Rossjohn, J. & Brooks, A. G. The major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with high affinity and 6GL1.
5. Pietra, G., Romagnani, C., Moretta, L. & Minigari, M. C. Recognition by subsets of NK and T cells. Curr. Pharm. Des. 15, 3336–3344 (2009).
6. Salerno-Goncalves, R., Fernandez-Vina, M., Levinsohn, D. M. & Szein, M. B. Identification of a human HLA-E-restricted CD8÷ T cell subset in volunteers immunized with Salmonella enterica serovar Typhi strain Ty21a typhoid vaccine. J. Immunol. 173, 5852–5862 (2004).
7. McLoon, M. et al. T cell recognition of Mycobacterium tuberculosis peptides presented by HLA-E derived from infected human cells. PLoS ONE 12, e0188228 (2017).
8. Harriff, M. J. et al. HLA-E presents glycopeptides from the Mycobacterium tuberculosis protein MPT32 to human CD8+ T cells. Sci. Rep. 7, 4622 (2017).
9. Grotzke, J. E. et al. The Mycobacterium tuberculosis phagosome is a HLA-I processing competent organelle. PLoS Pathog. 5, e1000374 (2009).
10. O’Callaghan, C. A. et al. Structural features impose tight peptide binding specificity in the non-classical MHC molecule HLA-E. Mol. Cell 1, 531–541 (1998).
11. Tomasec, P. et al. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gU40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. J. Immunol. 164, 5019–5022 (2000).
12. Ulbrecht, M. et al. Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. J. Immunol. 165, 4375–4385 (1998).
13. Lundegaard, C. et al. NetMHC-3.0: accurate web accessible predictions of peptide affinities of all known primate class I histocompatibility genes. J. Mol. Biol. 307, 184–202 (2001).
14. Romagnani, C. et al. HLA-E-restricted recognition of human cytomegalovirus by a subset of cytolytic T lymphocytes. Hum. Immunol. 65, 437–445 (2004).
15. Siehpahl, M. B. Cell-mediated immunity and antibody responses elicited by attenuated Salmonella enterica Serovar Typhi strains used as live oral vaccines in humans. Clin. Infect. Dis. 51, S15–S19 (2010).
16. Salerno-Goncalves, R., Wahid, R. & Szein, M. B. Ex vivo kinetics of early and long-term multifunctional human leukocyte antigen E-specific CD8+ cells in volunteers immunized with the Ty21a typhoid vaccine. Clin. Vaccine Immunol. 17, 1305–1314 (2010).
17. Goodridge, J. P., Burian, A., Lee, N. & Geraghty, D. E. HLA-E-F complex binding motif that is strikingly similar to HLA-A2. Hum. Immunol. 65, 531–541 (1998).
18. Pietra, G., Romagnani, C., Moretta, L. & Mingari, M. C. HLA-E and HLA-E-binding molecules are targets for human CD8 T cells with cytotoxic as well as MHC class I interaction. Tissue Antigens 72, 415–424 (2008).
19. Braud, V., Jones, E. Y. & McMichael, A. The major histocompatibility complex class Ib molecule HLA-E binds to the interface between innate and adaptive immunity. Tissue Antigens 72, 1164–1169 (1997).
20. Braud, V. et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 391, 795–799 (1998).
21. Hoare, H. L. et al. Subtle changes in peptide conformation profoundly affect recognition of the non-classical MHC class I molecule HLA-E by the CD94-NKG2A complex from natural killer cells. J. Biol. Chem. 278, 5082–5090 (2003).
22. Braud, V. M. et al. HLA-E binds to the binding motif that is strikingly similar to HLA-A2. J. Immunol. 160, 1967–1972 (1998).
23. Strong, R. K. et al. HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. J. Biol. Chem. 278, 1164–1169 (1997).
24. Lee, N. et al. HLA-E is a major ligand for the natural killer inhibitor receptor CD94/NKG2A. Proc. Natl Acad. Sci. USA 95, 5199–5204 (1998).
25. Lee, N., Goodlett, D. R., Ishitani, A., Marquardt, H. & Geraghty, D. E. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. J. Immunol. 160, 4951–4960 (1998).
26. Strong, R. K. et al. HLA-E ligands in MHC class I molecules. Nature 419, 498–499 (2002).
27. Braud, V. M. et al. HLA-E binds to the native killer cell receptors CD94/NKG2A, B and C. Nature 391, 795–799 (1998).
28. Hoare, H. L. et al. Subtle changes in peptide conformation profoundly affect recognition of the non-classical MHC class I molecule HLA-E by the CD94-NKG2A complex from natural killer cells. J. Biol. Chem. 277, 1297–1302 (2003).
29. Caccamo, N. et al. Human CD8 T lymphocytes recognize Mycobacterium tuberculosis antigens presented by HLA-E during active tuberculosis and express type 2 cytokines. Eur. J. Immunol. 35, 1069–1081 (2015).
30. Garcia, P. et al. Human T cell receptor-mediated recognition of HLA-E. Eur. J. Immunol. 32, 936–944 (2002).
31. Hansen, S. G. et al. Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E. Science 351, 714–720 (2016).
32. Heinzl, A. S. et al. HLA-E-dependent presentation of Mtb-derived antigen to human CD8+ T cells. J. Exp. Med. 196, 1473–1481 (2002).
33. Joosten, S. A. et al. Mycobacterium tuberculosis peptides presented by HLA-E molecules are targets for human CD8 T cells with cytotoxic as well as regulatory activity. PLoS Pathog. 6, e1000782 (2010).
34. Levinsohn, D. M. et al. Mycobacterium tuberculosis-specific CD8+ T cells preferentially recognize heavily infected cells. Am. J. Respir. Crit. Care Med. 168, 1346–1352 (2003).
35. Pietra, G. et al. HLA-E-restricted recognition of cytomegalovirus-derived peptides by human CD8+ T lymphocytes. Proc. Natl Acad. Sci. USA 100, 10896–10901 (2003).
46. Lo Monaco, E. et al. HLA-E: strong association with beta2-microglobulin and surface expression in the absence of HLA class I signal sequence-derived peptides. *J. Immunol.* 181, 5442–5450 (2008).

47. Springer, S., Doring, K., Skipper, J. C., Townsend, A. R. & Cerundolo, V. Fast association rates suggest a conformational change in the MHC class I molecule H-2Db upon peptide binding. *Biochemistry* 37, 3001–3012 (1998).

48. van Hateren, A., Bailey, A. & Elliott, T. Recent advances in Major Histocompatibility Complex (MHC) class I antigen presentation: Plastic MHC molecules and TAPBP-R-mediated quality control. *FI000Research* 6, 158 (2017).

49. Yanaka, S. et al. Peptide-dependent conformational fluctuation determines the stability of the human leukocyte antigen class I complex. *J. Biol. Chem.* 289, 3383–3389 (2014).

50. Jiang, J. et al. Crystal structure of a TAPBP-MHC-I complex reveals the mechanism of peptide editing in antigen presentation. *Science* 358, 1064–1068 (2017).

51. Thomas, C. & Tampe, R. Structure of the TAPBPR-MHC-I complex de

52. Boyle, L. H. et al. Tapasin-related protein TAPBP-R is an additional component of the MHC class I presentation pathway. *Proc. Natl Acad. Sci. USA* 110, 3465–3470 (2013).

53. Blee, A. et al. Structure of the human MHC-I peptide-loading complex. *Nature* 551, 523–528 (2017).

54. Purbhoo, M. A., Irvine, D. J., Huppa, J. B. & Davis, M. M. T cell killing does not require the formation of a stable mature immunological synapse. *Nat. Immunol.* 5, 524–530 (2004).

55. Theiler, J. et al. Epitope: a vaccine design tool applied to an HIV therapeutic vaccine and a pan-filovirus vaccine. *Sci. Rep.* 6, 33987 (2016).

56. Hansen, S. G. et al. Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. *Science* 328, 102–106 (2010).

57. Walter, T. S. et al. A procedure for setting up high-throughput nanolitre crystallization experiments. Crystallization workflow for initial screening, automated storage, imaging and optimization. *Acta Crystallogr. D Biol. Crystallogr.* 61, 651–657 (2005).

58. Evans, P. R. & Murshudov, G. N. How good are my data and what is the resolution? *Acta Crystallogr. D Biol. Crystallogr.* 66, 125–132 (2010).

59. Kabsch, W. *Xds.*

60. Evans, P. R. & Murshudov, G. N. How good are my data and what is the resolution? *Acta Crystallogr. D Biol. Crystallogr.* 69, 1204–1214 (2013).

61. Karplus, P. A. & Diederichs, K. Linking crystallographic model and data quality. *Science* 336, 1030–1033 (2012).

62. Vagin, A. & Teplyakov, A. Molecular replacement with MOLREP. *Acta Crystallogr. D Biol. Crystallogr.* 67, 235–242 (2011).

63. Wang, H. et al. Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. *Science* 328, 102–106 (2010).

64. Adams, P. D. et al. PHENIX: a comprehensive python-based system for macromolecular structure solution. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 62, 213–221 (2010).

65. Afonine, P. V. et al. Towards automated crystallographic structure refinement with phenix.refine. *Acta Crystallogr. D Biol. Crystallogr.* 68, 352–367 (2012).

66. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* 66, 486–501 (2010).

67. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* 66, 12–21 (2010).

68. Krissinel, E. & Henrick, K. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* 372, 774–797 (2007).

69. Krissinel, E. & Henrick, K. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallogr. D Biol. Crystallogr.* 60, 2256–2268 (2004).

70. Hansen, T. H., Connolly, J. M., Gould, K. G. & Fremont, D. H. Basic and translational applications of engineered MHC class I proteins. *Trends Immunol.* 31, 363–369 (2010).

Acknowledgements

The authors would like to thank the research staff who collected study samples, and Diamond Light Source (Didcot, UK) for access to beamlines I04 and I21 (MX14744) that contributed to the results presented here. We also wish to thank Pamela Bjorkman, Tim Elliott and Ton Schumacher for helpful discussions, and David W. Morrow and Abigail B. Ventura for technical assistance. This work was supported by grants from the BMGF OPP1133649, NIH/NIADD UM1 AI0645, NIH/NIADD U19 AI128741, UM1 AI126619, MRC MR/K012037/1, and MRC MR/M019837/1. J.B.S. and S.G.H. are supported by grant R21 AI127125. L.P. is supported by grants from NIH, NIADD (R01-A0094417, R37-AI054292, U19 AI095985, U19 AI096109, and P50-OI11092), and from the BMGF OPP1107409 and OPP1108533. V.J is funded by the EMBO long-term fellowship. E.Y.J. is supported by Cancer Research UK and MRC Programme Grants (C375/A17721 and MR/M000141/1), and Wellcome Trust grant 203141/Z/16/Z supporting the Wellcome Centre for Human Genetics.

Author contributions

L.C.W. performed binding and crystallographic experiments, interpreted data, and

helped prepare the manuscript. K.H., V.J., D.R., T.S.W. and E.Y.J. helped with crystallographic data analysis/data interpretation. T.S.W. helped with crystallography screen set-up. S.B. and I.B.P. performed single-chain trimer experiments and data interpretation. C.O.C. and P.B. helped with data evaluation. M.T. designed the UV peptide exchange peptide. S.G.H., S.A.J.M.G., J.S. and L.J.P. performed BCG vaccination studies, cellular experiments and helped with data interpretation. E.M. and K.F. constructed the RhCMV68–1/IV vaccine. A.J.Mc.M. and G.M.G. performed experiments, helped prepare the manuscript and supervised the project.

Additional information

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-05459-z.

Competing interests: L.J.P., S.G.H. and K.F. have a significant financial interest in Vir Biotechnology, Inc., a company that may have a commercial interest in the results of this research and technology. The potential individual and institutional conflicts of interest have been reviewed and managed by OHsu. All other authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2018