# Targeting MHC-E as a new strategy for vaccines and immunotherapeutics

Klaus Früh[1†], Persephone Borrow[2], Geraldine M. Gillespie[2], Andrew J. McMichael[2] & Louis J. Picker[1†]

[1]Vaccine & Gene Therapy Institute, Oregon Health & Science University, Beaverton, Oregon, USA.
[2]Centre for Immuno-Oncology, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.
[†]e-mails: fruehk@ohsu.edu; pickerl@ohsu.edu

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## Abstract

MHC-E is a highly conserved, non-polymorphic MHC protein that engages inhibitory and activating receptors on natural killer (NK) cells and T cells and can also present antigens to T cell receptors. NK cell responses driven by activating receptor interactions with MHC-E are implicated in controlling chronic viral infections and cancer. Immunotherapeutic targeting of interactions between MHC-E and inhibitory receptors to increase the activation of NK cells and T cells shows promise in improving antitumour immune responses. Furthermore, MHC-E-restricted $CD8^+$ T cells elicited by cytomegalovirus-based vaccines might, for certain infections and cancers, be more effective than $CD8^+$ T cells restricted by classical MHC class I or class II molecules. The ability of MHC-E to regulate or mediate both innate and adaptive immune responses independently of an

individual's MHC haplotype raises the possibility of new, universally effective vaccines and immunotherapies for infectious disease and cancer. Although the therapeutic exploitation of MHC-E is still in its infancy, recent advances in the understanding of MHC-E biology show enormous potential, as described in this Review.

## [H1] Introduction

The major histocompatibility complex (also known as the HLA complex in humans) has a key role in shaping cellular adaptive and innate immune responses. It is a multigenic chromosomal region that, in mammals, is typically divided into subregions encompassing genes encoding MHC class I (MHC-I) and MHC class II (MHC-II) molecules, proteins involved in antigen processing, ligands for innate receptors, mediators of inflammation such as complement components and innate cytokines (for example, tumour necrosis factor (TNF)), as well as proteins that have no known immunological functions[1]. The MHC-I region includes genes encoding classical MHC-Ia proteins (MHC-A, MHC-B and MHC-C), which present antigen-derived peptides to clonotypic T cell receptors (TCRs) of $CD8^+$ T cells, and genes encoding non-classical MHC-Ib proteins (MHC-E, MHC-F and MHC-G), which modulate both innate and adaptive immune cells through specific inhibitory and activating receptors[2]. The vast majority of $CD8^+$ T cell-targeting vaccines and immunotherapeutics aim to elicit and/or enhance responses to classical MHC-Ia-presented epitopes, the application and/or effectiveness of which are affected by MHC-Ia haplotype diversity. By contrast, MHC-Ib molecules such as MHC-E have limited polymorphism and broader functions, offering the potential for more-universal, 'off-the-shelf' therapeutics.

Although the therapeutic exploitation of MHC-E is still in its infancy, recent advances in the understanding of MHC-E biology show enormous potential. In this Review, we describe how MHC-E assembles with canonical 'VL9' epitopes and how the interaction of this peptide–MHC-E complex with innate immune receptors can be targeted therapeutically to improve innate and adaptive cellular immune responses to infected or cancer cells. In addition, we review how the presentation by MHC-E of non-canonical epitopes derived from pathogens or cancer antigens can elicit MHC-E-restricted T cells in vivo and can be targeted by MHC-E-restricted T cells elicited by cytomegalovirus (CMV)-based vaccine vectors.

## [H1] Properties of MHC-E

Both classical and non-classical MHC-I genes encode heavy chains that bind to β2-microglobulin (β2m), forming a heterodimer that associates with peptides. The MHC-Ib molecule MHC-E (HLA-E in humans) has the particular function of presenting conserved nonamer peptides known as 'VL9' peptides to CD94–NKG2A and CD94–NKG2C receptors, which regulate natural killer (NK) cells and a subset of $CD8^+$ T cells[3-5] (**Box 1**). VL9 peptides are contained within the cleavable signal sequence of MHC-Ia molecules (as well as the MHC-Ib molecule HLA-G), with the most frequent VL9 sequences in the human population being (VM[A/P]PRT[V/I][L/V/I//F]L)[6-8]. Notably, HLA-E itself does not contain a VL9 peptide. MHC-E is the most highly conserved MHC-I locus in primates, with HLA-E-like proteins being present across both old world and new world primates[9]. In humans and non-human primates, MHC-E has very limited polymorphism[9], with just two main alleles found in the human population that differ only in amino acid 107 (Arg in HLA-E∗0101; Gly in HLA-E∗01031 and HLA-E∗01032)[10]. This polymorphism is outside of the peptide-binding groove of HLA-E and does not affect its structure, although it does impact surface stability and expression levels[11,12]. Remarkably, a similar system is found in mice, whereby the non-polymorphic MHC-Ib protein $Qa\text{-}1^b$ similarly binds to conserved VL9-like peptides that are referred to as QDM (Qa-1 determinant modifier) and that are encoded in the signal peptides of mouse MHC-Ia molecules[6,13]. Thus, $Qa\text{-}1^b$ is the mouse homologue of HLA-E, and the ability to present MHC-Ia-derived peptides at the cell surface seems to be a highly conserved immune defence mechanism throughout evolution. Many pathogens as well as cancer cells downregulate MHC-Ia molecules to evade $CD8^+$ T cell-mediated control, but in turn this alerts the cellular immune system to 'missing self' disturbances in the MHC-Ia pathway through MHC-Ib[3,4]. Like classical MHC-Ia molecules, MHC-E is expressed on most nucleated cells[14]. However, the surface expression of MHC-E and presentation of VL9 peptides on most cells is usually very low unless induced by inflammatory signals such as interferons[15,16]. Moreover, HLA-E is rapidly internalized once at the cell surface, with the cytoplasmic tail of HLA-E directing it to late and recycling endosomes[17]. In the absence of interferon induction, HLA-E surface expression is most readily detected on leukocyte subsets such as T cells, NK cells, B cells, monocytes and macrophages, as well as endothelial cells.

## [H1] MHC-E-regulated innate immune responses

The MHC-E/β2m/VL9 complex modulates cellular innate and adaptive immune responses by engaging inhibitory and activating innate receptors expressed on NK cells and T cells. Pathogen targeting of these ligand/receptor interactions can result in immune evasion or enhanced immune control.

### *[H2] Signalling through CD94–NKG2A/NKG2C*

Surface-exposed trimeric complexes of peptide–MHC-E–β2m are ligands for the heterodimeric, type II transmembrane proteins CD94–NKG2A and CD94–NKG2C[3-5]. These receptors are an evolutionarily old lineage of C-type lectins found across all vertebrate species. Human CD94–NKG2A is an immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing inhibitory receptor, whereas CD94–NKG2C is an immunoreceptor tyrosine-based activatory motif (ITAM)-associating activating receptor[18-21] (**Fig. 1**). These receptors are widely expressed on NK cells and are also found on some $CD8^+$ memory T cells, although their expression patterns vary widely among individuals[22-24]. Structural analyses of the interaction between CD94–NKG2A and the HLA-E trimer have revealed the molecular basis for VL9 peptide specificity and have also indicated why NKG2A has a higher affinity than NKG2C for binding to this complex[12,25,26].

Recently, the structure of mouse CD94–NKG2A bound to Qa-$1^b$ in complex with the peptide AMAPRTLLL was solved[27]. This structure revealed subtle differences in the ligand–receptor interface compared with the human molecules, which explains the species specificity of the interaction of CD94–NKG2A with HLA-E or Qa-$1^b$. Based on these results, Qa-$1^b$ could be engineered for cross-reactivity with human CD94–NKG2A. Overall, however, this structure confirmed the remarkable conservation of the MHC-E system in presenting self-peptides derived from classical MHC-Ia molecules to CD94–NKG2A for the regulation of NK cell responses.

### *[H2] Signalling through LIR1 and LIR2*

Recently, it was shown that the inhibitory receptors leukocyte immunoglobulin-like receptor 1 (LIR1) and LIR2 (also known as LILRB1 and LILRB2) bind to Qa-$1^b$ and HLA-E independently of the bound peptide[15] (**Fig. 1**). LIR1 could thus be engaged by HLA-E molecules presenting non-VL9 peptides — for example, a peptide contained in the signal sequence of HSP60, which is upregulated during stress[28], or some other peptides identified in the human proteome that bind strongly to HLA-E[29]. In addition to HLA-E, LIR1 binds to both classical and other non-classical MHC-I molecules, as well as viral MHC-I-like molecules such as UL18 of human CMV (HCMV)[30]. The structural basis of the LIR–HLA-E interaction is presently unknown, although it is likely that it involves the α3-domain and β2m, as has been observed for other LIR–MHC complexes[30,31].

## *[H2] Modulation of NK cell and T cell responses*

Unlike CD8$^+$ αβ T cells, which are activated through signals from their αβ T cell receptor (TCR) engaging with peptide–MHC-I complexes, NK cells are regulated through signals from a broad array of activating receptors and inhibitory receptors. These receptors have important roles in NK cell development as well as regulating their activity in the periphery[32]. Similarly, γδ T cells can be activated through their γδ TCR engaging with non-MHC ligands or through activating receptors. In the blood, CD94–NKG2A is expressed by approximately half of human NK cells[33], by a very small percentage of circulating CD8$^+$ T cells (mostly previously activated cells) and by a distinct subset of γδ T cells (20–90% of the δ2 subset)[34]. In all of these cases, it is thought that signalling through NKG2A counteracts immediate effector function, such as cytolysis, of these cells in response to activation through the TCR or activating receptors. Thus, CD94–NKG2A functions as a negative feedback receptor after immune activation[15], which may be important for sustaining T cell function and avoiding exhaustion during chronic infection[35]. NKG2A has also been implicated in NK cell regulation and education, and in maintaining NK cell clonal expansion by avoiding activation-induced cell death[36,37]. NKG2A expression by γδ T cells increases their activation threshold upon signaling through their γδ TCR or through activating receptors; blocking NKG2A could potentially improve the antitumour functions of γδ T cells or their activity against chronic viral infections such as with HIV and hepatitis B virus (HBV)[34].

Only a small percentage of NK cells are reported to express both NKG2A and NKG2C, and the inhibitory receptor NKG2A binds with higher affinity to MHC-E so that the inhibitory signal generally overrides the activating signal if both receptors are expressed on the same NK cell[38]. Moreover, the ligation of CD94–NKG2A and CD94–NKG2C depends strongly on the specific VL9 sequence presented by MHC-E molecules[12]. Thus, polymorphism in the human VL9 sequence affects the threshold for NK cell activation versus inhibition[33]. Interestingly, yeast display analysis of HLA-E single-chain trimers presenting randomized peptides to CD94–NKG2A and CD94–NKG2C identified a peptide motif that was highly conserved between CD94–NKG2A-selected and CD94–NKG2C-selected libraries, as well as peptides that bound with higher affinity to NKG2A than NKG2C and vice versa, which might allow for NK cell responses to be specifically activated or inhibited depending on the peptide sequence bound to HLA-E[29]. It also seems that the recognition of VL9–HLA-E by CD94–NKG2C is more restricted in terms of the VL9 sequence compared with CD94–NKG2A[39,40].

### *[H2] Response to pathogens*

The percentage of NK cells that express the activating receptor NKG2C strongly depends on whether a person is infected with HCMV[36,41,42]. As these NKG2C$^+$ NK cells are selectively expanded in response to infection with certain pathogens, they are known as 'adaptive' or 'memory' NK cells[43], although it should be noted that NK cells with adaptive features can also be induced independently of NKG2C[44]. In addition to high levels of NKG2C, most adaptive NK cells have epigenetic changes, including hypermethylation that reduces expression of the transcription factor PLZF (also known as ZBTB16) and stochastically inhibits expression of the signalling adaptors FcεRIγ, SYK and/or EAT2 (also known as SH2D1B), and hypomethylation of *IFNG* and *TNF* regulatory regions that facilitates transcription of these cytokine genes[45-47]. As a result, adaptive NK cells respond poorly to stimulation with innate cytokines such as IL-12 and IL-18 (receptors for which are upregulated by PLZF), and certain SLAM proteins and natural cytotoxicity receptors, but produce high levels of interferon-γ (IFNγ) and TNF following ligation of the Fcγ receptor FcγRIII (also known as CD16), and mediate increased Fcγ receptor-dependent antibody-dependent cellular cytotoxicity (ADCC)[48,49].

The expansion of adaptive NKG2C$^+$ NK cell populations in individuals chronically infected with HCMV is partly explained by the presence of a VL9 mimic in the signal sequence of the HCMV glycoprotein UL40, which results in strong upregulation of HLA-E surface expression in virus-infected cells[50,51] (**Box 2**). A similar protein is encoded by non-human primate CMVs, for example Rh67 of rhesus macaque CMV (RhCMV)[52,53]. Interestingly, the polymorphism of the VL9 sequence of UL40 in different HCMV strains reflects the polymorphism of VL9 sequences in the HLA of the human population[33,54,55]. By contrast, the VL9 sequence in Rh67 is not polymorphic, likely mirroring the lack of polymorphism in the VL9 sequence of non-human primate MHC molecules[53]. Similarly to humans, adaptive NK cell populations expand in macaques upon CMV infection[56,57]. In addition to VL9 encoded in UL40, HLA-E-binding peptides encoded in the HCMV protein pp65 have been shown to activate adaptive NK cells *in vitro*, although it remains to be shown whether these peptides promote the expansion of adaptive NKG2C$^+$ NK cell populations *in vivo*[54,58].

Adaptive NK cells arise over time in chronically HCMV-infected individuals[42]. During acute infection, however, the main function of HCMV VL9 peptides is likely to support viral immune evasion through inhibtion of NKG2A$^+$ NK cells that represent the first line of defence prior to adaptive humoral and cellular immune responses[59]. The role of NKG2C$^+$ NK cells in controlling CMV in healthy individuals is not clearly established but there are some indications that HCMV

infection is better controlled in recipients of HCMV$^+$ transplants who have high levels of NKG2C$^+$ NK cells[60,61]. Moreover, a study of lung transplant recipients revealed correlations between viral VL9 variants that bind with low affinity to the HLA-E alleles of the patient and high levels of viremia over time[62]. In addition, NKG2C deficiency has been shown to be a risk factor for HCMV-mediated complications post-transplant[63].

In addition to HCMV, other pathogens have been reported to contain HLA-E-binding peptides. However, in most cases, these peptides lead to increased stimulation of NK cells, rather than NK cell evasion, as the peptide–HLA-E complex either does not engage NKG2A or, in some cases, can lead to the expansion of NKG2C$^+$ NK cells (**Box 2**).

## [H1] Targeting MHC-E-dependent innate responses in cancer

HLA-E is often more highly expressed in tumours than in healthy tissues[64-70] and even more so on circulating tumour cells that give rise to metastasis[71,72]. Concurrently, classical MHC-Ia molecules are often downregulated, particularly in solid tumours[73], whereas HLA-G can be upregulated[74]. Cancer cells expressing HLA-E can thus be targeted by adaptive, NKG2C$^+$ NK cells. On the other hand, NKG2A expression on tumor-infiltrating T cells and NK cells reduces their anti-tumour activity. This immune checkpoint is thus explored as a new target for cancer immunotherapy and as a means to increase the efficacy of cancer vaccines.

### *[H2] NKG2C$^+$ NK cells for cancer treatment*

Adaptive NKG2C$^+$ NK cells are activated by VL9–HLA-E complexes with minimal impact of the specific VL9 sequence that is bound (viral or endogenous)[54]. Consequently, adaptive NK cells that are present in HCMV$^+$ individuals have the capability of attacking not only HCMV-infected cells but also other cells that express high levels of VL9–HLA-E complexes, such as certain tumour cells. In addition, adaptive NK cells have a potent ability to mediate ADCC, potentially owing to their reduced expression of FcεRIγ, which enables an increased response to IgG through FcγRIII stimulation[75]. Such beneficial ‘side-effects’ of adaptive NK cells have been observed in patients with acute myeloid leukaemia (AML), a haematological malignancy in which cells express high levels of HLA-E[76]. In patients with AML who are treated with allogeneic haematopoietic stem cell grafts replete with T cells and NK cells, reactivation of HCMV resulting in the expansion of adaptive NK cell populations was found to correlate with a marked reduction in AML relapse[55,77-80]. Adaptive NK cells have greater resistance to apoptosis under inflammatory conditions than do other NK cell populations[47], which may also be advantageous in the setting of allogeneic

haematopoietic stem cell transplantation. NKG2C$^+$ NK cells obtained after HCMV reactivation or from induced pluripotent stem cells could also be directed towards AML cells *in vitro* using an anti-NKG2C–IL-15–anti-CD33 NK cell engager that directs NKG2C$^+$ NK cells to target CD33$^+$ AML cells while also providing a proliferation and survival signal through IL-15[81] (**Fig. 2a**)Furthermore, adaptive NK cells make an important contribution to the ADCC response triggered by therapeutic antibodies directed against surface antigens in blood cancers such as multiple myeloma[82].

Based on these findings, it was hypothesized that NKG2C$^+$ adaptive NK cells obtained from healthy, HCMV-seropositive 'super-donors' could be used as 'off-the-shelf' treatments for haematological cancers[83] or other tumours with high levels of HLA-E expression, such as some glioblastomas[84]. A GMP-compliant enrichment and expansion method for NKG2C$^+$ adaptive NK cells was recently described[85], and multiple clinical trials using infusion of 'off-the-shelf', adaptive NK cells have been conducted (NCT03081780, NCT03213964, NCT03319459) [43]. An alternative approach would be to use peptide ligands for HLA-E that specifically stimulate NKG2C$^+$ NK cells but not NKG2A$^+$ NK cells. Such NKG2C-specific peptide ligands were identified in unbiased screens of peptide libraries as well as in a screen for HCMV-derived peptides[26,29,58]. Potentially, one could use such NKG2C-specfic peptides to elicit adaptive NK cell responses *in vivo*.

***[H2] NKG2A as an immune checkpoint***

Although HLA-E expression renders cancer cells more susceptible to attack by NKG2C$^+$ NK cells, the majority of tumour-infiltrating NK cells express the inhibitory receptor NKG2A[86]. Thus, it is thought that HLA-E upregulation protects tumours against NK cell-mediated control. Indeed, genome-scale CRISPR screens using expanded NKG2A$^+$ NK cells showed that HLA-E expression is an important indicator of reduced tumour cell sensitivity to NK cell-mediated cytotoxicity[87]. The NKG2A–HLA-E interaction has been shown to be a key factor that determines NK cell-mediated cytotoxicity in many types of cancer, including multiple myeloma, chronic lymphocytic leukaemia and several solid tumours (renal, breast, liver, bladder and lung cancers, as well as neuroblastoma and glioblastoma)[88,89]. Moreover, in addition to NK cells, a large fraction of tumour-infiltrating CD8$^+$ T cells expresses NKG2A, often together with late differentiation and tissue residency markers[65,90-92]. IL-12 expressed in the tumour microenvironment seems to have a key role in the induction of NKG2A expression on CD8$^+$ T cells following TCR stimulation[93]. Therefore, HLA-E expression on cancer cells, together with IL-12 production in the tumour microenvironment, blunts the cytolytic capacity of tumour-infiltrating T cells. NKG2A-mediated inhibition of T cell activation was first reported for melanoma-specific CD8$^+$ T cells[94]. Since then,

many reports have shown that HLA-E–NKG2A functions as an immune checkpoint for tumour-specific $CD8^+$ T cells, in a similar manner to other immune checkpoints such as PDL-1–PD1[65,90,91,95-98]. Thus, blocking NKG2A has the potential to 'take the brakes off' T cells and NK cells within tumours[99] (**Fig. 2a,b**). However, in mouse cancer models, NKG2A blockade on its own had little effect on cancer progression. By contrast, addition of NKG2A-blocking antibodies increased the efficacy of other checkpoint inhibitors as well as cancer vaccines[24,86]. Similarly, blocking the HLA-E–NKG2A axis in clinical trials using the NKG2A-targeting monoclonal antibody monalizumab has so far had very little effect as a monotherapy (see ref.[88] for a list of clinical trials) but promising results were reported for the combination of monalizumab with a PD-L1-blocking antibody in patients with locally advanced (stage III), unresectable non-small cell lung cancer[100,101]. Results from an ongoing phase III clinical trial (ClinicalTrials.gov: NCT05221840) are anticipated at the end of 2025.

In addition to monalizumab, other NKG2A-targeting antibodies are being developed[102,103]. Furthermore, antibodies targeting VL9–HLA-E are also in development[104,105]. These may offer the advantage of both blocking NKG2A-mediated inhibition of NK cell and T cell responses and triggering NK cell-mediated ADCC of HLA-E-expressing tumour cells. Another approach to prevent VL9–HLA-E-mediated inhibition of tumour-targeting T cells and NK cells would be to target the VL9 processing and presentation pathway in tumour cells, for example using proteasome blockers, which have been shown to increase NK cell killing of cancer cells[106]. In mouse tumour models, it was recently shown that altering the HLA-E peptidome by deleting the endoplasmic reticulum (ER) aminopeptidase associated with antigen processing (ERAAP) in tumour cells prevented NKG2A engagement and sensitized tumours to NK cell-mediated control[107]. Moreover, a small-molecule inhibitor of exportin-1 that is approved for the treatment of multiple cancers affects HLA-E expression and enhances the effector function of NK cells against leukaemia cells[108]. One of the caveats of targeting VL9–HLA-E is that this would also block the interaction of VL9–HLA-E with the activating receptor NKG2C.

### *[H2] Modifying NKG2A expression on cell therapies*

The HLA-E–NKG2A inhibitory axis has also emerged as an important obstacle to effective adoptive cell therapy using genetically modified T cells or NK cells. Cytotoxic T cells carrying tumour antigen-specific TCRs or chimeric antigen receptors (CARs) show great promise for immunotherapy of cancer, and CAR T cells targeting B cell markers (mainly CD19) are approved for the treatment of refractory B cell malignancies[109]. However, CAR T cells have shown little

efficacy for the treatment of solid tumours, in part because of the immunosuppressive tumour microenvironment, which includes the upregulation of HLA-E[110]. Moreover, CAR T cells can undergo exhaustion upon re-introduction into the patient, resulting in the upregulation of inhibitory receptors such as NKG2A. In fact, exhausted CAR T cells have features similar to NK cells, including the upregulation of NKG2A[111]. As CAR T cells are created by genetic modifications *ex vivo*, their transition to an NK cell-like state in vivo could potentially be prevented by targeting key transcription factors controlling this transition or by targeting the inhibitory receptors that negatively regulate the activation of these cells[110]. Furthermore, as an alternative to CAR T cells, genetically engineered NK cells carrying CARs can be used that are additionally genetically modified to have reduced or absent expression of inhibitory receptors[112]. For example, deletion of *KLRC1* (which encodes NKG2A) using CRISPR–Cas9, together with lentivirus-mediated expression of a CD33-targeting CAR, resulted in CAR NK cells with increased killing activity against $CD33^+$ AML cells[113] (**Fig. 2c**). Moreover, these NKG2A-deleted CAR NK cells had increased IFNγ secretion, which in turn upregulates HLA-E expression and thus potentially renders cancer cells even more susceptible to control by adaptive NK cells stimulated through NKG2C. However, the silencing of NKG2A expression might lead to reduced survival of CAR NK cells as signalling through NKG2A also prevents activation-induced cell death[37]. **[Au: please clarify that the following A/C switch approach won't overcome the issue of AICD?]** An innovative alternative to deleting NKG2A is to turn NKG2A into an activating receptor by replacing the ITIM-motif-containing cytoplasmic tail with the cytoplasmic tail of NKG2C, which transmits activating signals through the ITAM motif of the DAP12 adaptor protein[114] (**Fig. 2c**). This 'A/C switch' approach takes advantage of the higher affinity of NKG2A for HLA-E compared with NKG2C. Both NK cells and $CD8^+$ T cells transduced with this NKG2A–NKG2C chimeric receptor killed HLA-E-expressing tumour cells *in vitro* or in mouse models, depending on the level of HLA-E expression[114]. As only cells with high levels of HLA-E expression are targeted, this approach is expected to have limited off-target effects. As such, chimeric NKG2A–NKG2C receptors represent a novel type of CAR specific for VL9–HLA-E.

## [H1] MHC-E-restricted adaptive T cell responses

In parallel to its function in modulating NK cell and T cell responses through innate receptors, HLA-E can also be targeted by the TCR of $CD8^+$ T cells, in a similar manner to TCR binding to classical HLA-Ia molecules, particularly when HLA-E presents peptides that are distinct from the endogenous VL9 peptides to which the T cells are expected to be immunologically tolerant[115,116].

### *[H2] MHC-E-restricted T cell responses elicited by pathogens [Au:OK?]*

A prominent example of a virus that elicits HLA-E-restricted T cells in humans is HCMV, because the viral VL9 sequence encoded by the UL40 protein of HCMV not only functions as a ligand for CD94–NKG2A and CD94–NKG2C but also can be a target for HLA-E-restricted $CD8^+$ T cells[116-120]. In fact, HCMV seems to induce long-lasting effector memory T cell responses to UL40–HLA-E that often parallel classical HLA-Ia-restricted responses with respect to frequency and phenotype.UL40-specific HLA-E-restricted T cells seem to be of particularly high frequency when there is a mismatch between the UL40-derived VL9 sequence and the endogenous VL9 leader sequences[118,120,121]. As such, these T cells have the potential to contribute to allograft rejection depending on the endogenous VL9 sequence present in the transplant[122,123]. UL40–HLA-E-specific T cells are not affected by the efficient inhibition of MHC-Ia-dependent antigen presentation by HCMV, which potentially might make them particularly important for controlling HCMV[119]. The peptide transporter associated with antigen processing (TAP)-independent loading of MHC-E by HCMV-encoded UL40 is conserved for RhCMV-encoded Rh67 and is mediated by protein sequences upstream of the VL9 sequence[53]. Owing to the limited sequence diversity of the UL40 VL9 peptide, the TCR diversity of UL40–HLA-E-specific T cells is also likely limited, albeit with more diversity than innate NK cells or NKT cells[115]. The role of UL40–HLA-E-specific T cells in controlling HCMV infection is still under investigation. A correlation between viral VL9 sequences and HCMV burden has not been observed in renal transplant recipients[124], whereas a mismatch between UL40-derived and endogenous VL9 peptides between children and mothers has been suggested to correlate with congenital CMV disease[125]. However, these studies are confounded by the dual role of UL40 in modulating both NK cell and T cell responses. Moreover, recent studies showed that UL40-reactive T cells with TCRs having high affinity for both UL40-derived and endogenous VL9 peptides co-expressed inhibitory NK receptors of the KIR2D family, resulting in weak functional responses, whereas low affinity TCR-expressing, HLA-E/VL9-specific T cells expressed NKG2C. [126]. Thus, HLA-E-restricted T cell recognition seems to be regulated by both central and peripheral mechanisms of immunological tolerance.

Further insight into the interaction of TCRs with HLA-E molecules presenting UL40-derived VL9 peptides was provided by two high-resolution structures[12,127,128]. Remarkably, TCR affinity and TCRβ-chain usage were dictated by single amino acids present at position 8 of the VL9 peptide. The interaction of the TCRs with HLA-E was found to be different from that of CD94–NKG2A and CD94–NKG2C, but similar to the binding of TCRs to HLA-Ia molecules. Although the two TCRs

were shown to have distinct docking modes on HLA-E, the energetic basis of the TCR interaction was found to be defined by a set of conserved HLA-E residues, with about half of the HLA-E contact residues being crucial for binding[12,127]. Together, these studies suggested that HLA-E can select T cells expressing distinct TCRs.

A substantial fraction of HLA-E molecules at the cell surface may be devoid of peptides[129], as also suggested recently by staining with antibodies that recognize peptide-free HLA-E[130]. This is likely the consequence of VL9 dissociating from HLA-E upon cell-surface egress. Unlike classical MHC-Ia molecules, HLA-E is quite stable without peptide[131] **[Au: it is only necessary to cite reference 132 here as this automatically links to the correction]**. In principle, 'empty' HLA-E molecules could bind a wide variety of non-VL9 peptides as the peptide-binding groove of HLA-E has some similarity with that of other HLA-Ia and HLA-Ib molecules[12,29,132]. In fact, some overlap between the peptidomes of HLA-E and HLA-A2.1 was observed[132-135]. Similarly to classical MHC-Ia molecules, HLA-E, Mamu-E (the rhesus macaque orthologue of HLA-E) and Qa-1$^{b}$ bind to distinct peptide motifs found in a wide range of cellular proteins[136]. However, in HLA-Ia-expressing cells, the abundant HLA-Ia-derived VL9 ligand generally outcompetes other cellular peptides that are either less frequent or bind only weakly to HLA-E[135]. By contrast, non-canonical (non-VL9) ligands can be eluted from HLA-E molecules expressed in cells that lack HLA-Ia molecules or in which TAP is inhibited[134,137-139]. In ERAAP-deficient mice or when mouse TAP is blocked by viral inhibitors, Qa-1$^{b}$ is loaded with an alternative set of peptides that can be recognized by CD8$^{+}$ T cells[140,141]. These novel host protein-derived, Qa-1$^{b}$-bound peptides give rise to semi-invariant CD8$^{+}$ T cell responses[142-144]. Inhibition of ERAAP by mouse CMV results in CD8$^{+}$ T cell responses targeting the same dominant Qa-1$^{b}$-restricted epitopes[145]. Thus, although HLA-E-restricted T cell responses are generally not a major part of the adaptive immune response, they can be induced during infection with specific pathogens that interfere with antigen presentation (**Supplementary Box 1**). In each of these cases, it is not entirely clear how non-VL9 peptides are acquired, with several, often pathogen-specific, pathways having been proposed (**Fig. 3**).

## [H1] Eliciting MHC-E-restricted T cells [Au:OK?]

The discovery that HLA-E can present non-VL9 peptides to CD8$^{+}$ T cells offers a unique opportunity to turn the upregulation of HLA-E that is used by pathogens and cancer cells to evade NKG2A$^{+}$ NK cell responses into a potential vulnerability. However, eliciting HLA-E-restricted CD8$^{+}$ T cells to specific pathogen or cancer antigens is a major challenge to vaccine development, despite the fact that certain vaccines seem to elicit some HLA-E-restricted T cells in addition to

conventional CD8⁺ T cells (**Supplementary Box 1**). Fortunately, a serendipitous finding in non-human primate models of CMV-vectored vaccines might offer a way to develop vaccines that elicit CD8⁺ T cells exclusively targeting HLA-E instead of classical HLA-Ia molecules[146-149].

### *[H2] CMV-based vaccines*

The initial idea of using CMV as a vector was based on the observation that CMV is unique in eliciting and maintaining very high frequencies of effector memory T cells[150,151]. It was hypothesized that using CMV to elicit such non-exhausted, tissue-resident T cells that are ready for rapid effector function could prevent the initial dissemination of difficult to control pathogens such as HIV[152]. Indeed, in the rhesus macaque model of HIV/AIDS, RhCMV-based vaccines expressing simian immunodeficiency virus (SIV) antigens protected about 60% of macaques against repeated, low-dose challenge with the highly pathogenic SIVmac239 strain[153,154]. This protection was not only superior to the protection afforded by conventional vaccine vectors, as shown by side-by-side comparison, but also fundamentally different in that SIV replication and spread were arrested early after SIV challenge and SIV was eventually cleared from the vast majority of protected monkeys[155-157]. Interestingly, the RhCMV–SIV vector did not elicit CD8⁺ T cell responses to the same epitopes as other SIV vaccines, despite the responses being extremely broad. Instead, about half of the CD8⁺ T cells were restricted by MHC class II whereas the remaining CD8⁺ T cells were restricted by MHC-E[158,159]. Importantly, these MHC-E-restricted CD8⁺ T cells were not directed against endogenous or virus-encoded VL9 peptides but against many different, unrelated SIV (and RhCMV) peptides, which suggests that they are fundamentally different from the CMV-VL9-specific, HLA-E-restricted T cells described above. These findings were both unprecedented and surprising because naturally infected rhesus macaques have RhCMV-specific CD8⁺ T cells that are restricted by MHC-Ia.

The recombinant vectors used in the SIV vaccine studies were all based on a RhCMV clone derived from strain 68-1, which, compared with wildtype RhCMV, contained multiple gene mutations, deletions and inversions[160]. Partial or complete repair of some of these deletions resulted in RhCMV vectors that elicited MHC-Ia-restricted T cell responses[158,159,161]. Thus, by genetically modifying the RhCMV vector backbone, it is possible to programme CD8⁺ T cell responses targeting different MHC molecules in a mutually exclusive manner, including MHC-E-restricted CD8⁺ T cells (MHC-II-restricted CD4⁺ T cells are not affected by any of the genetic changes in 68-1 RhCMV). As reviewed recently[146], extensive studies in this model have shown that RhCMV encodes both inhibitors and promoters of MHC-E-restricted T cell priming, including

several chemokine-like proteins that can prevent the induction of both MHC-II-restricted and MHC-E-restricted T cells[161]. Deleting these chemokine-like genes from wildtype-like genomes of either RhCMV or cynomolgus macaque CMV (CyCMV) enables the induction of unconventionally restricted T cells[162]. Importantly, homologous chemokine-like gene products that are encoded by HCMV had the same inhibitory function when inserted into RhCMV strain 68-1[161]. Moreover, the HCMV-specific MHC-I homologue UL18, which is a ligand for the inhibitory receptor LIR1, also prevents the induction of unconventionally restricted $CD8^+$ T cells when inserted into RhCMV strain 68-1[163]. Taken together, these results suggest that this type of T cell modulation is not limited to non-human primate CMVs.

The fact that both human and non-human primate CMVs encode multiple inhibitors of unvonventional (MHC-II-restricted and MHC-E-restricted) T cell responses raises the question of why these responses are induced in the first place (**Box 3**). Although this has not been answered as yet for MHC-II-restricted T cells, it seems that MHC-E-restricted T cell responses are a 'side-effect' of the viral VL9 peptide encoded in RhCMV Rh67 and HCMV UL40[164]. Deletion of Rh67, or even changing a single amino-acid in the VL9 peptide of Rh67, completely eliminated the induction of MHC-E-restricted $CD8^+$ T cells by RhCMV 68-1 while maintaining MHC-II-restricted $CD8^+$ T cells. Importantly, UL40 was able to substitute for Rh67 in supporting MHC-E-restricted responses, again supporting the conservation of these mechanisms in humans. Why and how viral VL9 is required for the induction of MHC-E-restricted T cells that target a broad array of non-VL9 peptides is not entirely clear, but as MHC-E remains in the ER in the absence of Rh67 or UL40 owing to viral inhibition of TAP (which prevents loading with endogenous peptides), it is likely that viral VL9 is required for ER egress of MHC-E but ultimately is replaced by non-VL9 peptides (**Fig. 3**). However, as eliminating the viral TAP inhibitor Rh185 (or US6) together with the MHC-targeting viral proteins Rh178, Rh182 (or US2), Rh184 (or US3) and Rh189 (or US11) did not restore the ability of Rh67-deleted RhCMV 68-1 to elicit MHC-E-restricted T cells[164], it seems that endogenous VL9 cannot substitute for viral VL9. Thus, either the oversupply of viral VL9 or additional functions of Rh67 and UL40[53] are required for the induction of MHC-E-restricted $CD8^+$ T cells. The multitude of viral gene products that prevent this side-effect of viral VL9 expression is reminiscent of the well-known multi-gene evasion of classically restricted $CD8^+$ T cells by CMV, indicating that there is strong evolutionary pressure on the virus to avoid both classical and non-classical $CD8^+$ T cell responses[165,166]. However, whereas the stimulation of MHC-Ia-restricted $CD8^+$ T cells is prevented in *cis* by intracellular proteins that block MHC-Ia-dependent antigen presentation, the induction of MHC-E-restricted $CD8^+$ T cells by CMV-infected cells seems to be

prevented in *trans*, by the secretion of chemokine-like proteins or surface expression of the MHC-I-like HCMV protein UL18 which suppresses responses through the inhibitory receptor LIR1 expressed on T cells.

The ability to programme T cell responses restricted by MHC-E, MHC-II or MHC-Ia by gene deletions in CMV vectors was further expanded by the insertion of targeting sites for cell type-specific microRNAs into essential viral genes, thus limiting viral replication in the corresponding cell type. RhCMV 68-1 carrying target sites for myeloid cell-specific microRNA, and thus unable to replicate in myeloid cells, lost the ability to elicit MHC-E-restricted T cell responses, resulting in 'MHC-II only' vectors, whereas targeting sites for endothelial cell-specific microRNAs resulted in 'MHC-E only' vectors[167]. Interestingly, inclusion of both myeloid cell-specific and endothelial cell-specific microRNA target sites in a RhCMV 68-1 vector reverted the $CD8^+$ T cell responses to MHC-Ia, which suggests that conventional MHC-Ia restriction is the default priming mode when cell type-specific unconventional priming is prevented[167]. Using different RhCMV backbones it was then possible to determine which $CD8^+$ T cell programmes are most efficient in protecting against SIV. Results from multiple studies showed that only vectors that elicit MHC-E-restricted $CD8^+$ T cells, either alone or together with MHC-II-restricted $CD8^+$ T cells, protect against SIVmac239 challenge[161,164,167,168]. This was the first demonstration that MHC-E-restricted $CD8^+$ T cells, in the absence of MHC-Ia-restricted $CD8^+$ T cells, are protective in a vaccine.

Vaccines using RhCMV or CyCMV vectors have been shown to protect not only against SIV but also against *Mycobacterium tuberculosis* (*Mtb*), the malaria parasite *Plasmodium knowlesi* and a highly pathogenic avian influenza virus[169-171]. Unlike for SIV, MHC-E-restricted T cells were not superior to MHC-Ia-restricted T cells in protecting against *Mtb*, and protection against influenza virus correlated with $CD4^+$ T cell responses rather than $CD8^+$ T cell responses. For malaria, it has not yet been established whether 'MHC-Ia only' vectors would be protective. The result for *Mtb* suggests that HLA-E-restricted $CD8^+$ T cells that are naturally induced by *Mtb* (**Supplementary Box 1**) might not be protective. MHC-E-restricted T cells were also elicited in rhesus macaques against HBV[172] and against well-established cancer antigens[69]. The latter finding was particularly striking as many cancer antigens are self-antigens to which it is generally difficult to elicit strong MHC-Ia-restricted $CD8^+$ T cell responses owing to central tolerance. Importantly, in both cases it was shown that MHC-E-restricted T cells in rhesus macaques respond to human cells expressing the target antigen together with HLA-E, which is consistent with the high level of conservation of the peptide-binding groove between Mamu-E and HLA-E[12].

HLA-E-directed vaccines eliciting T cells to specific epitopes or entire antigens derived from pathogens or cancer cells could be advantageous over traditional T cell-based vaccines because particular pathogens or cancers have not been selected to escape these types of T cells and might thus be susceptible to their control particularly when HLA-E is upregulated as a means to limit NK cell-mediated control (**Box 4**). This is exemplified in the RhCMV–SIV model, where MHC-E-restricted T cells confer a distinct type of protection from any other vaccine. Given the largely unsuccessful history of conventional HIV vaccines, a vaccine that induces MHC-E-restricted responses might offer hope as an alternative strategy for preventing the continued spread of HIV in human populations. Similarly, the upregulation of HLA-E observed in many cancers might render these particularly vulnerable to control by HLA-E-restricted T cells.

However, developing vaccines that elicit HLA-E-restricted T cells in humans is not without its challenges. Vaccines based on CMV face the obstacle that CMVs are highly species-specific with respect to infection and the ability to persist, as well as in their ability to elicit MHC-E-restricted $CD8^+$ T cells[162]. As such, HCMV-based HIV vaccines designed with genetic modifications homologous to the modifications enabling protection of non-human primates by CMV–SIV vaccines are currently in clinical testing (NCT05854381, NCT04725877). Alternatively, vaccines based on non-human primate CMVs could be tested in humans provided that the species-specific limitations of MHC-E-restricted T cell priming can be lifted, a goal of ongoing studies **[Au: how might this be achieved?]**. Another possibility may be to develop DNA or mRNA vaccines that emulate the CMV-induced alterations in antigen presentation pathways and compartments that favour priming of MHC-E-restricted T cells. It is encouraging that other vaccine moieties have recently been shown to elicit MHC-E-restricted T cells, including mRNA and adenovirus-based vaccines[173,174]. However, it is unlikely that these approaches will be able to elicit T cell responses that are dominated by MHC-E-restricted T cells and that target a broad array of epitopes. As such, traditional vaccine-based induction of MHC-E-restricted T cells might be limited to specific epitopes. A particular challenge to such epitope-based vaccines is the low binding efficacy for MHC-E of non-VL9 peptides[131,175-177]. Whereas VL9 peptides stabilize HLA-E molecules, the binding of non-VL9 peptides is often not detectable in diverse assays[178].

### *[H2] Therapeutic potential of MHC-E-restricted T cells*

The mounting evidence that non-polymorphic HLA-E molecules presenting peptides other than VL9 can be targeted by HLA-E-restricted T cells offers exciting opportunities for novel types of

immunotherapy. The limited polymorphism of HLA-E is a particularly attractive feature for the development of 'off-the-shelf' TCR-based immunotherapies targeting infectious diseases or cancers (**Box 4**). The use of traditional TCR-transfected T cells or TCR-containing soluble immunoreagents such as bispecifics is limited to recipients who express the matching HLA-Ia allele to which that TCR is restricted. By contrast, HLA-E-restricted TCRs do not have this limitation and could therefore be used universally, regardless of the recipient's HLA type. As with traditional TCR-based reagents, off-target effects of HLA-E-restricted TCRs might occur if a given target antigen is expressed in non-cancerous tissues or a given pathogen epitope-specific TCR cross-reacts with epitopes from host proteins, although the low level of HLA-E expression in healthy tissues in the absence of inflammation may limit off-target TCR triggering. Despite their potential advantages, developing HLA-E-targeting TCRs is challenging as many of the traditional rules of TCR–MHC interactions do not seem to apply to HLA-E-restricted TCR recognition. As discussed above, almost all non-VL9 peptides do not bind very strongly to HLA-E. This makes it difficult to identify these peptides by traditional immunopeptidomic approaches, and also limits their usefulness in identifying specific T cells and TCRs by tetramer or dextramer staining. A more successful approach seems to be the bioinformatic prediction of HLA-E-binding peptides from specific antigens, followed by validation of peptide–HLA-E binding, then the cloning (from infected individuals or *in vitro*-primed cultures) of T cells and TCRs that recognize these peptides in the context of HLA-E, or screening of synthetic TCR libraries to identify peptide–HLA-E-reactive TCRs[179-182]. However, the TCR–MHC-E interaction also seems to be of low affinity and even affinity maturation does not overcome this issue, thus limiting the usefulness of soluble TCR-based reagents[183]. Tailoring these approaches to allow for the targeting of low-affinity interactions will need to be accomplished to successfully apply 'off the shelf' TCR-based approaches.

## [H1] Concluding remarks

**[Au: please add a short final paragraph to summarize the key themes of the Review.]**

During the last decade, HLA-E has moved from an obscure immunoregulatory detail to a center stage in immunotherapeutics due to a series of findings summarized in this review. The dual nature of HLA-E as a ligand for innate receptors and antigen presenting protein offers a myriad of possibilities for therapeutic and prophylactic interventions. While most approaches are still in preclinical stages, several new treatments and vaccines are now being tested in clinical trials for infectious diseases as well as cancer. The work of many laboratories has made this progress possible and we expect that the next couple of years will generate new and exciting pre-clinical and clinical data involving HLA-E.

**Figure 1 | Innate and adaptive receptors for HLA-E.** Leukocyte immunoglobulin-like receptor 1 (LIR1) and LIR2 (also known as LILRB1 and LILRB2) are inhibitory receptors that bind to HLA class I (HLA-I) molecules, including HLA-E, irrespective of bound peptide (where X = any or no peptide). CD94–NKG2A and CD94–NKG2C are inhibitory and activating receptors, respectively, that specifically recognize VL9 peptides presented by HLA-E–β2-microglobulin (β2m). HLA-E-specific T cell receptors (TCRs) recognize either foreign VL9 peptides, such as from cytomegalovirus, or non-VL9 peptides presented by HLA-E. ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif.

**Figure 2 | Targeting HLA-E-expressing cancer cells or infected cells. a,** Natural killer (NK) cells. Tumour cells or infected cells expressing VL9–HLA-E complexes are targets for adaptive NKG2C$^+$ NK cells but inhibit NKG2A$^+$ NK cells. The inhibitory HLA-E–NKG2A axis can be disrupted by antibodies targeting NKG2A or HLA-E, or by blocking HLA-E-dependent presentation of VL9 peptides (not shown).. Adaptive NKG2C$^+$ NK cells can control HLA-E-expressing cancer cells directly. They can also be targeted to HLA-E-negative cancer cells by cytokine (IL-15)-armored bispecific antibodies that bind both NKG2C and a cancer antigen such as CD33. **b**, T cells. T cell receptors (TCRs) from HLA-E-restricted T cells recognizing non-VL9 peptides can be used to generate bispecific fusion proteins with anti-CD3 antibodies that redirect CD3-expressing T cells to cells expressing the specific HLA-E–non-VL9 complex. HLA-E-expressing cancer cells can also be directly targeted by in vivo-primed HLA-E-restricted CD8$^+$ T cells recognizing non-VL9 peptides. Tumour-infiltrating T cells that are HLA-Ia restricted and that express NKG2A can be ‘unblocked’ by disrupting the NKG2A–HLA-E interaction with antibodies targeting NKG2A or HLA-E. **c**, Genetically engineered cells. Transfecting NK cells or T cells with a fusion protein consisting of the extracellular domain of NKG2A and the intracellular, activation domain of NKG2C (termed an ‘A/C switch’) activates NK cells (or T cells) in the presence of HLA-E–VL9-expressing cells. These chimeric NKG2A–NKG2C receptors represent a novel type of chimeric antigen receptor (CAR). The activity of CAR T cells can be improved by deleting *KLRC1* (which encodes NKG2A) using CRISPR–Cas9. TCRs from HLA-E-restricted non-VL9-specific T cells can be introduced to generate T cells that target specific tumour antigens or infectious disease antigens.

**Figure 3 | Loading of HLA-E with non-VL9 peptides.** HLA-E is rapidly internalized from the cell surface by endocytosis, a process that requires the cytoplasmic tail of HLA-E. HLA-E may lose the bound VL9 peptide prior to, during or after endocytosis. ‘Empty’ HLA-E molecules can

acquire non-VL9 peptides, which may bind to HLA-E much more weakly than does VL9. Unlike peptide loading in the endoplasmic reticulum (ER), this peptide exchange likely is not a dedicated, chaperoned process —for example, it is not dependent on the peptide transporter associated with antigen processing (TAP) — but rather is a random binding process that is supported by the stability of the peptide-binding groove of HLA-E in the absence of bound peptide. Such non-VL9–HLA-E complexes need to be recycled back to the cell surface to be recognized by HLA-E-restricted T cells. The source and processing pathway of non-VL9 peptides are not known but this likely involves endocytic, HLA-E-containing vesicles gaining some access to cellular degradation compartments, such as autophagosomes and lysosomes (in a similar manner to peptide loading on MHC class II molecules), before being recycled back to the cell surface. It has also been proposed that Golgi-located proteases, particularly furin, might be involved in generating non-VL9 peptides. These could be intercepted during recycling of HLA-E via the trans-Golgi network (TGN). The generation of non-VL9 peptides in recycling compartments could be further exacerbated in virus-infected and bacteria-infected cells; bacterial phagosomes have been implicated in loading of HLA-E with non-VL9 peptides, and cytomegalovirus (CMV) creates a mutivesicular viral assembly complex while inhibiting lysosomal degradation of autophagosomal content.

**Box 1 | Assembly and intracellular transport of HLA-E**

HLA class Ia (HLA-Ia) molecules are type I transmembrane proteins; their leader sequences are cleaved by the signal peptidase (SPase) during translocation into the endoplasmic reticulum (ER) through the SEC61 complex (see the figure). The VL9 peptide is contained within the cleaved leader squence protein fragment, which is further processed by the signal peptide peptidase (SPPase) at the ER membrane and by the proteasome upon release into the cytosol[184-186]. In a similar manner to peptides that are presented by classical HLA-Ia molecules, VL9 peptides are transported from the cytosol into the lumen of the ER by the peptide transporter associated with antigen processing (TAP) and loaded onto HLA-E–β2-microglobulin (β2m) complexes in a tapasin-dependent manner[6,16]. In addition, the ER aminopeptidase associated with antigen processing (ERAP1 and ERAP2 in humans; ERAAP in mice) is required for VL9 presentation as ERAAP-knockout mice present a different set of peptides on Qa-$1^b$ (the mouse homologie of HLA-E)[141]. Similarly, deletion of ERAP1 and ERAP2 sensitized human cancer cells to killing by natural killer cells that were otherwise inhibited by HLA-E[107]. In mouse cells, presentation of the VL9-like QDM peptide by Qa-$1^b$ was shown to be dependent on each member (SPase, SPPase, TAP1, TAP2, Tapasin, Erp57 and ERAAP) of the peptide-loading complex[15]. In general, it seems that the

supply of endogenous VL9 peptides is limited, such that most HLA-E molecules are ‘waiting’ in the ER for peptide[5,17,187]. Upon peptide loading, the trimeric VL9–HLA-E–β2m complex is transported to the cell surface via the Golgi. However, unlike classical HLA-Ia molecules, HLA-E is turned over rapidly at the cell surface as a result of internalization signals in its cytoplasmic tail[17]. Similarly, Qa-1$^{b}$ is rapidly removed from the surface of mouse cells[15]. The oversupply of HLA-E together with its rapid turnover from the cell surface presumably provides a real-time monitor of the presence of HLA-Ia-derived VL9 inside the cell to facilitate continuous sensing by innate immune receptors.

**Box 2 | Modulation of the MHC-E pathway by pathogens**

By promoting the engagement of innate receptors (NKG2A and NKG2C) on NK cells and T cells through HLA-E, various pathogens either evade or are controlled by cellular immune responses.

**[bH1] Cytomegalovirus**

In human cytomegalovirus (HCMV)-infected cells, the peptide transporter associated with antigen processing (TAP) is blocked by the viral protein US6[188,189], whereas tapasin, which facilitates peptide loading onto MHC molecules, is inhibited by viral protein US3[190]; together, US6 and US3 prevent endogenous VL9 peptides from being loaded onto HLA-E. This limitation is overcome by HCMV UL40, an endoplasmic reticulum (ER)-resident viral glycoprotein containing a VL9 mimic in its signal sequence, which results in strong upregulation of surface HLA-E in virus-infected cells[50,51]. UL40-derived VL9, similarly to endogenous VL9, is processed by the signal peptidase and signal peptide peptidase but its loading onto HLA-E is independent of TAP and tapasin[50,53,191]. The UL40 VL9 peptide is likely further trimmed by ER aminopeptidases prior to loading onto HLA-E.

**[bH1] Human immunodeficiency virus**

HIV contains the HLA-E-binding peptide AA9 in Gag protein, but the AA9–HLA-E complex does not engage NKG2A[192]. Additional HLA-E-binding peptides have been reported in Gag and other HIV proteins, although their binding to HLA-E is typically extremely weak[12,176,193]. Notably, people living with HIV (PLWH) have an expansion of adaptive NKG2C$^{+}$ natural killer (NK) cell populations that correlates with HIV viral loads[194,195-197]. However, as PLWH are almost all HCMV-seropositive, this likely reflects greater HCMV-driven NKG2C$^{+}$ NK cell expansion in the context of reduced HCMV control rather than NK cell expansion in response to HLA-E-binding peptides derived from HIV proteins[198-200].

### [bH1] SARS coronavirus

HLA-E is upregulated on SARS-CoV-2-infected lung epithelium or on epithelial cell lines transfected with Spike protein or non-structural protein 13 (NSP13) of SARS-CoV-2, both of which contain HLA-E-binding peptides[179,201,202]. The NSP13–HLA-E peptide complex does not engage the inhibitory receptor NKG2A, and this correlates with increased control of SARS-CoV-2-infected cells *in vitro*[202]. By contrast, target cells transfected with Spike protein inhibited the degranulation of NKG2A$^+$ NK cells whereas they activated adaptive NKG2C$^+$ NK cells[201]. This was also observed for SARS-CoV-2-infected cell lines. HLA-E upregulation or NK cell degranulation was not observed with Spike proteins containing mutations in the P5 position of peptide YLQPRTFLL. However, a direct interaction between NKG2C and YLQPRTFLL–HLA-E could not be demonstrated[179]. Nevertheless, an HCMV-independent, but transient, increase in the number of adaptive NK cells was observed in acutely SARS-CoV-2-infected individuals[201], and SARS-CoV-2 infection or vaccination of PLWH skews NK cells towards a more adaptive phenotype[203]. This is consistent with earlier reports that adaptive NK cells are associated with control of SARS-CoV-2 in macaque models of infection[204].

### [bH1] Other viruses

HLA-E-binding peptides that inhibit NK cell and T cell responses by signalling through NKG2A have also been identified in Torque teno virus (TTV), a non-pathogenic negative-strand DNA virus that is the most abundant eukaryotic virus of the human virome[205], as well as in Epstein–Barr virus[206]. Moreover, influenza-derived HLA-E-binding peptides are reported to drive the expansion of adaptive NKG2C$^+$ NK cell populations *in vitro*[200], and infection of cells with Puumala virus upregulates HLA-G expression, which provides a source of VL9-containing leader peptides that drive expansion of NKG2C$^+$ NK cell populations[207].

**Box 3 | Key questions regarding cytomegalovirus-induced, MHC-E-restricted T cells**

**Genetically modified vaccine vectors derived from rhesus and cynomolgus macaques are the first vaccine platform that enables the induction of MHC-E restricted CD8+ T cells to any inserted antigen including antigens from other pathogens or tumour-associated antigens. Moreover, only CMV-vectors capable of eliciting MHC-E-restricted CD8+ T cells protect macaques against SIV challenge. These findings raise the questions including how CMV achieves the induction of these unconventional T cells and why SIV is more susceptible to them than to conventional T cells.**

**[bH1] What enables certain CMV vectors to elicit MHC-E-restricted T cells?**

In addition to strongly driving the egress of MHC-E from the endoplasmic reticulum (ER) in infected cells, cytomegaloviruses (CMVs) reorganize secretory organelles and block autophagocytosis, resulting in a multi-vesicular assembly complex. MHC-E molecules that are endocytosed from the cell surface might acquire other peptides in this compartment upon loss of VL9 and might be transported back to the cell surface to provide large populations of non-VL9-loaded MHC-E (**Fig. 3**). As infection of myeloid-lineage cells is required for MHC-E-restricted $CD8^+$ T cell priming[167], it is also conceivable that this process is particularly efficient in infected monocytes, macrophages and dendritic cells.

**[bH1] Why does CMV encode multiple inhibitors of MHC-E-restricted $CD8^+$ T cells?**

Multiple viral chemokine-like proteins need to be deleted from rhesus or cynomolgus macaque CMV (RhCMV, CyCMV) for them to elicit MHC-E-restricted $CD8^+$ T cells, and each of these chemokine homologues, as well as thei HCMV relatives, individually prevents their induction[161]. In addition, the MHC-I-like human CMV (HCMV) protein UL18 prevents the induction of MHC-E-restricted $CD8^+$ T cells when inserted into chemokine-deleted RhCMV[163]. It is not clear why CMVs go to such great lengths to prevent the induction of these unconventional T cells. However, whereas CMV-infected cells become ‘invisible’ to MHC-Ia-restricted $CD8^+$ T cells owing to their inhibition of MHC-Ia-dependent antigen presentation through various mechanisms, this is not the case for MHC-E-restricted $CD8^+$ T cells because surface expression of MHC-E is upregulated by the viral VL9-containing proteins Rh67 and UL40[164]. Thus, MHC-E-restricted T cells might limit the dissemination of CMVs that lack the ability to prevent them from being induced or recognizing infected cells.

**[bH1] What is the epitope-specificity of MHC-E-restricted T cells?**

Several pieces of evidence suggest that the induction of MHC-E-restricted T cells by RhCMV strain 68-1 is not the result of naive T cell priming but rather the reprogramming of existing memory T cells. For example, the viral protein UL18 blocks their induction by binding to LIR1[163], an inhibitory receptor that is found on memory but not naive T cells[208]. Upon reprogramming, unconventionally restricted T cells recognize a vast repertoire of peptides, most of which have no measurable binding to MHC-E[12]. Moreover, so-called ‘supertopes’ are recognized by MHC-E-restricted T cells in every animal[158,159]. Taken together, these observations suggest that these T

cells may recognize multiple peptides rather than being specific for single peptide–MHC-E complexes.

### [bH1] Why do MHC-E-restricted CD8⁺ T cells arrest and clear SIV?

There are three non-mutually exclusive possibilities for why MHC-E-restricted CD8⁺ T cells can mediate simian immunodeficiency virus (SIV) replication arrest whereas MHC-Ia-restricted and MHC-II-restricted CD8⁺ T cells do not[146]. First, MHC-E is upregulated on SIV-infected CD4⁺ T cells whereas MHC-Ia is downregulated and MHC-II is only intermittently expressed[209]. Thus, MHC-E-restricted CD8⁺ T cells may be more efficient at recognizing early-infected cells, leading to the efficient interception of nascent infection. Second, MHC-E-restricted CD8⁺ T cells elicited by RhCMV-68-1–SIV vaccination are highly cross-reactive for multiple SIV epitopes, which might allow for more efficient recognition of nascent infection as each T cell can be activated by multiple ligands. Third, SIV-specific MHC-E-restricted CD8⁺ T cells are of relatively low avidity compared with MHC-Ia-restricted and MHC-II-restricted CD8⁺ T cells and thus likely have a different functional output upon epitope recognition, including β-chemokine production. It is possible that these SIV-inhibiting β-chemokines might be more efficient at blocking SIV spread than a full cytotoxic response, which would kill some infected cells but also create an inflammatory milieu that would bring in more CD4⁺ target cells and enhance their infectivity[210]. The low avidity of SIV-specific MHC-E-restricted CD8⁺ T cells may also reduce the risk of functional T cell exhaustion.

### [bH1] Why can MHC-E-restricted T cells recognize cells expressing non-VL9 targets?

The widespread ability of MHC-E-restricted T cells to recognize non-VL9 peptides on target cells strongly suggests that some MHC-E molecules are loaded with non-VL9 peptides even in the presence of endogenous VL9. Owing to their generally low binding strength, together with the low abundance of individual peptides, these non-VL9 peptides are not detected by immunopeptidomic experiments and do not efficiently elicit direct or cross-priming of T cell responses, but they can be detected by T cells and trigger functional responses. The fact that MHC-E is stable in the absence of bound peptides, unlike classical MHC-Ia, and is rapidly recycled from the cell surface[17] (**Fig. 3**) may explain this background presentation of non-VL9 antigens.

### Box 4 | Advantages of HLA-E-targeted vaccines and immunotherapeutics

**The discovery that HLA-E routinely presents non-VL9 peptides together with the monomorphic nature of HLA-E offers opportunities for new vaccines and**

**immunotherapuetics that overcome some of the shortcomings of traditional approaches that target antigens presented by polymorphic HLA-Ia molecules.**

## [bH1] Vaccines

Conventional T cell vaccines attempt to mimic the immunity elicited by natural infection. They are limited by MHC-haplotype specificity and evasion mechanisms such as downregulation of HLA-Ia and upregulation of inhibitory signals, including HLA-E. In the context of cancer vaccines, T cells specific for self-antigens are subject to central and peripheral tolerance. Such immunological tolerance is difficult to overcome with vaccination. Moreover, the presentation of T cell epitopes derived from tumour neoantigens is highly dependent on the specificity of HLA-Ia alleles, thus requiring personalized vaccines.

By contrast, vaccines that elicit HLA-E-restricted T cells are HLA-Ia haplotype-independent, thus potentially resulting in less variability of protection. Such vaccines take advantage of the upregulation of HLA-E that is routinely observed on neoplastic and infected cells, thus converting an immune evasion strategy to limit natural killer cell responses into a vulnerability that can be targeted by T cells. Pathogens and cancer cells that are selected for evasion of conventionally HLA-I-restricted T cells are likely to be less capable of evading HLA-E-restricted T cells.

## [bH1] Immunotherapeutics

T cells transduced with HLA-Ia-restricted T cell receptors (TCRs) targeting specific epitopes derived from pathogens or tumour neoantigens are limited to specific HLA-Ia alleles and are expensive to generate as they require the genetic manipulation and expansion of autologous T cells from a given patient. Conventionally restricted, ‘off-the-shelf’ T cell libraries targeting various viruses are less expensive to generate but are still limited to use in individuals who share at least one HLA-Ia allele[211]. The efficacy of conventionally restricted T cell therapies is further limited by HLA-Ia downregulation, which is often observed for pathogen-infected cells and cancer cells. The same limitations apply for bispecific antibodies consisting of a TCR-like portion specific for a given peptide–HLA-Ia complex and a costimulatory antibody portion.

By contrast, HLA-E-restricted TCRs are universal and can be used as ‘off-the-shelf’ therapies either in the context of bispecific antibodies or TCR-transfected T cells. Conceivably, a library of TCRs and bispecific antibodies targeting common HLA-E-restricted epitopes derived from

neoantigens or pathogen antigens could be generated. Thus, treatments could be tailored to given mutations or infections by selecting from previously generated reagents.

# References

**[Please ensure that references are cited sequentially in the following order: main text, tables, figure legends and then boxes. The numbered references should be listed at the end of the article in the format:**

1. Author, A. B. & Author, B. C. Title of the article. *Nat. Cell Biol*. **6**, 123–131 (2001).

**(with journal abbreviation italic, and volume bold). If there are six or more authors to a reference, only the first author should be listed followed by 'et al.'. For more details on reference format please consult the Guidelines to Authors. Please note that doi numbers can be remoed if volume and page numbers are available. I have corrected the first 5 references as an example. I have also updated the**

**details for references 17, 32, 34, 53, 61, 68, 74, 88, 103, 105, 107, 114, 115, 121, 124, 148, 158, 162, 163, 165, 174, 180 and 206; please check. ]**

**[Au: For references that are particularly worth reading (5-10% of the total), please provide a single bold sentence beneath each that indicates the significance of the work.]**


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**Author contributions**

The authors contributed equally to all aspects of the article.

**Competing interests**

Oregon Health & Science University (OHSU), L.J.P. and K.F. have a substantial financial interest in Vir Biotechnology, Inc., a company that may have a commercial interest in the results of this research and technology. L.J.P. and K.F. are inventors of technology licensed to Vir Biotechnology. These potential individual and institutional conflicts of interest have been reviewed and managed by OHSU. A.J.M. and G.M.G. are inventors on HLA-E-related patents owned by Oxford University Innovation. A.J.M. and G.M.G. are cofounders of E-Biotherapy Ltd, a dormant company with a potential commercial interest in this research and technology.

**Peer review information**

*Nature Reviews Immunology* thanks [Referee#1 name], [Referee#2 name] and [Referee#3 name] for their contribution to the peer review of this work.

**Supplementary information**

Supplementary information is available for this paper at https://doi.org/10.1038/s415XX-XXX-XXXX-X

## Table of Contents

The dual nature of non-polymorphic MHC-E as a ligand for innate receptors and antigen presenting protein raises the possibility of new, universally effective vaccines and immunotherapies for infectious disease and cancer that are independent of an individual's MHC haplotype.

## Author notes

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Fig 1

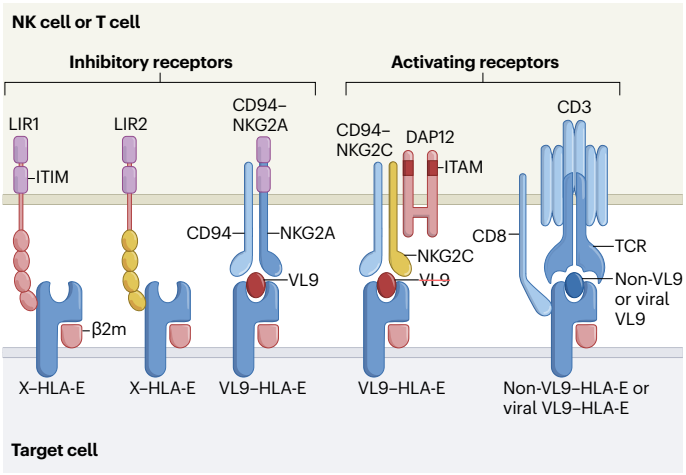

NK cell or T cell
Inhibitory receptors
Activating receptors
LIR1
ITIM
LIR2
CD94–
NKG2A
CD94–
NKG2C
DAP12
ITAM
CD3
CD94
NKG2A
NKG2C
CD8
TCR
VL9
VL9
Non-VL9
or viral
VL9
β2m
X–HLA-E
X–HLA-E
VL9–HLA-E
VL9–HLA-E
Non-VL9–HLA-E or
viral VL9–HLA-E
Target cell

Fig 2

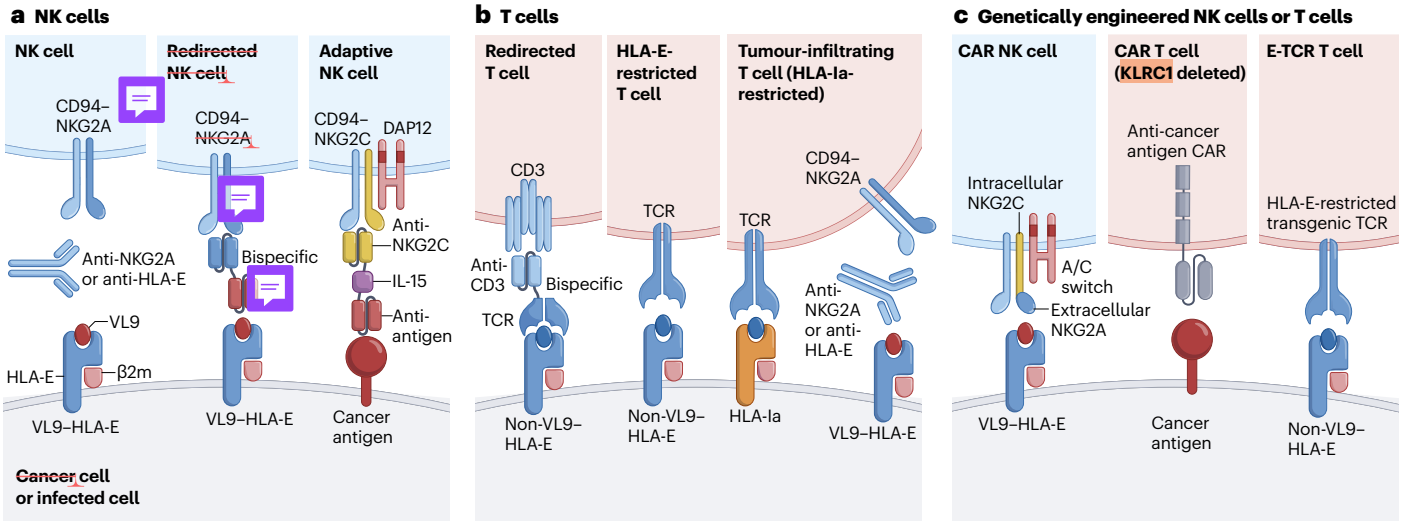
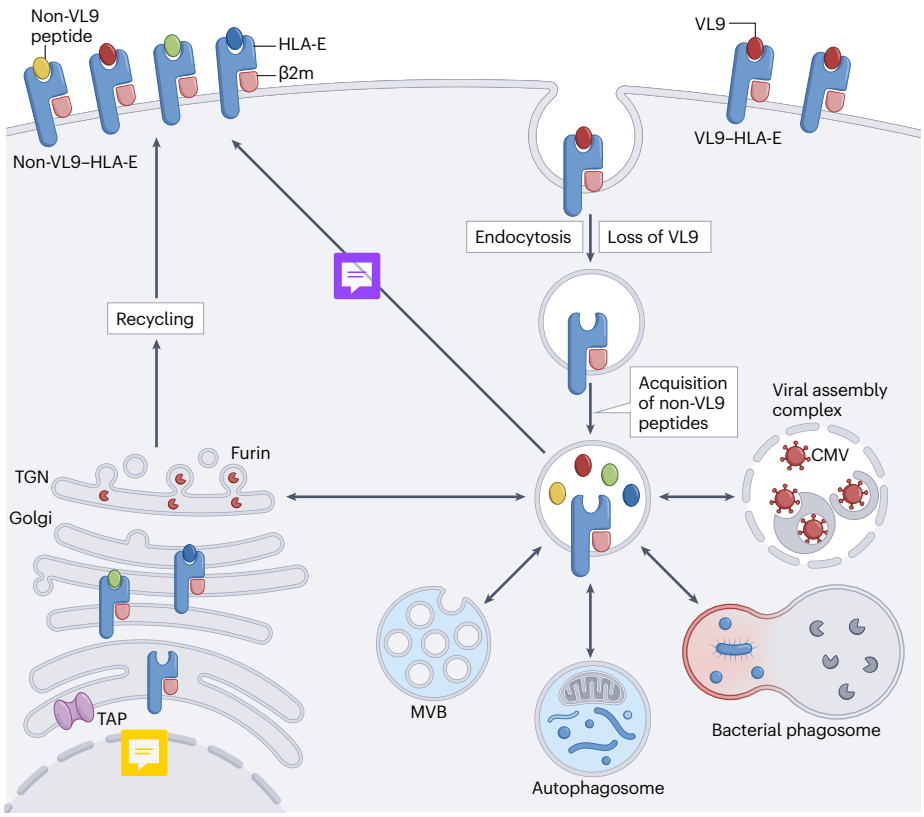
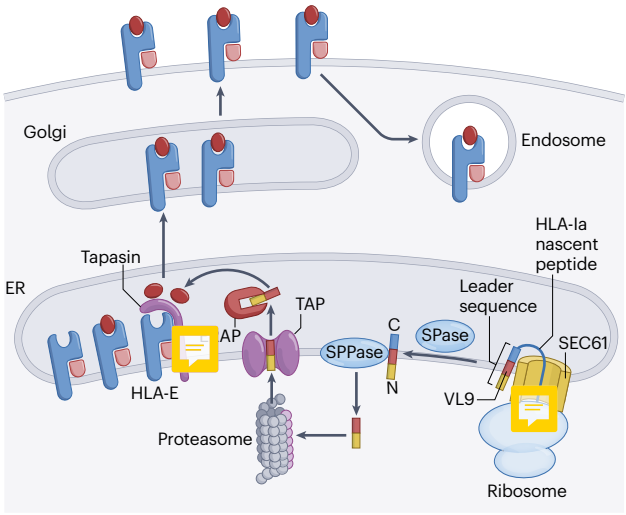

a NK cells
NK cell
Redirected NK cell
Adaptive NK cell
CD94–NKG2A
CD94–NKG2A
CD94–NKG2C
DAP12
Anti-NKG2A or anti-HLA-E
Bispecific
Anti-NKG2C
IL-15
Anti-antigen
VL9
HLA-E
β2m
VL9–HLA-E
VL9–HLA-E
Cancer antigen
Cancer cell or infected cell
b T cells
Redirected T cell
HLA-E-restricted T cell
Tumour-infiltrating T cell (HLA-Ia-restricted)
CD3
Anti-CD3
Bispecific
TCR
TCR
TCR
CD94–NKG2A
Anti-NKG2A or anti-HLA-E
Non-VL9–HLA-E
Non-VL9–HLA-E
HLA-Ia
VL9–HLA-E
c Genetically engineered NK cells or T cells
CAR NK cell
CAR T cell (KLRC1 deleted)
E-TCR T cell
Intracellular NKG2C
A/C switch
Extracellular NKG2A
Anti-cancer antigen CAR
HLA-E-restricted transgenic TCR
VL9–HLA-E
Cancer antigen
Non-VL9–HLA-E

Fig 3


Non-VL9 peptide
HLA-E
β2m
Non-VL9–HLA-E
VL9
VL9–HLA-E
Endocytosis
Loss of VL9
Recycling
Acquisition of non-VL9 peptides
Viral assembly complex
CMV
Furin
TGN
Golgi
TAP
MVB
Autophagosome
Bacterial phagosome

Box 1


Golgi
Endosome
HLA-Ia
nascent
peptide
Tapasin
ER
Leader
sequence
TAP
C
SPase
AP
SPPase
SEC61
HLA-E
N
VL9
Proteasome
Ribosome