





HLA-E: exploiting pathogen-host interactions for vaccine development

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Summary

Viruses, when used as vectors for vaccine antigen delivery, can induce strong cellular and humoral responses against target epitopes. Recent work by Hansen *et al.* describes the use of a cytomegalovirus-vectored vaccine, which is able to generate a stable effector-memory T cell population at the sites of vaccination in rhesus macaques. This vaccine, targeted towards multiple epitopes in simian immunodeficiency virus (SIV), did not induce classical CD8⁺ T cells. However, non-canonical CD8⁺ T cell induction occurred via major histocompatibility complex (MHC) class II and MHC-E. The MHC-E-restricted T cells could recognize broad epitopes across the SIV peptides, and conferred protection against viral challenge to 55% of vaccinated macaques. The human homologue, human leucocyte antigen (HLA)-E, is now being targeted as a new avenue for vaccine development. In humans, HLA-E is an unusually oligomorphic class Ib MHC molecule, in comparison to highly polymorphic MHC class Ia. Whereas MHC class Ia presents peptides derived from pathogens to T cells, HLA-E classically binds defined leader peptides from class Ia MHC peptides and down-regulates NK cell cytolytic activity when presented on the cell surface. HLA-E can also restrict non-canonical CD8⁺ T cells during natural infection with various pathogens, although the extent to which they are involved in pathogen control is mostly unknown. In this review, an overview is provided of HLA-E and its ability to interact with NK cells and non-canonical T cells. Also discussed are the unforeseen beneficial effects of vaccination, including trained immunity of NK cells from bacille Calmette-Guérin (BCG) vaccination, and the broad restriction of non-canonical CD8⁺ T cells by cytomegalovirus (CMV)-vectored vaccines in pre-clinical trials.

Keywords: cytomegalovirus, HLA-E, trained immunity, vaccines

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Introduction to human leucocyte antigen-E (HLA-E)

Sequence, structure and function

HLA-E is a non-classical, class Ib major histocompatibility complex (MHC) molecule located on chromosome 6, and is expressed throughout the majority of nucleated tissues in humans [1]. Evolutionarily older than the HLA class Ia molecules (HLA-A, -B and -C), HLA-E distorts the traditional boundaries between fast-acting innate and memory-driven adaptive immunity [2]. In contrast to

the highly polymorphic HLA class Ia [3], HLA-E is relatively more conserved, reducing the capacity for highly varied antigen recognition and peptide binding [4]. This reduced polymorphism is also thought to confer tight constraints on binding of specific, hydrophobic epitopes [5]. This alone contravenes the dogma that the high polymorphism of the classical major histocompatibility complex facilitates the recognition of (theoretically) 10¹⁵ epitopes, which forms the basis of adaptive immunity in vertebrates [6].

Twenty-seven HLA-E alleles have been reported to date, but most are found infrequently or as non-functional proteins [7–9]. HLA-E exists predominantly in the human population as two alleles, HLA-E*0101 and HLA-E*0103, varying by an arginine or a glycine at amino acid position 107, respectively [10,11]. These alleles are found at near-equal frequencies within the population [12], and probably evolved prior to divergence of humans and primates and the emergence of HLA class Ia genes [13,14]. Both HLA-E*0101 and HLA-E*0103 alleles have a relatively low expression on the cell surface [15]. Although there is no structural difference of the peptide binding groove between these alleles, HLA-E*0103 has a higher affinity for peptides than HLA-E*0101, and is able to stabilize and up-regulate at the cell surface much more efficiently [10,16,17]. There is also variation in the peptide-binding affinities of HLA-E*0101 and HLA-E*0103 in the absence of HLA class Ia and tapasin. In the absence of HLA class Ia peptides, for example due to viral down-regulation, both alleles bind a diverse and mutually exclusive range of cellular-derived peptides, which promote cell surface stability [18].

Peptide binding and cell surface presentation

Unlike HLA class Ia molecules, the rigidity of the HLA-E binding groove confers preferential avidity for a limited range of peptides. In a healthy cell setting, HLA-E binds conserved leader peptides from HLA class Ia molecules [10,19]. The highly hydrophobic binding groove is ideally suited to bind a 9 amino acid 'leader peptide', typically VMAPRTL(L/V/I)L (VL9, Table 1) [20]. Although VL9 is the optimal peptide for binding to HLA-E, the affinity of the HLA-E : peptide complex is defined by specific amino acid anchors; methionine (M) at position 2 and leucine (L) at position 9 [21], with ancillary anchors at P3, P6 and P7 [22]. Higher-affinity epitopes bind further into the HLA-E binding groove, improving stability at the cell surface for longer [23]. There are also several reported non-MHC derived self-peptides that contain a leader sequence able to up-regulate HLA-E on the cell surface (Table 2). HSP60 and adenosine triphosphate (ATP)-binding cassette protein multi-drug-resistance protein (MRP)7 both contain putative leader peptides which demonstrate HLA-E cell-surface stabilization. However,

Table 1. Example human leucocyte antigen (HLA)-E VL9 epitopes from HLA class Ia peptides [4,19,22,25,35,84]

Allele	Sequence
HLA-A*0201	VMAPRTLVL
HLA-A*01	VMAPRTLLL
HLA-B*07	VMAPRTVLL
HLA-B*27	VTAPRTVLL
HLA-C*07	VMAPRALLL
HLA-G*01	VMAPRTLFL

the immunological function that these peptides exert from HLA-E up-regulation is currently unknown [18].

HLA-E orthologues

HLA-E has defined orthologues within mammals, and is unusually conserved in function across the evolution of adaptive immune genes [24]. In mice and rats, the corresponding HLA-E orthologues are Qa-1b and RT-BM1, respectively [25], and Mamu-E is the HLA-E homologue in the rhesus macaque (*Macaca mulatta*) [24]. Primate MHC is more polymorphic than human MHC, and Mamu-E itself exhibits further polymorphism than human HLA-E, with at least 33 functional alleles identified in rhesus macaque populations [24,26]. However, Mamu-E is still the most conserved of all the class I MHC loci in macaques. HLA-E and Mamu-E share 88% amino acid identity, especially within the peptide binding region of the protein [27], and there is conservation in function between rhesus macaques, cynomolgus macaques (*M. fascicularis*) and humans [26,27]. Mamu-E is also able to bind a wider range of peptides than HLA-E, although it still preferentially binds the canonical VL9 peptide [5].

HLA-E function in a healthy cell – the fringe of innate and adaptive immunity

HLA class Ia nascent peptides are cleaved by signal peptidase, and are assembled and translocated in the endoplasmic reticulum (ER) of a cell via the peptide-loading complex consisting of transporter associated with antigen processing (TAP), tapasin and calreticulin [28]. HLA-E epitopes from these peptides are cleaved by signal peptide peptidase, and bind to nascent HLA-E within the ER,

Table 2. Reported HLA-E peptides found in pathogens and self-peptides [18,36,61,74,100,101]

Pathogen	Gene product	HLA-E leader peptide
HCMV	UL40	VMAPRTL(I/V/L)L
Hepatitis C virus	Core	YLLPRRGPR
Epstein–Barr virus	BZLF1	SQAPLPCVL
HIV	P24	AISPRTLNA
<i>Mycobacterium tuberculosis</i>	Mtb14, P49, Mtb44	RMAATAQVL, RMPPLGHEL, RLPKAPLL
<i>Salmonella typhimurium</i>	GroEL	GMQFDRGYL
serovar Typhi		
Self-peptides		
n.a.	HSP60	QMRPVSRL
n.a.	ATP-binding cassette protein MRP7	ALALVRMLI

HLA = human leucocyte antigen; HCMV = human cytomegalovirus; HSP = heat shock protein; ATP = adenosine triphosphate; MRP = multi-drug-resistance protein; n.a. = not available.

also via interaction with TAP and tapasin [25]. On the cell surface, HLA-E is stabilized by association with β 2-microglobulin [28], and predominantly interacts with CD94/NKG2 receptors on natural killer (NK) cells [15].

HLA-E and NK cells

Although classified as part of the innate immune system, NK cells span the traditional boundaries of innate and adaptive immunity through generation of memory-like phenotypes and adaptation to infection [29,30]. They are defined through expression of the cell-surface molecule CD56 [31], and are vital for protection against viral pathogens, especially herpesviruses [19]. NK cells target infected cells directly through the up-regulation of inflammatory markers on the cell surface, or indirectly through their down-regulation of MHC class Ia [32]. HLA-E, when stabilized with HLA class Ia-derived peptides, exerts a regulatory function upon NK cells expressing the dimeric receptors CD94 and NKG2A or NKG2C [22,33,34]. This interaction is dependent on the amino acid residues at positions 5 and 8 of the HLA-E-bound leader peptide [35]. NKG2A is a C-type lectin-like receptor containing an inhibitory immunoreceptor tyrosine-based inhibition motif (ITIM) sequence [36], which recruits Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1/2 tyrosine phosphatases [37] and prevents a release of cytotoxic granules containing interferon (IFN)- γ and tumour necrosis factor (TNF)- α [38]. The HLA-E : class Ia peptide complex can also bind the activating NK cell receptor CD94/NKG2C [39], albeit with an approximately sixfold lower affinity [36,40]. One exception is the HLA class Ib molecule HLA-G, the VL9 peptide of which has phenylalanine at position 8, and interacts with a much higher affinity with CD94/NKG2C molecules [35]. HLA-G*01 is expressed during development of the placental trophoblast and has the ability to activate cytolytic CD94/NKG2C NK cells, potentially acting to maintain balance of tissue growth [35]. CD94/NKG2 NK cell receptors have homologues in macaques and homoplasious molecules in mice [36], all of which interact with the corresponding orthologue of HLA-E. Up-regulation of HLA-E by MHC class Ia peptides facilitates the passive monitoring of epitope presentation and TAP function within the cell; pathogen-induced cessation of HLA class Ia synthesis prevents HLA-E cell-surface expression, and leads to destruction of the cell through 'missing self' activation of NKG2C⁺ NK cells [41].

HLA-E and infection

Herpesvirus infection

Herpesviruses exhibit convergently evolved mechanisms that alter MHC presentation of viral antigens to the

host adaptive immune system. Cytomegalovirus (CMV) is a highly species tropic β -herpesvirus, and has evolved and diversified in tandem with its mammalian hosts [42]. Human CMV (HCMV) is prevalent in 60–100% of a given population [43]. HCMV manifests a lifelong latent infection, with subclinical presentation and immune control in immunocompetent individuals [1,44]. Infection is established within salivary gland epithelial cells [45], and disseminates throughout the host during latency [46].

HCMV has evolved the ability to evade the host adaptive immune system through manipulation of HLA expression. The HCMV US2-11 protein(s) down-regulate HLA class Ia molecules on the cell surface [47], preventing presentation of viral epitopes to canonical CD8⁺ T cells (reviewed in Table 3). US2 and US11 induce translocation of HLA class Ia towards the proteasome [48,49], US6 binds and changes TAP conformation to prohibit peptide binding to HLA class Ia [50] and US3 prevents peptide stabilization in the binding groove of HLA class Ia through direct binding to tapasin [51]. Furthermore, UL18 is a homoplasious protein with similar function to HLA class Ia molecules. UL18 complexes with β 2-microglobulin on the cell surface and binds with high affinity to inhibitory leucocyte immunoglobulin-like receptor 1 on T cells, down-regulating their cytotoxic activity [52] (Fig. 1).

Of most relevance to HLA-E is UL40, a 221 amino acid glycoprotein containing a 37 amino acid signal sequence with an HLA-E binding VL9 leader peptide that is identical to the leader peptide of HLA-C*03 (Tables 1 and 2) [1,19,47]. Although the function of the UL40 protein is unknown, the UL40 leader peptide binds to nascent HLA-E in the endoplasmic reticulum in a TAP-independent manner, working synergistically with the US6 family of HCMV gene products that inhibit TAP function [1,53]. Crucially, the UL40-VL9 leader peptide is sufficient to up-regulate HLA-E expression on the cell surface [54], whereby it prevents NK cell cytolysis of infected cells through interaction with the inhibitory CD94/NKG2A molecule [1]. This viral-mediated up-regulation of HLA-E by UL40 can overcome the reduction of the VL9 leader peptide from down-regulation and proteolysis of HLA class Ia peptides by HCMV. This, in turn, prevents CD94/NKG2A NK cell-mediated killing of HCMV-infected cells, despite the lack of HLA class Ia-derived leader peptide. Loss of UL40 in the CMV genome leads to CD94/NKG2C NK cell-mediated cytolysis of infected cells [54].

During the course of CMV infection, a subset of NK cells with low CD56 and high NKG2C expression (CD56^{dim}NKG2C^{bright}) are vastly expanded in approximately 50% individuals, and do not contract during latent infection. This NK subset has the potential to

Table 3. The HCMV and RhCMV gene products involved in MHC manipulation [47–51]

Gene product in HCMV	Gene product in RhCMV	Effect on MHC expression
Down-regulation of MHC class Ia on the cell surface		
US2, US11	Rh182, Rh189	Retrotranslocation of MHC class Ia from endoplasmic reticulum to cytoplasm, for degradation in the proteasome
US6	Rh185	Alters TAP conformation and peptide binding to MHC class Ia groove
US3	Rh184	Interacts with tapasin and prevents peptide binding to MHC class Ia groove
Up-regulation of MHC class Ib/prevention of cytotoxic responses		
UL18	Not present	HLA class Ia functional homologue that can bind inhibitory LIR1 T-cell receptor
UL40	Rh67	Stabilizes and up-regulates MHC-E at the cell surface

HLA = human leucocyte antigen; HCMV = human cytomegalovirus; LIR1 = leucocyte immunoglobulin-like receptor 1; MHC = major histocompatibility complex; RhCMV = rhesus cytomegalovirus; TAP = transporter associated with antigen processing.

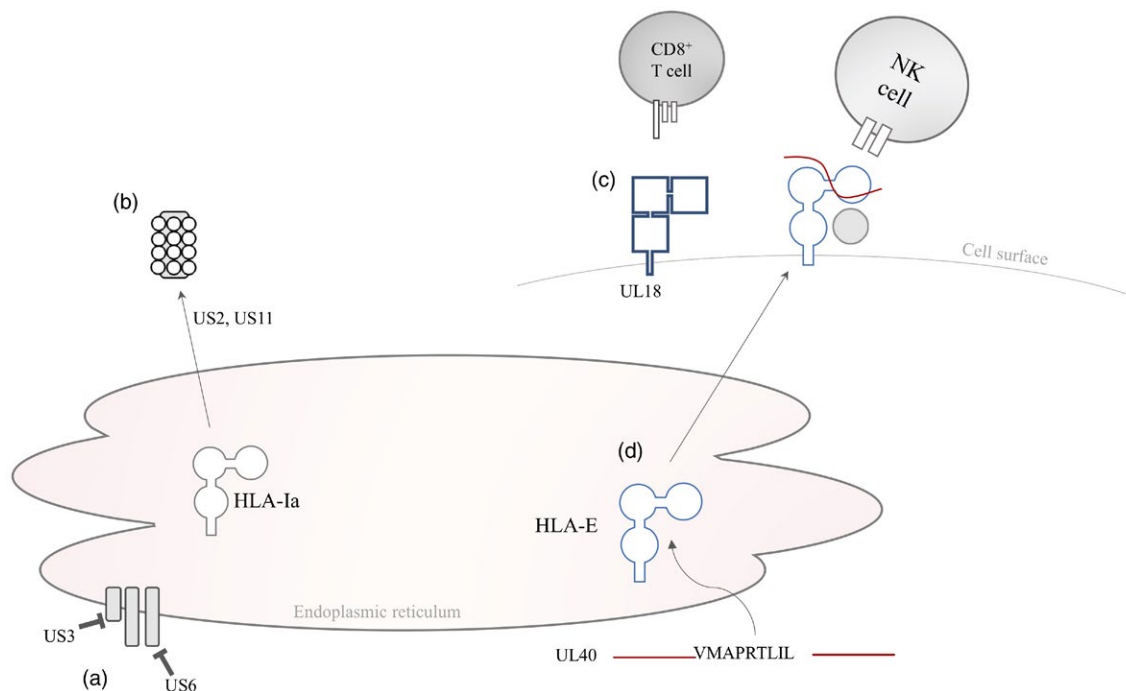


Fig. 1. Manipulation of human leucocyte antigen (HLA) molecules by human cytomegalovirus (HCMV). (a) US3 and US6 prevent peptide binding to the HLA class Ia groove via interaction with tapasin and transporter associated with antigen processing (TAP), respectively. (b) US2 and US11 direct nascent HLA class Ia to the proteasome. (c) UL18 acts as a functional homologue of HLA-E, and binds to the inhibitory leucocyte immunoglobulin-like receptor 1 (LIR1) on T cells. (d) UL40 contains a VL9 leader peptide, which binds and stabilizes HLA-E on the cell surface to interact with inhibitory CD94/NKG2A receptors on natural killer (NK) cells [1,47–52].

control the virus through increased cytotoxic activity [55]. CD56^{dim}NKG2C^{bright} NK cells may also express CD57, a marker of maturation and terminally differentiated NK cells [56,57], which potentially induce a form of innate ‘memory’ towards persistent infections such as HCMV.

HIV

Similarly, human immunodeficiency virus (HIV) causes a lifelong and latent infection, which is fatal if left untreated. HIV contains a putative HLA-E binding leader peptide in the p24 gene product (AA9, Table

2). Although AA9 can stabilize HLA-E on the cell surface, it is not sufficient to initiate interaction with CD94/NKG2 molecules on NK cells [58]. Therefore, CD94/NKG2A⁺ NK cells are not inhibited by HLA-E expression, and facilitate cytolytic killing of HIV-infected cells [59]. In HIV infection, there is an expansion of the NKG2C^{bright} NK compartment, although this has been correlated with HCMV co-infection and not as a response to HIV [60].

Mycobacterium tuberculosis

Tuberculosis afflicts roughly one-third of the world's population, and is caused by persistent latent infection by the bacterium *Mycobacterium tuberculosis* (*Mtb*) [61]. The current licensed vaccine against *Mtb* infection is bacillus Calmette–Guérin (BCG), a live-attenuated vaccine derived from *M. bovis* [62]. NK cells form a substantial part of the innate immune response generated from this vaccine, and produce inflammatory cytokines in response to infection [63]. Although understanding of NK cell function during *Mycobacteria* infection is limited, NK cells in the *Mtb* granuloma exert cytotoxic pressure through production of granzyme and perforin [64], as well as restriction of bacterial growth through direct contact with infected cells via cytotoxic NKG2D⁺ NK cells [32]. HLA-E : *Mtb* peptide complexes are not recognized by CD94/NKG2 molecules, so do not control activation or inhibition of NK cells through these receptors [65].

NK cells and trained immunity

NK cells (and other innate lymphoid cells such as macrophages and monocytes) display 'trained immunity' in response to BCG vaccination [66]. Trained immunity induces a lasting anti-pathogen response to secondary, unrelated antigen exposure [62], which in NK cells correlates with increased proinflammatory cytokine production against new pathogens [67,68]. In infants, BCG vaccination correlates with increased weight gain, reduced mortality and reduced infection ability of other *Mycobacteria* species [66]. In immunodeficient SCID mice lacking T and B cells, similar protection is observed after BCG vaccination towards *Schistosoma* and *Candida* infection [66,69]. The proinflammatory effect of BCG is also commonly used to treat urothelial cell carcinoma [70]. Trained immunity differs from innate memory, as the heightened response is not specific to the original pathogen, although is stronger compared to antigen-naïve innate cells [62] and occurs as a result of histone methylation at the H3K4me1 locus of innate immune cells, inducing a lasting enhanced level of NK cell activation and cytokine production [56,63,71].

HLA-E and T cell restriction

HLA-E-mediated presentation of pathogen-derived peptides to T cells has been observed during infection with CMV, *Mtb* and *Salmonella enterica* [72–74], and recently in CMV-vectored vaccines against simian immunodeficiency virus (SIV) [5].

Mycobacterium tuberculosis

In humans and mice, CD4⁺ and CD8⁺ T cells are vital for control of *Mtb* infection [75]. Unusually, for any known pathogen, there is a large population of CD8⁺ T cells restricted by HLA-E induced by infection [76], possibly enhanced by the up-regulation of HLA-E on the surface of *Mtb*-infected phagosomes [65,70]. Multiple peptides within the *Mtb* genome can be presented (including peptides from p49 and Mtb44 proteins, Table 2) [61,72,74]. Their varying amino acid length implies that HLA-E has higher peptide binding plasticity than solely the VL9 peptide [75]. These non-canonical T cells contribute to the majority of T cells present during active *Mtb* infection [77], and overshadow T cells restricted by canonical HLA class Ia epitope presentation [78,79]. *Mtb* antigens presented by HLA-E to CD8⁺ T cells can induce either a cytotoxic or regulatory phenotype, consequently inhibiting *Mtb* pathogenesis and growth in infected macrophages [7,78]. HLA-E : *Mtb*-restricted T cells from active TB infection express a type 2 cytokine profile with increased interleukin (IL)-4 and IL-10 production, and assist B cells with antibody and cytokine production to inhibit *Mtb* growth [65,76,77].

Cytomegalovirus

Classically restricted HCMV-targeting CD8⁺ T cells are critical in the control of HCMV infection, and constitute up to 10% of the circulating T cell population during active infection [46,48,80], typically directed towards epitopes in the pp65 and IE peptides [81]. Infection with HCMV also establishes a 'memory inflation' population of CD8⁺ effector memory T cells (T_{EM}), which are defined by their large expansion after infection, terminally differentiated phenotype (CD57⁺) and their expression of CX3CR1 [81–83]. In CMV-seropositive individuals, a proportion of CD8⁺ T cells are HLA-E-restricted, long-lasting and express a T_{EM} phenotype [83], although interact with the T cell receptor (TCR) with much lower affinity than HLA class Ia-bound peptides. These T cells recognize the UL40-VL9 epitope presented by HLA-E, and may arise from permanent exposure to HCMV epitopes, which perpetuate a stronger response comparable to canonically restricted CD8⁺ T cells [83,84].

Utilizing unusual immune phenotypes for vaccine development

The role of HLA-E in NK cell activation has been well studied; however, the unforeseen property of HLA-E to restrict non-canonical T cells has revealed interesting potential for developments in vaccine design and efficacy.

CMV-vectored vaccines for SIV

Cytomegalovirus, when used as a vaccine vector in rhesus macaques, induces the restriction of non-classical T cells by Mamu-E. This has been demonstrated in a rhesus macaque CMV-vectored vaccine against SIV, the primate homologue of HIV [85]. SIV, like HIV, is a lentivirus that permanently infects the host through integration into the genome. SIV has been used as a close model for HIV, and causes similar pathogenesis including loss of CD4⁺ T cells, AIDS-like illness and eventually death in infected macaques [86].

CMV as a virus, and as a vaccine vector, can generate swift T_{EM} cell responses at the site of exposure [87,88]. In contrast, many non-viral-vectored vaccines activate a slower-acting T central memory (T_{CM}) response in the secondary lymphoid organs [89]. In these studies, rhesus macaque cytomegalovirus strain 68-1 (RhCMV 68-1) was used as a vaccine vector, in accordance with the highly species tropic nature of cytomegaloviruses. The SIV genes gag+rev/nef/ tat+env+pol were expressed in this RhCMV vector [87,90]. In all vaccinated macaques, this vaccine generated robust, long-lasting CD4⁺ and CD8⁺ T_{EM} responses to all SIV peptides, contrasting with predominantly T_{CM} responses from adenovirus-vectored vaccines containing the same peptides. Fifty-five per cent of RhCMV : SIV vaccinated macaques generated completely protective immune responses when challenged 59 weeks later with intrarectal infection of highly pathogenic SIVmac239 [88]. These atypical CD8⁺ T cell responses were able to recognize broad SIV epitopes, alongside occasional 'supertopes' (epitopes recognized by all macaques) within the vaccine, regardless of the MHC haplotype of the macaques [90]. Furthermore, classically defined SIV epitopes were not recognized by the T cells of any macaque. Protection was also conferred irrespective of the route of SIV infection, and could be transferred to SIV-seronegative macaques after adoptive transfer of haematolymphoid cells prior to SIVmac239 challenge [86].

The RhCMV : SIV vaccine restricts non-canonical CD8⁺ T cells

In macaques vaccinated with the RhCMV : SIV vaccine, MHC class II-restricted T cells made up 65% of this non-canonical response, and the remaining 35% were restricted by Mamu-E. No canonically restricted CD8⁺ T cells were produced in response to this vaccine [5,90]. The

Mamu-E-restricted SIVgag-specific response was so broad that an average of 20 SIVgag epitopes were recognized per vaccinated macaque, in contrast to ~13 SIVgag epitopes from canonical CD8⁺ T cell restriction [90]. This broad response in the non-canonical CD8⁺ T cell compartment has not been previously observed in any preclinical vaccine trial. It may be due to the deletion of Rh157.4 and Rh157.5, which encode an orthologue of the HCMV pentameric glycoprotein receptor complex encoded by UL128 and UL130, which enables non-fibroblast tropism [5,90]. Similarly to HCMV, normal RhCMV infection only activates canonically restricted T cells [91], and due to the differences between the vaccine and wild-type RhCMV there is no pre-existing immunity against this RhCMV-vectored vaccine in macaques [92].

RhCMV contains an MHC-E-binding VL9 sequence

Through this RhCMV-vectored SIV vaccine, Mamu-E exhibits the ability to overcome the classical paradigm of MHC class Ia T cell restriction, and in doing so generates an enormous breadth of non-canonical T cell responses. This, in theory, can offer equal protection to all vaccinated individuals, regardless of MHC genotype, and suggests that Mamu-E can bind a much broader range of peptides beyond the canonical VL9 leader peptide [5]. The VL9 peptide in this vaccine is provided by the RhCMV gene Rh67, which is loaded into Mamu-E in a TAP-independent manner. Rh67 initiates Mamu-E up-regulation at the cell surface in a convergent fashion to HCMV-UL40. It is hypothesized that the Rh67-VL9 peptide can bind and stabilize Mamu-E deep in the rigid, hydrophobic binding groove, acting as a chaperone and allowing a broader range of SIVgag epitopes to bind higher up in the binding groove and interact with Mamu-E-restricted T cells. Although cytomegaloviruses have high species tropism, Rh67 can stabilize and up-regulate HLA-E in human cells, suggesting that the function of the ancestral MHC-E gene has remained conserved [5]. This suggests that in the RhCMV : SIV vaccine, Rh67 is facilitating the broad peptide presentation by stabilization of Mamu-E on the cell surface, allowing presentation of antigen peptides to non-classical CD8⁺ T cells. Furthermore, several MHC-altering genes in RhCMV and HCMV share surprising functional similarity through preventing cell-surface presentation of MHC class Ia molecules [48] (Table 3). Overall, the convergent evolution of RhCMV-Rh67 and HCMV-UL40, and other genes involved in MHC down-regulation, suggests that the mechanism of HLA-E stabilization is conserved between cytomegalovirus species, and therefore a HCMV-vectored vaccine in humans may function in the same way.

CMV-vectored vaccines for tuberculosis

Recently, Hansen *et al.* [93] published a preclinical trial testing three RhCMV strain 68-1-vectored vaccines

expressing six or nine proteins from *Mtb*. All vaccines induced IFN- γ - and TNF-producing T_{EM} cells that are vital in protection against *Mtb* infection, and induced complete or partial protection in > 40% vaccinated macaques. One vaccine, using the original RhCMV68-1 vector from the SIV vaccine challenge, elicited unconventionally restricted MHC class II- and MHC-E-restricted T cells. However, two new '68-1.2 RhCMV'-vectored vaccines only exhibited a canonical T cell response in vaccinated macaques. All three vaccines, however, obtained the same levels of efficacy during challenge trials, suggesting that, contrary to earlier reports [61,65], Mamu-E-restricted T cell responses are not vital for control of *Mtb* infection [93]. Natural *Mtb* infection induces both cytotoxic and regulatory-like HLA-E-restricted T cells in humans. HLA-E-restricted regulatory T cells are likely to be beneficial in containing the *Mycobacterium* and preventing dissemination [61,76]. However, HLA-E-mediated peptide presentation may not be as beneficial to *Mtb* vaccine function if the induction of regulatory CD8⁺ T cells reduces the progression of disease, but does not establish a cytolytic environment targeted to *Mtb* infection. It will also be interesting to see if, in future studies, this *Mtb* vaccine can generate similar trained immunity in innate and NK cells, as is seen in the current BCG vaccine.

CMV vaccines in human clinical trials

Phase I clinical trials of CMV vaccines in humans were not able to elicit the same broad epitope response as RhCMV-vectored vaccines. The vaccine, created from chimeric Towne and Toledo fibroblast-adapted strains, was aimed at inducing immunity against HCMV infection, rather than for use as a viral vector. This vaccine did not contain the pentameric glycoprotein complex, facilitating non-fibroblast tropism in a similar fashion to the RhCMV vector. However, vaccination did not elicit any non-canonically restricted T cells in the human participants [94–96]. There is also reasonable concern regarding the use of CMV as a vaccine in CMV-seropositive individuals, thus creating a 'superinfection' serostatus, and possibly negating the immunogenic effect of the vaccine. Although this proved unproblematic in macaques, which have a RhCMV seropositivity of > 90% in captive populations [97], it is still uncertain what effect this will have in human clinical trials, given the ability of HCMV to reduce vaccine efficacy [98].

Future directions for utilizing pathogens to enhance vaccine efficacy

The dual functionality of HLA-E, through its ability to inhibit NK cells and activate non-classical CD8⁺ T cells,

has made it an intriguing target of research for both understanding the immunology behind pathogen infection and improving vaccine design. Although it is attractive to think that the results obtained by Hansen *et al.* [5,87,88] in the SIV field could lead to the creation of a non-conventional T cell stimulating vaccine in humans, there are still fundamental questions that must be answered to assess the potential for and safety of developing a vaccine that can induce HLA-E restricted T-cells. It is also important to acknowledge the existence of self-peptides, including HSP60, that contain potential HLA-E leader peptides [99]. These peptides are able to up-regulate HLA-E, although their immunological function is not well understood, and therefore may have an impact on vaccine efficacy.

However, as HLA-E is emerging as an important aspect of the host response to several pathogens, understanding how it can restrict T cells via non-conventional mechanisms will continue to be an important avenue of vaccine development, and also improve fundamental understanding of how HLA-E borders innate and adaptive immunity. Furthermore, the important contribution of NK cells towards vaccine efficacy has still to be fully elucidated.

In conclusion, trained immunity and the restriction of non-canonical CD8⁺ T cells by CMV-vectored vaccines are just two examples of unexpected effects caused by vaccination, which are impacting future vaccine design. If viral-vectored vaccines can be developed to induce HLA-E-restricted T cells in human patients, it may pave the way for the development of vaccines with broad, fast-acting and best-placed immunogenicity against many pathogens.

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H. R. S. and T. L. conceptualized and wrote the review; G. B. and S. B. contributed to the review structure, contents and proofreading.

References

- 1 Tomasec P, Braud VM, Rickards C *et al.* Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science* 2000; **287**:1031.
- 2 Rodgers JR, Cook RG. MHC class Ib molecules bridge innate and acquired immunity. *Nat Rev Immunol* 2005; **5**:459–71.
- 3 Little AM, Parham P. Polymorphism and evolution of HLA class I and II genes and molecules. *Rev Immunogenet* 1999; **1**:105–23.
- 4 Braud V, Jones EV, McMichael A. The human major histocompatibility complex class Ib molecule in HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. *Eur J Immunol* 1997; **27**:1164–9.
- 5 Hansen SG, Wu HL, Burwitz BJ *et al.* Broadly targeted CD8⁺ T cell responses restricted by major histocompatibility complex E. *Science* 2016; **351**:714–20.
- 6 Wooldridge L, Ekeruche-Makinde J, van den Berg HA *et al.* A single autoimmune T cell receptor recognizes more than a million different peptides. *J Biol Chem* 2012; **287**:1168–77.
- 7 Prezzemolo T, van Meijgaarden KE, Franken KLMC *et al.* Detailed characterization of human *Mycobacterium tuberculosis* specific HLA-E restricted CD8. *Eur J Immunol* 2018; **48**:293–305.
- 8 Grimsley C, Kawasaki A, Gassner C *et al.* Definitive high resolution typing of HLA-E allelic polymorphisms: identifying potential errors in existing allele data. *Tissue Antigens* 2002; **60**:206–12.
- 9 Felício LP, Porto IO, Mendes-Junior CT *et al.* Worldwide HLA-E nucleotide and haplotype variability reveals a conserved gene for coding and 3' untranslated regions. *Tissue Antigens* 2014; **83**:82–93.
- 10 Strong RK, Holmes MA, Li P, Braun L, Lee N, Geraghty DE. HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. *J Biol Chem* 2003; **278**:5082–90.
- 11 Veiga-Castelli LC, Castelli EC, Mendes CT *et al.* Non-classical HLA-E gene variability in Brazilians: a nearly invariable locus surrounded by the most variable genes in the human genome. *Tissue Antigens* 2012; **79**:15–24.
- 12 Grimsley C, Ober C. Population genetic studies of HLA-E: evidence for selection. *Hum Immunol* 1997; **52**:33–40.
- 13 Sawai H, Kawamoto Y, Takahata N, Satta Y. Evolutionary relationships of major histocompatibility complex class I genes in simian primates. *Genetics* 2004; **166**:1897–907.
- 14 Geraghty DE, Stocksleader M, Ishitani A, Hansen JA. Polymorphism at the HLA-E locus predates most HLA-A and -B polymorphism. *Hum Immunol* 1992; **33**:174–84.
- 15 Braud VM, Allan DS, O'Callaghan CA *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998; **391**:795–9.
- 16 Tripathi P, Agrawal S. The role of human leukocyte antigen E and G in HIV infection. *AIDS* 2007; **21**:1395–404.
- 17 Lauterbach N, Wieten L, Popeijus HE *et al.* Peptide-induced HLA-E expression in human PBMCs is dependent on peptide sequence and the HLA-E genotype. *Tissue Antigens* 2015; **85**:242–51.
- 18 Celik AA, Kraemer T, Huyton T, Blasczyk R, Bade-Döding C. The diversity of the HLA-E-restricted peptide repertoire explains the immunological impact of the Arg107Gly mismatch. *Immunogenetics* 2016; **68**:29–41.
- 19 Ulbrecht M, Martinozzi S, Grzeschik 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* 2000; **164**:5019–22.
- 20 O'Callaghan CA, Tormo J, Willcox BE *et al.* Structural features impose tight peptide binding specificity in the nonclassical MHC molecule HLA-E. *Mol Cell* 1998; **1**:531–41.
- 21 Joosten SA, Sullivan LC, Ottenhoff TH. Characteristics of HLA-E restricted T-cell responses and their role in infectious diseases. *J Immunol Res* 2016; **2016**:2695396.
- 22 Hoare HL, Sullivan LC, Clements CS *et al.* Subtle changes in peptide conformation profoundly affect recognition of the non-classical MHC class I molecule HLA-E by the CD94-NKG2 natural killer cell receptors. *J Mol Biol* 2008; **377**:1297–303.
- 23 Lampen MH, Hassan C, Sluijter M *et al.* Alternative peptide repertoire of HLA-E reveals a binding motif that is strikingly similar to HLA-A2. *Mol Immunol* 2013; **53**:126–31.
- 24 Dambaeva SV, Bondarenko GI, Grendell RL, Kravitz RH, Durning M, Golos TG. Non-classical MHC-E (Mamu-E) expression in the rhesus monkey placenta. *Placenta* 2008; **29**:58–70.
- 25 van Hall T, Oliveira CC, Joosten SA, Ottenhoff TH. The other Janus face of Qa-1 and HLA-E: diverse peptide repertoires in times of stress. *Microbes Infect* 2010; **12**:910–8.
- 26 Wu HL, Wiseman RW, Hughes CM *et al.* The role of MHC-E in T cell immunity is conserved among humans, rhesus macaques, and cynomolgus macaques. *J Immunol* 2018; **200**:49–60.
- 27 Boyson JE, McAdam SN, Gallimore A *et al.* The MHC E locus in macaques is polymorphic and is conserved between macaques and humans. *Immunogenetics* 1995; **41**:59–68.
- 28 Braud VM, Allan DS, Wilson D, McMichael AJ. TAP- and tapasin-dependent HLA-E surface expression correlates with the binding of an MHC class I leader peptide. *Curr Biol* 1998; **8**:1–10.
- 29 Romee R, Foley B, Lenvik T *et al.* NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). *Blood* 2013; **121**:3599–608.
- 30 Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med* 1989; **320**:1731–5.

- 31 Cook KD, Waggoner SN, Whitmire JK. NK cells and their ability to modulate T cells during virus infections. *Crit Rev Immunol* 2014; **34**:359–88.
- 32 Esin S, Batoni G. Natural killer cells: a coherent model for their functional role in *Mycobacterium tuberculosis* infection. *J Innate Immun* 2015; **7**:11–24.
- 33 Kaiser BK, Pizarro JC, Kerns J, Strong RK. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc Natl Acad Sci USA* 2008; **105**:6696–701.
- 34 Petrie EJ, Clements CS, Lin J *et al.* CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J Exp Med* 2008; **205**:725–35.
- 35 Llano M, Lee N, Navarro F *et al.* HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. *Eur J Immunol* 1998; **28**:2854–63.
- 36 Cheent KS, Jamil KM, Cassidy S *et al.* Synergistic inhibition of natural killer cells by the nonsignaling molecule CD94. *Proc Natl Acad Sci USA* 2013; **110**:16981–6.
- 37 García P, Llano M, de Heredia AB *et al.* Human T cell receptor-mediated recognition of HLA-E. *Eur J Immunol* 2002; **32**:936–44.
- 38 Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol* 2013; **31**:227–58.
- 39 Lauterbach N, Wieten L, Popeijus HE, Voorter CE, Tilanus MG. HLA-E regulates NKG2C+ natural killer cell function through presentation of a restricted peptide repertoire. *Hum Immunol* 2015; **76**:578–86.
- 40 Sullivan LC, Clements CS, Beddoe T *et al.* The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition. *Immunity* 2007; **27**:900–11.
- 41 López-Botet M, Bellón T, Llano M, Navarro F, García P, de Miguel M. Paired inhibitory and triggering NK cell receptors for HLA class I molecules. *Hum Immunol* 2000; **61**:7–17.
- 42 Yan G, Zhang G, Fang X *et al.* Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. *Nat Biotechnol* 2011; **29**:1019–23.
- 43 Powers C, Früh K. Rhesus CMV: an emerging animal model for human CMV. *Med Microbiol Immunol* 2008; **197**:109–15.
- 44 Wilkinson GW, Tomasec P, Stanton RJ *et al.* Modulation of natural killer cells by human cytomegalovirus. *J Clin Virol* 2008; **41**:206–12.
- 45 Sathiyamoorthy K, Chen J, Longnecker R, Jardetzky TS. The COMPLEXity in herpesvirus entry. *Curr Opin Virol* 2017; **24**:97–104.
- 46 Gillespie GM, Wills MR, Appay V *et al.* Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. *J Virol* 2000; **74**:8140–50.
- 47 Mazzarino P, Pietra G, Vacca P *et al.* Identification of effector-memory CMV-specific T lymphocytes that kill CMV-infected target cells in an HLA-E-restricted fashion. *Eur J Immunol* 2005; **35**:3240–7.
- 48 Pande NT, Powers C, Ahn K, Früh K. Rhesus cytomegalovirus contains functional homologues of US2, US3, US6, and US11. *J Virol* 2005; **79**:5786–98.
- 49 Wiertz EJ, Jones TR, Sun L, Bogoy M, Geuze HJ, Ploegh HL. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* 1996; **84**:769–79.
- 50 Hengel H, Koopmann JO, Flohr T *et al.* A viral ER-resident glycoprotein inactivates the MHC-encoded peptide transporter. *Immunity* 1997; **6**:623–32.
- 51 Park B, Kim Y, Shin J *et al.* Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. *Immunity* 2004; **20**:71–85.
- 52 Chapman TL, Heikeman AP, Bjorkman PJ. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* 1999; **11**:603–13.
- 53 Heatley SL, Pietra G, Lin J *et al.* Polymorphism in human cytomegalovirus UL40 impacts on recognition of human leukocyte antigen-E (HLA-E) by natural killer cells. *J Biol Chem* 2013; **288**:8679–90.
- 54 Wang EC, McSharry B, Retiere C *et al.* UL40-mediated NK evasion during productive infection with human cytomegalovirus. *Proc Natl Acad Sci USA* 2002; **99**:7570–5.
- 55 Muntasell A, Vilches C, Angulo A, López-Botet M. Adaptive reconfiguration of the human NK-cell compartment in response to cytomegalovirus: a different perspective of the host-pathogen interaction. *Eur J Immunol* 2013; **43**:1133–41.
- 56 Netea MG. Training innate immunity: the changing concept of immunological memory in innate host defence. *Eur J Clin Invest* 2013; **43**:881–4.
- 57 Min-Oo G, Lanier LL. Cytomegalovirus generates long-lived antigen-specific NK cells with diminished bystander activation to heterologous infection. *J Exp Med* 2014; **211**:2669–80.
- 58 Nattermann J, Nischalke HD, Hofmeister V *et al.* HIV-1 infection leads to increased HLA-E expression resulting in impaired function of natural killer cells. *Antivir Ther* 2005; **10**:95–107.
- 59 Davis ZB, Cogswell A, Scott H *et al.* A Conserved HIV-1-derived peptide presented by HLA-E renders infected T-cells highly susceptible to attack by NKG2A/CD94-bearing natural killer cells. *PLOS Pathog* 2016; **12**:e1005421.
- 60 Brunetta E, Fogli M, Varchetta S *et al.* Chronic HIV-1 viremia reverses NKG2A/NKG2C ratio on natural killer cells in patients with human cytomegalovirus co-infection. *AIDS* 2010; **24**:27–34.

- 61 Joosten SA, van Meijgaarden KE, van Weeren PC *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* 2010; **6**: e1000782.
- 62 Gardiner CM, Mills KH. The cells that mediate innate immune memory and their functional significance in inflammatory and infectious diseases. *Semin Immunol* 2016; **28**:343–50.
- 63 Della Chiesa M, Marcenaro E, Sivori S, Carlomagno S, Pesce S, Moretta A. Human NK cell response to pathogens. *Semin Immunol* 2014; **26**:152–60.
- 64 Garand M, Goodier M, Owolabi O, Donkor S, Kampmann B, Sutherland JS. Functional and phenotypic changes of natural killer cells in whole blood during *Mycobacterium tuberculosis* infection and disease. *Front Immunol* 2018; **9**:257.
- 65 Caccamo N, Pietra G, Sullivan LC *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* 2015; **45**:1069–81.
- 66 Blok BA, Arts RJ, van Crevel R, Benn CS, Netea MG. Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. *J Leukoc Biol* 2015; **98**:347–56.
- 67 Rusek P, Wala M, Druszczyńska M, Fol M. Infectious agents as stimuli of trained innate immunity. *Int J Mol Sci* 2018; **19**.
- 68 Kleinnijenhuis J, Quintin J, Preijers F *et al.* BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clin Immunol* 2014; **155**:213–9.
- 69 Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 2011; **9**:355–61.
- 70 Buffen K, Oosting M, Quintin J *et al.* Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. *PLOS Pathog* 2014; **10**:e1004485.
- 71 Fanucchi S, Fok ET, Dalla E *et al.* Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. *Nat Genet* 2019; **51**:138–50.
- 72 Heinzel AS, Grotzke JE, Lines RA *et al.* HLA-E-dependent presentation of Mtb-derived antigen to human CD8+ T cells. *J Exp Med* 2002; **196**:1473–81.
- 73 Salerno-Gonçalves R, Fernandez-Viña M, Lewinsohn DM, Sztein MB. 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* 2004; **173**:5852–62.
- 74 Walters LC, Harlos K, Brackenridge S *et al.* Pathogen-derived HLA-E bound epitopes reveal broad primary anchor pocket tolerability and conformationally malleable peptide binding. *Nat Commun* 2018; **9**:3137.
- 75 McMurtrey C, Harrieff MJ, Swarbrick GM *et al.* T cell recognition of *Mycobacterium tuberculosis* peptides presented by HLA-E derived from infected human cells. *PLOS ONE* 2017; **12**:e0188288.
- 76 van Meijgaarden KE, Haks MC, Caccamo N, Dieli F, Ottenhoff TH, Joosten SA. Human CD8+ T-cells recognizing peptides from *Mycobacterium tuberculosis* (*Mtb*) presented by HLA-E have an unorthodox Th2-like, multifunctional, Mtb inhibitory phenotype and represent a novel human T-cell subset. *PLOS Pathog* 2015; **11**:e1004671.
- 77 Harrieff MJ, Wolfe LM, Swarbrick G *et al.* HLA-E presents glycopeptides from the *Mycobacterium tuberculosis* protein MPT32 to human CD8. *Sci Rep* 2017; **7**:4622.
- 78 Bian Y, Shang S, Siddiqui S *et al.* MHC Ib molecule Qa-1 presents *Mycobacterium tuberculosis* peptide antigens to CD8+ T cells and contributes to protection against infection. *PLOS Pathog* 2017; **13**:e1006384.
- 79 McMichael AJ, Picker LJ. Unusual antigen presentation offers new insight into HIV vaccine design. *Curr Opin Immunol* 2017; **46**:75–81.
- 80 Sylwester AW, Mitchell BL, Edgar JB *et al.* Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *J Exp Med* 2005; **202**:673–85.
- 81 Klenerman P. The (gradual) rise of memory inflation. *Immunol Rev* 2018; **283**:99–112.
- 82 Gordon CL, Lee LN, Swadling L *et al.* Induction and maintenance of CX3CR82-intermediate peripheral memory CD8. *Cell Rep* 2018; **23**:768–82.
- 83 Jouand N, Bressollette-Bodin C, Gérard N *et al.* HCMV triggers frequent and persistent UL40-specific unconventional HLA-E-restricted CD8 T-cell responses with potential autologous and allogeneic peptide recognition. *PLOS Pathog* 2018; **14**:e1007041.
- 84 Hoare HL, Sullivan LC, Pietra G *et al.* Structural basis for a major histocompatibility complex class Ib-restricted T cell response. *Nat Immunol* 2006; **7**:256–64.
- 85 Sharp PM, Shaw GM, Hahn BH. Simian immunodeficiency virus infection of chimpanzees. *J Virol* 2005; **79**:3891–902.
- 86 Hansen SG, Piatak M, Ventura AB *et al.* Immune clearance of highly pathogenic SIV infection. *Nature* 2013; **502**:100–4.
- 87 Hansen SG, Vieville C, Whizin N *et al.* Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* 2009; **15**:293–9.
- 88 Hansen SG, Ford JC, Lewis MS *et al.* Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* 2011; **473**:523–7.
- 89 Small JC, Haut LH, Bian A, Ertl HC. The effect of adenovirus-specific antibodies on adenoviral vector-induced, transgene product-specific T cell responses. *J Leukoc Biol* 2014; **96**:821–31.
- 90 Hansen SG, Sacha JB, Hughes CM *et al.* Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science* 2013; **340**:1237874.
- 91 Hansen SG, Powers CJ, Richards R *et al.* Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. *Science* 2010; **328**:102–6.

- 92 Früh K, Picker L. CD8⁺ T cell programming by cytomegalovirus vectors: applications in prophylactic and therapeutic vaccination. *Curr Opin Immunol* 2017; **47**:52–6.
- 93 Hansen SG, Zak DE, Xu G *et al.* Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine. *Nat Med* 2018; **24**:130–43.
- 94 Murray SE, Nesterenko PA, Vanarsdall AL *et al.* Fibroblast-adapted human CMV vaccines elicit predominantly conventional CD8 T cell responses in humans. *J Exp Med* 2017; **214**:1889–99.
- 95 Adler SP, Manganello AM, Lee R *et al.* A Phase 1 study of 4 live, recombinant human cytomegalovirus Towne/Toledo chimera vaccines in cytomegalovirus-seronegative men. *J Infect Dis* 2016; **214**:1341–8.
- 96 Suárez NM, Lau B, Kemble GM *et al.* Genomic analysis of chimeric human cytomegalovirus vaccine candidates derived from strains Towne and Toledo. *Virus Genes* 2017; **53**:650–5.
- 97 Hansen SG, Strelow LI, Franchi DC, Anders DG, Wong SW. Complete sequence and genomic analysis of rhesus cytomegalovirus. *J Virol* 2003; **77**:6620–36.
- 98 Falconer O, Newell ML, Jones CE. The effect of human immunodeficiency virus and cytomegalovirus infection on infant responses to vaccines: a review. *Front Immunol* 2018; **9**:328.
- 99 Michaëlsson J, Teixeira de Matos C, Achour A, Lanier LL, Kärre K, Söderström K. A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition. *J Exp Med* 2002; **196**:1403–14.
- 100 Jensen PE, Sullivan BA, Reed-Loisel LM, Weber DA. Qa-1, a nonclassical class I histocompatibility molecule with roles in innate and adaptive immunity. *Immunol Res* 2004; **29**:81–92.
- 101 Araújo RC, Dias FC, Bertol BC *et al.* Liver HLA-E expression is associated with severity of liver disease in chronic hepatitis C. *J Immunol Res* 2018; **2018**:2563563.