Modulation of innate and adaptive immunity by cytomegaloviruses

Richard Berry[1,2,3]*, *Gabrielle M. Watson*[1,2,3], *Stipan Jonjic*[4], *Mariapia A. Degli-Esposti*[1,5,6] *and Jamie Rossjohn*[1,2,3,7]

Abstract | The coordinated activities of innate and adaptive immunity are critical for effective protection against viruses. To counter this, some viruses have evolved sophisticated strategies to circumvent immune cell recognition. In particular, cytomegaloviruses encode large arsenals of molecules that seek to subvert T cell and natural killer cell function via a remarkable array of mechanisms. Consequently, these 'immunoevasins' play a fundamental role in shaping the nature of the immune system by driving the evolution of new immune receptors and recognition mechanisms. Here, we review the diverse strategies adopted by cytomegaloviruses to target immune pathways and outline the host's response.

Although many infections are associated with severe disease symptoms, viruses that have a long-term evolutionary relationship with their host species are typically relatively benign. This is best exemplified by the herpesviruses, a group of large, doubled-stranded DNA viruses that establish lifelong infections within their hosts that are characterized by prolonged periods of latency interspersed with cycles of reactivation and dissemination. To survive undetected, herpesviruses have developed an array of immunomodulatory molecules — termed 'immunoevasins' — that serve to dampen many innate and adaptive immune pathways. Considerable research attention has focused on immune evasion by a group of betaherpesviruses, collectively referred to as cytomegaloviruses (CMVs). Individual CMVs are highly species specific and unable to replicate in organisms that are even closely related to their hosts; however, as a genus they infect a broad range of species, including humans[1], mice[2], rats[3] and several non-human primates, such as chimpanzees[4], rhesus macaques[5] and owl monkeys. Mouse CMV (MCMV) has been particularly useful in unravelling the complex interplay that exists between viral immunoevasins and the host immune system[6] and, alongside human CMV (HCMV), will be the primary focus of this Review.

HCMV is also an important human pathogen that places a significant health burden on society. By using a stealthy approach, HCMV has become incredibly widespread, infecting 45–100% of the worldwide population, with seroprevalence being dependent on age, ethnicity, sex and socioeconomic status[7]. While primary HCMV infection is generally asymptomatic in healthy individuals, the virus is a major cause of morbidity and death in immunocompromised individuals. For example, individuals with AIDS or transplant recipients can experience a range of HCMV-mediated complications, including hepatitis, pneumonia, retinitis and other opportunistic infections[8]. Moreover, HCMV infection contracted during pregnancy can result in transmission to the developing fetus, which is associated with severe and permanent sequelae, including vision impairment, mental retardation and hearing loss, as well as an increased risk of death[8]. Such congenital CMV infection is the most common non-genetic cause of birth defects and disabilities in industrialized nations[9]. Thus, in addition to advancing our fundamental understanding surrounding virus–host immunobiology, dissecting the basis for CMV immune evasion also has the potential to inform as to the most effective strategies to alleviate viral pathogenesis.

The immune response to CMV infection

The control of CMV infection requires the concerted activities of both innate and adaptive immune effectors[10]. Within the innate immune system, natural killer (NK) cells act as the first line of defence and play an important role in limiting early CMV infection, both in humans and in genetically resistant mouse strains such as C57BL/6 (REF.[11]). This is particularly evident in individuals who lack or exhibit impaired NK cell function, who are highly susceptible to herpesvirus infections[12]. Commensurate with their function as rapid responders, NK cells are primed for attack via the constitutive transcription of genes encoding cytokines (primarily interferon-γ (IFNγ))[13] and cytotoxic molecules (perforin and granzymes)[14], thereby allowing the rapid secretion of these molecules on target cell engagement. Although NK cells can be activated without the need for prior antigen exposure, they can also mediate antigen-specific memory responses towards CMV infection[15] and other

*e-mail: richard.berry@monash.edu

https://doi.org/10.1038/s41577-019-0225-5

Paired receptors
Closely related receptors that bind to the same or similar ligands but trigger opposing functional effects (for example, stimulatory versus inhibitory).

viral infections[16]. NK cell specificity is governed by the integration of signals received from various germline-encoded, paired receptors that detect alterations in the expression of ligands on the surface of potential target cells. Accordingly, NK cell receptors constitute a major target for many viral immunoevasins.

Cytotoxic $CD8^+$ T cells are essential to limit viral replication during the later stage of acute CMV infection in mice[17], whereas $CD4^+$ T cells limit viral replication at sites of chronic infection, principally the salivary glands[18,19]. The protective function of CMV-specific $CD4^+$ T cells appears to require both cytolysis[20] and cytokine secretion, with IFNγ reported as the key antiviral cytokine[21,22]. In humans, data from patients who have undergone haematopoietic stem cell transplant showing that recovery from CMV disease correlates with reconstitution of the $CD8^+$ T cell pool[23–27] have provided correlative evidence for the role of $CD8^+$ T cells in controlling HCMV reactivation. A correlation between IFNγ-secreting $CD4^+$ T cells and protection from CMV disease has also been reported[24,28–30]. Importantly, mouse studies have also highlighted how crosstalk between the innate immune system and the adaptive immune system — including between dendritic cells and NK cells[31,32], and between T cells and NK cells[33] — is crucial for maximal control of MCMV infection.

Despite these carefully coordinated responses, complete clearance of CMV by the immune system invariably fails. Herein, we examine the viral molecules that counter these host defences and detail the mechanisms by which CMVs are able to establish lifelong infections within their hosts.

CMV-encoded immunoevasin families

At ~ 235 kilobases in size, HCMV harbours the largest, and potentially the most variable, genome of any human virus described to date (BOX 1). Of the ~170 open reading frames, most genes (~70%) are dispensable for viral replication in vitro[34] and many of these have been suggested to modulate host immunity[35–37]. These putative immunoevasin genes are primarily (but not exclusively) clustered into tandem arrays or families, whose members typically harbour one or more signature motifs and/or possess a common underlying structural architecture (FIG. 1). Notably, most immunoevasin families exhibit sequence homology to proteins encoded within their host, indicating that they may have arisen by a process of gene capture and subsequent expansion driven by immune selective pressure. However, despite sharing a common ancestor, immunoevasins within a given family have often evolved to be highly divergent, in terms of primary amino acid sequence, structure and molecular mechanism of action.

The genome of the low-passage clinical HCMV strain Merlin includes four established families of immunoevasins, three of which are type I integral membrane proteins. These include a small group of MHC class I (MHC-I) homologues (the UL18 family), of which at least one member (UL18) associates with peptide and β_2-microglobulin (β_2M), and a larger cluster of contiguous genes within the US region (US2–US11) that are primarily involved in interfering with MHC-I cell surface expression[38]. Although often divided into two subgroups (US2 family, US2 and US3; US6 family, US4–US11) sequence and structural analysis indicates that each of these proteins includes a single immunoglobulin domain[39]. An additional group of genes (the RL11 family, including RL5A, RL6, RL11–RL13, UL1 and UL4–UL11) located at the opposite end of the genome may adopt immunoglobulin-like folds[35], although whether this putative assignment is correct remains to be formally demonstrated. The proteins encoded by these genes are characterized by the 'RL11 domain', which is defined by a region of variable length (65–82 residues) that includes a conserved tryptophan and two cysteine residues[35]. Although the function of most members of the RL11 family have yet to be determined, RL11–RL13 can bind to IgG and inhibit Fc receptor (FcR) activation, and as a group they are highly variable among HCMV isolates[40], indicating they may be under immune selective pressure. The final cluster of HCMV immunoevasins (US12 family, US12–US21) spans a group of ten tandemly arranged genes that encode proteins that are predicted to include seven transmembrane segments and share low-level sequence identity to the cellular transmembrane BAX inhibitor motif-containing protein (TMBIM) family. Despite being architecturally dissimilar to other immunoevasins, the US12 family has recently been described as a major new hub of immune regulation, whose members are involved in the regulation of NK cell ligands, adhesion molecules and cytokine receptors[36,41].

HCMV immunoevasin families are conserved to differing extents in other primate CMVs[35]. For example, rhesus CMVs appears to possess orthologues of many of the US6 family genes[42] as well as an additional viral protein (Rh178) that interferes with translation of MHC-I heavy chains in a signal peptide-dependent manner[43]. However, direct homologues of HCMV immunoevasins do not exist in MCMV, despite the collinearity of their genomes[2] (FIG. 1). This disparity suggests that each viral species has evolved molecules that are tailored towards the immune system of its respective host. Nevertheless, a number of parallels between HCMV and MCMV immunoevasins are clearly evident. MCMV encodes two large families of immunoevasins located at opposite ends of the genome[2]. At the extreme right is the m145 family (m17 and m145–m158), a group of relatively well-studied cell surface glycoproteins whose

Author addresses

[1]Infection and Immunity Program, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia.
[2]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia.
[3]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, Australia.
[4]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
[5]Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia.
[6]Centre for Experimental Immunology, Lions Eye Institute, Perth, Western Australia, Australia.
[7]Institute of Infection and Immunity, Cardiff University School of Medicine, Cardiff, UK.

Box 1 | **Impact of CMV variability and host genetics**

Analysis of human cytomegalovirus (HCMV) has been confounded by the extreme variability exhibited among different clinical isolates and the rapid emergence of HCMV mutants during in vitro culture, even in low-passage strains[198]. For example, the extensively used laboratory strains AD169 and Towne harbour significant deletions at the right end of the unique long (U_L) region that is now known to encode at least two immunoevasin genes (*UL141* and *UL142*)[130,132,199], in line with the increased vulnerability of these strains to natural killer (NK) cell attack[200]. These issues have been somewhat resolved by the development of a technology to repress RL13 and UL131A during virus propagation[201]; this enables the analysis of HCMV in the absence of the confounding effects associated with in vitro adaptation[202]. Numerous HCMV strains exist, and superinfection with multiple strains is common[203–206]. Hence, it is important that future studies use multiple repaired HCMVs that match clinical isolates.

With respect to mouse CMV (MCMV) infection, the vast majority of research has been performed with the Smith and K181 viral strains. Although the genome of MCMV is more stable than that of HCMV, genetic variation exists between these commonly used strains and wild-derived MCMV isolates[207]. This is particularly evident in genes that encode immunoevasin proteins, and these polymorphisms can directly impact the outcome of viral infection[52,107,161]. MCMV strain variability can have considerable impact on therapeutic approaches; for example, it affects the capacity of immunoglobulin therapy to prevent reactivation in a preclinical model of bone marrow transplantation[208]. It is therefore important to consider CMV strain variability in future studies.

MCMV infection pathogenesis is also highly dependent on host genetics. C57BL/6 mice are unique in that they express the activating Ly49H receptor that recognizes the m157 immunoevasin. This mouse strain has been widely used to study NK cell responses to CMV infection, including adaptive features of NK cells[15,16]. Infection of BALB/c mice has many features of HCMV infection pathogenesis and is a useful model to study CMV-induced disease[209]. Understanding how CMV strain variability and host genetics affect the outcome of infection is an important aspect of future research.

members adopt MHC-I-like folds, although none have been shown to associate with peptides or β_2M[44]. These proteins exhibit a variety of distinct functions that range from downregulation of self (MHC-I)[45] or induced-self (NKG2D) ligands[46–48] to acting as decoys for missing-self (Ly49) receptors[49]. At the left of the genome is a less well characterized group of related molecules (m02–m16, collectively referred to as the m02 family) that adopt a fold that is loosely related to that of the V-type immunoglobulin domain[50,51]. While little is known regarding most of the m02 family members, those whose function has been elucidated (m04, m06 and m12) appear to be involved in subversion of 'missing-self' recognition[52–54]. Furthermore, although great progress has been made in uncovering the identity and function of many immunoevasins, a large number of putative immunoevasin genes remain uncharacterized.

V-type immunoglobulin domain
A compact protein module comprising two β-sheets arranged into a β-sandwich fold. 'V' refers to 'variable' indicating a subclass of immunoglobulin domains that possess nine β-strands and resemble those located within the variable portion of antibodies.

'Missing-self' recognition
A term used to describe how the downregulation of self-molecules, which act as ligands for inhibitory receptors, can trigger natural killer cell activation.

Sec61 complex
A dynamic multiprotein channel that, in eukaryotic cells, is located within the endoplasmic reticulum membrane. It mediates the membrane insertion and translocation of most proteins that reside in the endomembrane system or are destined for secretion.

Strategies to dampen the immune response

Having co-evolved alongside their hosts over millennia, CMVs have developed numerous strategies to restrict host immunity. Here we primarily focus on viral modulators of T cell and NK cell receptors, although additional mechanisms, such as suppression of immune cell signalling pathways (reviewed in REF.[55]), have also been documented. In BOX 2 we propose three main classes of immunoevasins on the basis of their similarity to the endogenous ligand and their molecular mode of action. These classes are 'molecular mimics', 'convergent immunoevasins' and 'alternative binders'. In the following sections, we have grouped immunoevasins on the basis of how they interfere with the immune system. However, this is an oversimplification of what is an inherently complex system. For instance, several immunoevasins can act together to target a single host molecule or pathway, and a single immunoevasin may exhibit multiple distinct functions. For an overview that takes these considerations into account, see TABLE 1, which provides a summary of the origin, molecular targets and function of CMV-encoded immunoevasins, including several that are not discussed in the text due to space limitations.

Modulation of antigen presentation

$CD8^+$ T cells are critical for the detection and elimination of CMV-infected cells via their expression of T cell receptors (TCRs), which directly recognize viral peptides presented by MHC-I molecules[56]. In infected cells, antigenic peptides derived from proteasomal processing of viral proteins are transported from the cytosol to the endoplasmic reticulum (ER), loaded on to MHC-I and exported to the cell surface via the secretory pathway. Accordingly, CMV interferes with almost all stages of the MHC-I antigen presentation pathway to avoid $CD8^+$ T cell recognition (FIG. 2a).

MHC downregulation by the HCMV US6 family. Most MHC-I-targeting immunoevasins (including four US6 family members in HCMV, namely US2, US3, US6 and US11) are ER-resident type I membrane proteins that possess an immunoglobulin-like luminal domain, a transmembrane domain and a cytosolic tail. However, despite these structural similarities, they differ in regard to their target specificity, molecular requirements and mechanistic basis for MHC-I downregulation (FIG. 2a). For example, the luminal domains of US2 and US11 recognize overlapping and distinct subsets of HLA allomorphs[37,39,57–59] and mediate dislocation of these MHC-I heavy chains from the ER to the cytosol, resulting in their efficient proteasomal degradation[60,61]. However, while US2-mediated translocation is dependent on the cytoplasmic tail[62] and occurs via the Sec61 complex[61], US11 translocation requires the cytoplasmic tail of MHC-I and is dependent on a glutamine residue within the US11 transmembrane domain that mediates recruitment of MHC-I heavy chains into the dislocation complex via derlin 1 (REFS[63,64]). Moreover, US2 appears to have broader specificity, and can interact with other non-MHC molecules (for example, nectin 2 and integrin-α)[65] and MHC-I-like molecules (for example, HLA-DRα, HLA-DMα, hereditary haemochromatosis protein (HFE) and CD1d)[66–69]. Additionally, US10 appears to specifically target the non-classical MHC-I molecule HLA-G via its characteristic shortened cytoplasmic tail[70], although it has also been implicated in delaying the maturation of classical MHC-I[71]. Intriguingly, US10-mediated downregulation of HLA-G surface expression occurs via an unidentified degradation pathway that is distinct from that utilized by US2 and US11 (REF.[70]).

HCMV interference with the peptide loading complex. The import of peptides into the ER and their loading onto MHC-I is coordinated by a dynamic, multisubunit apparatus termed the 'peptide loading complex'. In addition to the nascent MHC-I heterodimer, the

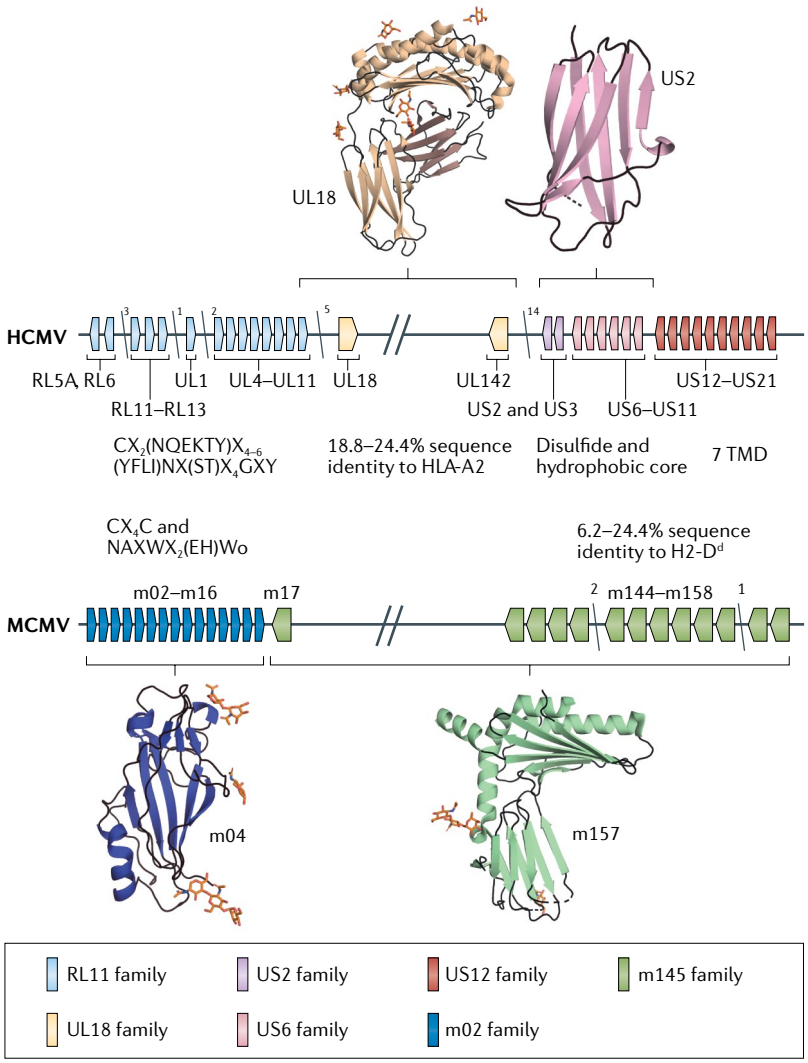


Fig. 1 | **Overview of the major HCMV and MCMV immunoevasin families.** A simplified linear representation of the mouse cytomegalovirus (MCMV) and human cytomegalovirus (HCMV) genomes is depicted with the major immunoevasin families coloured according to the key. Single black lines indicate the number of unrelated genes that are interspersed among immunoevasin clusters. Double black lines indicate the conserved essential genes located within the central region of the genome. Immunoevasins that are not ascribed to any particular family have been omitted for clarity. Characteristic sequence motifs and representative structures are shown for each immunoevasin family depicted, where available. Structures were derived from the Protein Data Bank entries 3D2U (UL18), 1IM3 (US2), 4PN6 (m04) and 2NYK (m157). Glycan chains are shown as orange sticks. For sequence motifs, X indicates any amino acid, o indicates a hydrophobic amino acid and alternative residues are shown in parentheses. TMD, transmembrane domain.

peptide loading complex comprises a peptide translocating channel (peptide transporter involved in antigen processing 1 (TAP1) and TAP2 subunits), two chaperones (tapasin and calreticulin) and an oxidoreductase (ERp57)[72]. HCMV interferes with this assembly via US3, which retains MHC-I in the ER by binding to tapasin and inhibiting tapasin-dependent peptide loading[73]. Some MHC-I can still be detected on the cell surface in US3-expressing cells[74], consistent with observations that US3-mediated downregulation of MHC-I does not lead to NK cell-mediated cytotoxicity[75]. These findings can be reconciled by the fact that not all MHC-I allotypes require tapasin for peptide loading[73]. The US6 glycoprotein also prevents peptide loading on MHC-I, but does so by inhibiting the entry of peptides into the ER by binding to the TAP1 subunit[76,77]. In doing so, US6 prevents TAP1 from undergoing ATP-driven large-scale conformational changes that are required for peptide translocation from the cytosol into the ER[78].

MCMV regulation of MHC-I. MCMV also encodes two glycoproteins that downregulate MHC-I surface expression (FIG. 2a). These include an immunoglobulin-like molecule, m06, that reroutes newly assembled MHC-I complexes to the lysosome[54]. MHC-I association is mediated by the luminal and transmembrane domain of m06 whereas a dileucine motif within the cytoplasmic tail is responsible for altered trafficking[54]. In addition, a distinct MHC-I-like molecule, m152, selectively blocks transport of MHC-I through the ER–Golgi intermediate compartment (ERGIC)/*cis*-Golgi compartment[45]. This function resides within the ER luminal domain of m152 (REF.[79]), which includes a 43 amino acid linker sequence that anchors it (and any associated MHC-I molecule) to the ER via an interaction with TMED10, an endogenous member of the p24 family[80].

Escape from 'missing-self' recognition

Although an effective means to avoid T cell responses, the downregulation of MHC-I or other 'self' markers of cell health triggers the activation of NK cells via a process termed 'missing-self recognition'[81]. Within this context, under steady-state conditions NK cells are rendered inactive due to the interaction of inhibitory NK cell receptors with self-ligands that are broadly expressed on the surface of healthy cells. However, downregulation of self-ligands on infected target cells results in a loss of inhibitory signalling and promotes NK cell-mediated lysis. Accordingly, CMVs have evolved a number of strategies that serve to limit NK cell activation, primarily by providing surrogate ligands that engage inhibitory NK cell receptors (FIG. 2b), although many of these also trigger stimulatory NK cell receptors (discussed later).

An HCMV homologue of MHC-I. The first identified NK cell surrogate ligand was UL18, an HCMV-encoded MHC-I homologue that associates with peptide and β_2M[82–84]. UL18 binds to the inhibitory receptor leukocyte immunoglobulin-like receptor subfamily B member 1 (LIR1) with extremely high affinity, approximately 1000-fold tighter than that of its endogenous ligands, which include a range of classical and non-classical MHC-I molecules[85]. While surface expression of UL18 dampens LIR1$^+$ NK cell-mediated cytotoxicity[86,87] in line with its proposed function as an immunoevasin, UL18 can enhance NK cell killing[88], although this latter effect may be LIR1 independent[86]. Despite its structural similarity to MHC-I[89] and its dependence on TAP for surface expression[90], UL18 is resistant to the action of the US6 family of immunoevasins described earlier[91], perhaps due to its extensive glycosylation state, which is considered to shield most of the protein surface from unwanted interactions[89]. Intriguingly, UL18 counteracts US6-mediated TAP blockade, thereby allowing it to

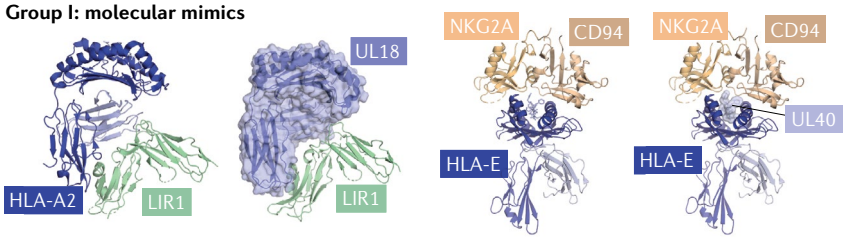
access TAP-dependent peptides[90]. However, at the same time, UL18 interferes with the physical association of MHC-I with TAP to maintain the blockade of MHC-I peptide loading.

MCMV and rat CMV surrogate ligands. Other CMV species use similar strategies to target distinct receptor systems that exist within their hosts. For example, MCMV encodes an MHC-I-like protein, m157, which

Box 2 | The molecular basis for viral immune evasion

Here, we assign immunoevasins into one of three separate groups on the basis of whether their fold and/or binding site is conserved with that of the endogenous ligand (see the figure).

Group I: molecular mimics



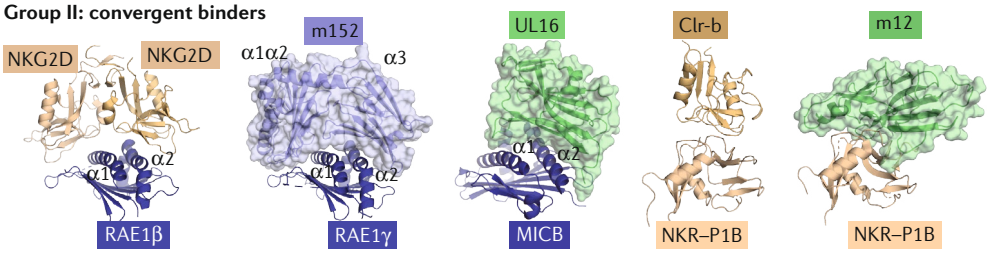
Group 1: molecular mimics

The molecular mimics are immunoevasins whose fold and docking mode are similar to those of the endogenous ligand (see the figure). This group includes the human cytomegalovirus-encoded MHC class I (MHC-I)-like protein UL18, which despite sharing only ~25% sequence identity with MHC-I, binds to the receptor leukocyte immunoglobulin-like receptor subfamily B member 1 (LIR1) in an almost identical manner to that of HLA-A2 (REF.[89]). However, subtle differences within UL18 at the LIR1-binding interface result in an interaction that is of substantially higher affinity[85], which might allow only minute quantities of UL18 to exert a robust immunomodulatory effect. Although the structure of the UL40 signal peptide has not been determined per se, we have also included UL40 in this group since it differs from the HLA-G peptide that was present in the CD94–NKG2A–HLA-E structure by only a single residue[210].

Group 2: convergent immunoevasins

Convergent immunoevasins are structurally unrelated to the endogenous ligand but use the same binding site (see the figure).

Group II: convergent binders

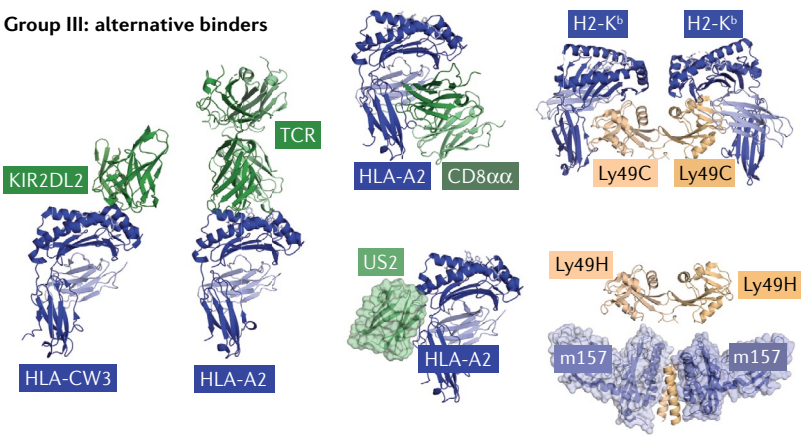


For example, while the two C-type lectin-like domains of the NKG2D homodimer sit on top of the α1 and α2 domains of its self-ligands, respectively[211–214], m152 binds with comparable affinity to a similar site on retinoic acid early-inducible protein 1γ (RAE1γ), despite being a monomeric MHC-I-like molecule[215]. To achieve this, m152 lies across RAE1γ such that its α1α2 platform-like and α3-like domains occupy positions similar to those of each of the NKG2D monomers. In addition, the Ig-like molecule UL16 occupies only one of the NKG2D-binding sites,yet binds with high affinity to MHC-I polypeptide-related sequence B (MICB), UL16-binding protein 1 (ULBP1) and ULBP2 (REF.[216]). Thus, two structurally diverse immunoevasins encoded by distinct viral species have evolved to target the same binding site as NKG2D, but do so using completely unrelated structural scaffolds. Also within this class is the Ig-like m12 immunoevasin, which engages the same surface on NKR-P1B as the endogenous C-type lectin-like ligand Clr-b[52]. However, despite their similar binding site, recent data indicate that m12 and Clr-b recognition are governed by quite distinct mechanisms[217]. More specifically, while m12 uses a 'polar claw'-style docking mode that permits a high-affinity interaction with NKR-P1B, Clr-b binding is extremely weak and requires additional avidity effects conferred by oligomerization of NKR-P1B[217].

Group 3: alternative binding

The third group of immunoevasins possess folds that are similar to those of the endogenous binding partners of their targets but recognize a completely different (alternative) binding site (see the figure). For example, although a variety of Ig-based receptors bind to MHC-I either above (for example, the T cell receptor (TCR)[56] or killer cell immunoglobulin-like receptors (KIR)[175]) or below (for example, CD8)[218] the peptide-binding platform, US2 targets a distinct site located at the junction of the peptide-binding groove and the α3 domain[39]. Even more remarkable is the docking mode of m157, which despite adopting an MHC-I-like fold, does not bind to the C-type lectin domain of Ly49 receptors, but instead 'tackles its legs' by targeting an aromatic peg motif located within the helical Ly49 stalk[162]. These examples in particular, highlight the difficulties associated with drawing conclusions regarding an immunoevasin's mechanism of action from sequence or structural similarities.

Group III: alternative binders



In the figure, where available, structures of CMV immunoevasins bound to their molecular targets are shown next to the corresponding endogenous receptor–ligand interaction. Host-encoded molecules are shown as ribbons and immunoevasins are depicted with a transparent surface. Molecules are coloured according to their fold: blue (MHC-like), green (immunoglobulin-like), wheat (C-type lectin-like). Structures were derived from the following Protein Data Bank entries: 3D2U (UL18–LIR1), 1P7Q (HLA-A2–LIR1), 3CDG (CD94–NKG2A–HLA-E), 4G59 (RAE1γ–m152), 2WY3 (MICB–UL16), 4PP8 (RAE1β–NKG2D), 5TZN (NKR-P1B–m12), 6E7D (NKR-P1B–Clr-b), 1IM3 (HLA-A2–US2), 1EFX (HLA-CW3–KIR2DL2), 1AKJ (HLA-A2–CD8), 1BD2 (HLA-A2–TCR), 4JO8 Ly49H–m157) and 3C8K (Ly49C–H2-Kᵇ). The position of the Ly49H lectin-like domain within the Ly49H–m157 complex has been modelled on the basis of available data.

Table 1 | **Summary of CMV immunoevasins that manipulate immune responses**

Immunoevasin	CMV species	Fold	Target	Mechanism	Refs
Modulation of antigen presentation					
US2[a]	HCMV	Ig	MHC-I, MHC-II, HFE, CD1d	Proteasomal degradation	65,69,71–73
US3	HCMV	Ig	MHC-I–tapasin	ER retention	77
US6	HCMV	Ig	MHC-I–TAP	Prevents peptide translocation into the ER	80,81
US10	HCMV	Ig	MHC-I, HLA-G	Delayed maturation of MHC-I, degradation of HLA-G	74,75
US11	HCMV	Ig	MHC-I	Proteasomal degradation	37,61,62,64,67
pp71 (UL82)	HCMV	Unknown	MHC-I	May block surface expression	202
pp65 (UL83)[a]	HCMV	Tegument protein	MHC-II	Lysosomal degradation	203
miR-376a[a]	HCMV	MicroRNA	HLA-E	Blocks surface expression	204
miR-US4-1	HCMV	MicroRNA	ERAP1	Blocks processing of viral peptides	205
m06	MCMV	Ig-like	MHC-I	Lysosomal degradation	59
m152[a]	MCMV	MHC-like	MHC-I	ER retention	50,83
Rh178	RhCMV	Unknown	MHC-I	Blocks translation	48
Surrogate ligands for inhibitory receptors					
UL18	HCMV	MHC-like	LIR1	Direct binding	89–91,93
UL40[b]	HCMV	Peptide	CD94–NKG2A, LIR1	Promotes surface expression of HLA-E and UL18	103–107,206
m04[b]	MCMV	Ig-like	MHC-I	Escorts MHC-I to the cell surface	55,58,110
m12[b]	MCMV	Ig-like	NKR-P1A, NKR-P1B, NKR-P1C	Direct binding	57
m157[b]	MCMV	MHC-like	Ly49C, Ly49I, Ly49H	Direct binding	54,161,162
RCTL[b]	RCMV	Lectin-like	NKR-P1A, NKR-P1B	Direct binding	100
Prevention of activating receptor signalling					
US2[a]	HCMV	Ig	Nectin 2	Proteasomal degradation	69
US9	HCMV	Ig	*MICA*008*	Proteasomal degradation of NKG2D ligand	119
US12	HCMV	7-TM	ULBP2	Downregulation of NKG2D ligands	36
US13	HCMV	7-TM	MICA, MICB, ULBP2	Downregulation of NKG2D ligands	36
US18	HCMV	7-TM	MICA, B7-H6	Lysosomal degradation of activating receptor ligands	36,46,146
US20	HCMV	7-TM	MICA, MICB, B7-H6, ULBP2	Lysosomal degradation of activating receptor ligands	36,46,146
UL16	HCMV	Ig-like	MICB, ULBP1, ULBP2, ULBP6	Intracellular retention of NKG2D ligands	120,207,208
UL141[a]	HCMV	Ig-like	Nectin 2/nectin-like protein 5	ER retention of activating receptor ligands	40,41,132
UL142	HCMV	MHC-like	MICA, ULBP3	Intracellular retention of NKG2D ligands	117,209
UL148A	HCMV	Unknown	MICA	Lysosomal degradation of NKG2D ligand	118
pp65 (UL83)	HCMV	Potential dUTPase	NKp30	Dissociates CD3ζ adaptor module	145
miR-UL112	HCMV	microRNA	MICB	Downregulates expression of NKG2D ligand	121
m20.1	MCMV	Unknown	Nectin-like protein 5	ER retention and degradation of DNAM1 ligand	130
m138[a]	MCMV	Ig-like	H60, MULT1, B7-1, RAE1ε	Endocytosis and lysosomal degradation of NKG2D ligands	124,126,210
m145	MCMV	MHC-like	MULT1	Blocks surface expression of NKG2D ligand	52
m152[a]	MCMV	MHC-like	RAE1	ER retention of NKG2D ligand	189
m154	MCMV	MHC-like	CD48	Proteasomal and lysosomal degradation of 2B4 ligand	141
m155	MCMV	MHC-like	H60	Redirection to proteasome of NKG2D ligand	53
Rh159	RhCMV	Unknown	MICB, MICA	Retention of NKG2D ligands	211
A43	OMCMV	Ig	2B4	Binds to 2B4 and blocks its interaction with CD48	143

Table 1 (cont.) | **Summary of CMV immunoevasins that manipulate immune responses**

Immunoevasin	CMV species	Fold	Target	Mechanism	Refs
Fc receptor decoys					
RL11	HCMV	Ig-like	IgG1–IgG4	Block FcγR activation and ADCC	151,153
UL119	HCMV	Ig-like	IgG1–IgG4	Block FcγR activation and ADCC, lysosomal degradation	151,155,156
RL12	HCMV	Ig-like	IgG1, IgG2	Binds to Fcγ	152
RL13	HCMV	Ig-like	IgG1, IgG2	Internalizes Fcγ to endosomes	152
m138[a]	MCMV	Ig-like	IgG	Binds to cell surface Fcγ	157
Others					
US2[a]	HCMV	Ig	Integrin-α	Degradation	69
UL11	HCMV	Ig-like	CD45	Direct binding	212,213
UL141[a]	HCMV	Ig-like	TRAILR1 and TRAILR2	Retains death receptors in the ER to prevent apoptosis	40
m166	MCMV	ND	TRAIL	Suppresses TRAIL death receptor expression	214

7-TM, seven-transmembrane; ADCC, antibody-dependent cellular cytotoxicity; CMV, cytomegalovirus; DNAM1, DNAX accessory molecule 1; dUTPase; deoxyuridine triphosphatase; ER, endoplasmic reticulum; ERAP1, endoplasmic reticulum aminopeptidase 1; HCMV, human CMV; HFE, hereditary haemochromatosis protein; Ig, immunoglobulin; LIR1, leukocyte immunoglobulin-like receptor subfamily B member 1; MCMV, mouse CMV; MHC-I; MHC class I; MHC-II, MHC class II; MIC, MHC class I polypeptide-related sequence; MULT1, mouse UL16-binding protein-like transcript 1; ND, not defined; OMCMV, owl monkey CMV; RAE1ε, retinoic acid early-inducible protein 1ε; RCMV, rat CMV; RCTL, rat C-type lectin-like immunoevasin; RhCMV; rhesus CMV; TAP; peptide transporter involved in antigen processing; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; TRAILR, tumour necrosis factor-related apoptosis-inducing ligand receptor; ULBP, UL16-binding protein. [a]Immunoevasins that fit into more than one category. [b]Inhibitory receptor surrogate ligands that are also recognized by activating receptors.

manipulates the Ly49 family, the major class of MHC-I-binding NK cell receptors found in mice. Despite not binding to peptide or β_2M[92], m157 nevertheless is capable of engaging inhibitory Ly49C and Ly49I receptors in certain susceptible mouse strains[49] and this interaction dampens NK cell-mediated lysis (FIG. 2b). A similar function is performed by the m12 immunoevasin to subvert NKR-P1B-mediated missing-self recognition[52]. Here, m12 functions to replace the endogenous NKR-P1B ligand, Clr-b, whose expression is rapidly downregulated following MCMV infection[93] in part due to the action of the *ie3* gene product, m122, that represses the *Clec2d* promoter[94]. A parallel system also exists in rat cytomegalovirus, where a rat C-type lectin-like immunoevasin serves as a decoy ligand for the inhibitory receptor NKR-P1B to protect infected cells from NK cell attack[95]. While m12 is an immunoglobulin-like molecule, rat C-type lectin-like immunoevasin is encoded by a spliced C-type lectin-like gene with similar intron–exon structure to rodent *Clec2d* genes, and encodes a protein with 60% amino acid identity to rat Clr-b[96]. These examples highlight the versatility of the 'gene capture and adapt' approach that is one of the most prominent features of CMV immune evasion.

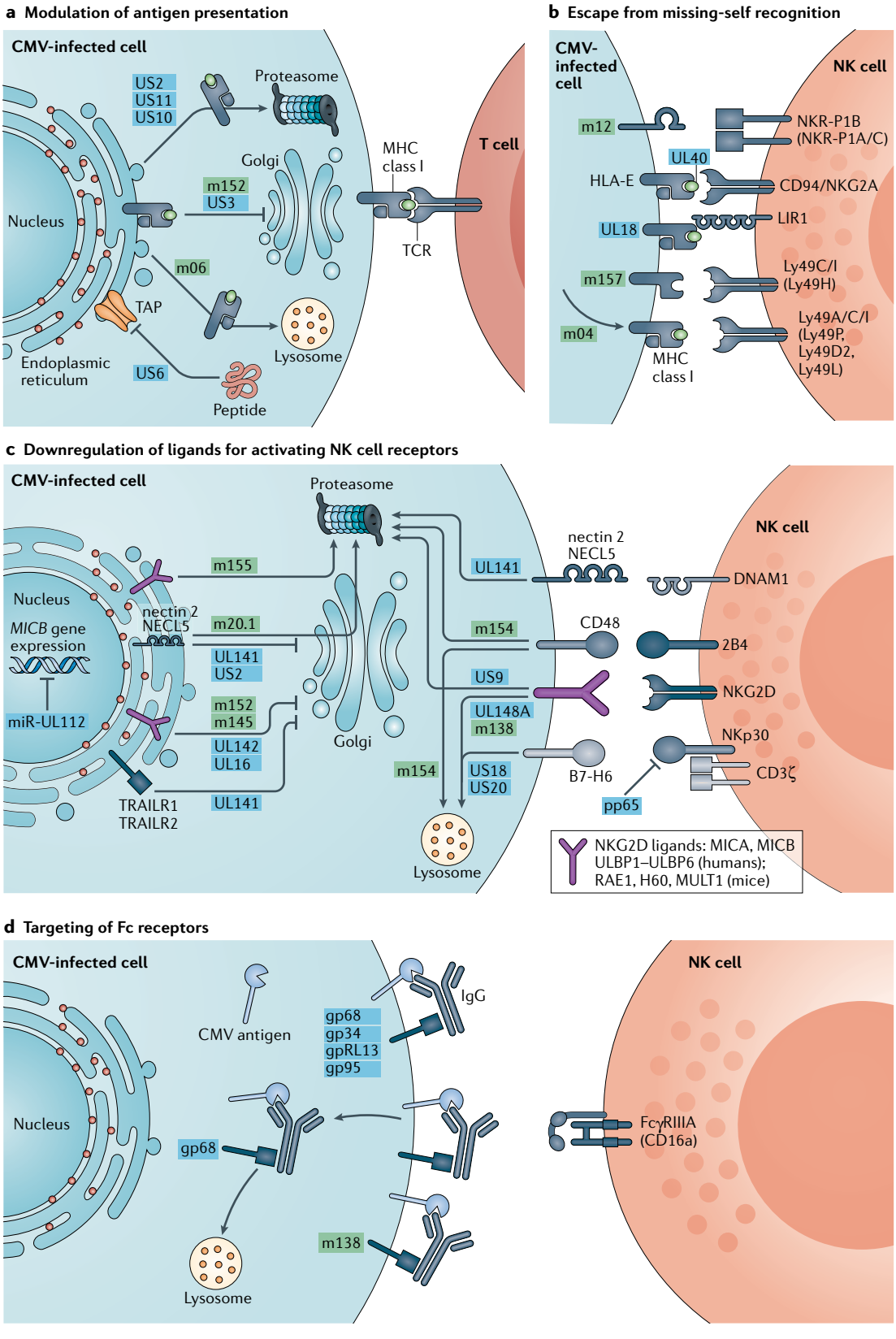
HCMV mimicry of an MHC-I signal peptide. In addition to receptors that recognize MHC-I directly, NK cells also monitor MHC-I surface expression indirectly via a heterodimer comprising CD94 coupled to an inhibitory or activating NKG2 family member[97]. CD94–NKG2 heterodimers bind to the non-classical MHC-I molecule HLA-E, which presents a nonamer peptide derived from the leader sequence of other MHC-I molecules[98]. Accordingly, downregulation of classical MHC-I ablates recognition of HLA-E by CD94–NKG2, triggering NK cell activation. However, the signal peptide of the HCMV-encoded UL40 glycoprotein is either identical or very similar in sequence to the endogenous HLA-E ligand[99,100] (FIG. 2b). Indeed, several groups have demonstrated that UL40 triggers enhanced expression of HLA-E that protects infected cells from NK cell-mediated lysis via interactions with the CD94–NKG2A inhibitory receptor[99–103]. Importantly, unlike conventional HLA-E peptides, loading of the UL40 signal peptide onto HLA-E is TAP independent[99], thereby allowing HLA-E surface expression to be maintained or even upregulated despite the action of US6. However, although UL40 can effectively bypass CD94–NKG2A-mediated missing-self reactivity, some $CD8^+$ T cells are able to distinguish between the subtle changes evident between these self and non-self peptides[104,105].

In addition to encoding viral surrogate ligands, MCMV also seeks to avoid missing-self recognition by 'rescuing' some MHC-I molecules from m06-mediated degradation. In particular, the m04 glycoprotein forms complexes with β_2M-associated MHC-I in the ER and escorts them to the cell surface, where they can be engaged by inhibitory Ly49 receptors[53,106] (FIG. 2b). The interplay between m04 and m06 may be important in maintaining a 'goldilocks' balance of MHC-I on the cell surface; sufficient quantities to overcome missing-self recognition but not enough to trigger a T cell response. m04 is highly variable in sequence among field isolates[107], but nevertheless binds to a broad range of mouse MHC-I molecules via its immunoglobulin-like domain[50]. However, MHC-I–m04 complexes are exported from the ER only in the presence of an additional viral factor, MATp1 (REF.[108]). Notably, MCMV-infected cells do not trigger NK cell activation despite having dramatically diminished MHC-I surface levels, indicating that inhibitory Ly49 receptors are triggered more strongly by m04–MHC-I complexes than by MHC-I alone[53]. These results imply that NK cell inhibition can be achieved through complex formation between MHC-I and viral protein(s)

rather than by peptides or pathogen-encoded self-like decoy molecules, and may explain the long-standing puzzle why licensed NK cells that are sensitive to changing levels of MHC-I are still inhibited in many mouse strains on MCMV infection[109].

Downregulation of activating ligands

Missing-self recognition is not the only mechanism by which NK cells detect infection. Indeed, NK cells express various activating receptors that recognize self-molecules whose expression is low or absent under



normal homeostatic conditions but becomes upregulated on cellular stress. This type of 'induced-self' recognition is a key mechanism by which NK cells detect viral infection. As such, CMV devotes considerable resources towards interfering with this process, predominantly via the downregulation of stress-induced ligands (FIG. 2c).

NKG2D. One of the most prominent receptors for 'induced-self' ligands in mice and humans is NKG2D, a homodimeric C-type lectin-like activating receptor that is expressed on NK cells and some types of T cells. The significance of NKG2D signalling in the control of CMV infection is best illustrated by the fact that both MCMV and HCMV downregulate surface expression of NKG2D ligands in a systematic and redundant manner[110]. Thus, although CMV infection triggers a dramatic upregulation in the transcription of genes encoding NKG2D ligands, increased NKG2D ligand surface expression is not detected on infected cells, and mouse strains that harbour intact NK cells expressing NKG2D fail to control MCMV infection during the acute stage[10]. However, deletion of any of the MCMV immunoevasins described below sensitizes the virus to NK cell-dependent control in vivo[111].

NKG2D ligands are distantly related to MHC-I and include MHC-I polypeptide-related sequence A (MICA), MICB and the UL16-binding proteins (ULBP1–ULBP6) in humans[112]. HCMV dampens the cell surface expression of all of these ligands throughout both early and late stages of infection via the combined action of several immunoevasin proteins. For example, US9, US18, US20, UL142 and UL148A can target various MICA allomorphs, either through promoting their degradation within the lysosome or proteasome or by retaining them in the *cis*-Golgi compartment[41,113–116] (FIG. 2c). In addition, UL16 retains MICB, ULBP1 and ULBP2 within the ER and *cis*-Golgi compartment[117]. As well as through protein-based immunoevasins, HCMV also downregulates MICB via a virally encoded microRNA sequence, miR-UL112, that binds to the 3′ untranslated region of the MICB mRNA to dampen gene expression[118].

In mice, the NKG2D ligands include mouse UL16-binding protein-like transcript 1 (MULT1), minor histocompatibility protein 60 (H60A–H60C) and five members of the retinoic acid early-inducible protein 1 family (RAE1α–RAE1ε)[119]. Each of these are also targeted by MCMV immunoevasins, primarily those encoded within the m145 superfamily of MHC-I-like molecules (FIG. 2c). For example, m145 dampens surface expression of MULT1 by an unknown mechanism[47], whereas m152 downregulates RAE1 by retaining it in the ER–Golgi intermediate compartment and *cis*-Golgi compartment[120]. Notably, not all RAE1 isoforms are equally prone to downregulation by m152 (REF.[121]). In particular, RAE1δ is a more resistant form, which may be because this isoform lacks the PLWY motif that is found in RAE1α, RAE1β, and RAE1γ[122]. In contrast, m155 targets H60 after it exits from the ER–Golgi intermediate compartment/*cis*-Golgi compartment[46] via a proteasome-dependent mechanism[48]. Downregulation of NKG2D ligands by MCMV is not limited to MHC-I-like immunoevasins however. Other studies have shown that an immunoglobulin-based MCMV immunoevasin, m138, downregulates surface levels of H60 and MULT1, in the latter case via interference with clathrin-mediated recycling, resulting in degradation of MULT1 within lysosomes[123].

DNAX accessory molecule 1. DNAX accessory molecule 1 (DNAM1; also known as CD226) is an immunoglobulin-based activating receptor that is expressed on the surface of mouse and human NK cells and T cells, as well as on human $CD4^+$ T cells and monocytes[124]. DNAM1 recognizes certain nectin and nectin-like adhesion molecules, including nectin 2 (also known as CD112) and nectin-like protein 5 (NECL5; also known as CD155 and PVR)[125,126]. In a similar fashion to the NKG2D ligands, *NECL5* gene transcription is upregulated upon MCMV[127] and HCMV[128] infection, potentially as a side effect of a strong transactivating activity of the IE1 and IE2 proteins that are required for productive viral replication[128]. However, despite this, nectin 2 and NECL5 surface expression is suppressed upon HCMV and MCMV infection[127,129]. In HCMV, these effects have been attributed to UL141, which curiously downregulates these closely related molecules by distinct mechanisms, namely by retaining the immature form of NECL5 in the ER[130] while instead targeting nectin 2 for proteasomal-mediated degradation[129] (FIG. 2c). The multitasking nature of UL141 is further highlighted by its capacity to use a distinct molecular surface to bind and retain the death receptors tumour necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAILR1) and TRAILR2 in the ER, thereby preventing apoptosis and allowing survival of virus-infected cells[131,132]. Nectin 2 is also downregulated by another multifunctional immunoevasin, US2, which acts in concert with UL141 to retain nectin 2 in the ER and promote its translocation to the cytosol and degradation[65].

In contrast, the MCMV molecule m20.1 is solely responsible for NECL5 downregulation in mice, while another currently unidentified viral mediator targets nectin 2 (REF.[127]). Importantly, the inhibitory receptors CD96 and TIGIT compete with DNAM1 for binding to NECL5 (REFS[133–137]). Therefore, the ultimate effect on immune cell activation is dependent on the relative expression levels of each receptor and ligand, and their relative affinity. Within this context, it is interesting that mutant viruses lacking m20.1 were attenuated in vivo, indicating that the activating DNAM1 receptor plays a dominant role[127]. These effects were abolished by

◀ Fig. 2 | **Overview of CMV immune evasion strategies.** Representations of immunoevasins that modulate antigen presentation (part **a**), mediate escape from missing-self recognition (part **b**), downregulate ligands for activating natural killer (NK) cell receptors (part **c**) and inhibit Fc receptor signalling (part **d**) are provided. Host proteins are coloured in grey and black. Human cytomegalovirus (CMV)-encoded and mouse CMV-encoded immunoevasins are indicated in blue and green labels, respectively. DNAM1, DNAX accessory molecule 1; LIR1, leukocyte immunoglobulin-like receptor subfamily B member 1; MIC, MHC class I polypeptide-related sequence; MULT1, mouse UL16-binding protein-like transcript 1; NECL5, nectin-like protein 5; RAE1, retinoic acid early-inducible protein 1; TAP, peptide transporter involved in antigen processing; TCR, T cell receptor; TRAILR, tumour necrosis factor-related apoptosis-inducing ligand receptor; ULBP, UL16-binding protein.

depletion of mononuclear phagocytes, which express high levels of the stimulatory DNAM1 receptor, but not the inhibitory receptors TIGIT and CD96.

NK cell receptor 2B4. A member of the SLAM family of receptors, 2B4 is a transmembrane protein that functions as an activating NK cell receptor in humans[138] and exhibits both inhibitory and activating properties in mice[139,140]. In CMV infection, 2B4 appears to serve mainly as an activating receptor, and in keeping with this, the m154 protein of MCMV targets the 2B4 ligand CD48 for degradation, most likely via both proteasome-mediated and lysosome-mediated mechanisms[141] (FIG. 2c). CD48 is also downregulated in HCMV-infected cells, suggesting the existence of a putative viral immune evasion strategy targeting the 2B4 pathway[142]. Recently, owl monkey CMV was found to encode a ligand for 2B4, A43, that exhibits high sequence identity to host CD48 (REF.[143]). Unusually, A43 is shed from the cell surface, allowing it to act as a soluble factor that binds and masks 2B4 to impede NK cell-mediated viral control.

Natural cytotoxicity receptors. Although natural cytotoxicity receptors (NCRs) are key receptors on NK cells, not much is known about their cellular ligands and whether they are regulated by CMV[144]. Of the three human NCRs (NKp30, NKp44 and NKp46), only NKp30 is known to be targeted by CMV-encoded immunoevasins. Namely, HCMV tegument protein pp65 binds NKp30, leading to dissociation from its signalling adaptor, the CD3ζ chain, and resulting in the inhibition of NK cell cytotoxicity[145] (FIG. 2c). In addition, the HCMV proteins US18 and US20 downregulate NKp30 ligand B7-H6 to compromise NK cell function[146]. Mice express only one NCR (NKp46) but very little is known about its cellular ligands. There is some evidence that MCMV downregulates NCR ligand expressed on MCMV-infected fibroblasts, but the viral inhibitor remains unknown[147].

Targeting of Fc receptors

NK cells also target infected cells by a process termed 'antibody-dependent cellular cytotoxicity'. Here, FcRs — typically FcγRIIIa (also known as CD16a) on NK cells — recognize the Fc region of IgG bound to infected cells and stimulate an effector immune response[148]. However, CMV reinfection of seropositive hosts occurs, despite the presence of high levels of CMV-specific IgG[149], suggesting the existence of mechanisms to overcome the antiviral activity of protective antibodies. Indeed, several CMV immunoevasins have been reported to bind to the Fc region of IgG and prevent triggering of host FcγR (FIG. 2d).

The ability of HCMV-infected cells to bind to the Fcγ chain of IgG was first described more than 40 years ago[150]. To date, four HCMV glycoproteins have been shown to exhibit Fcγ-binding capacity, three of which (gp34, gpRL13 and gp95) are encoded within the RL11 gene cluster, while the fourth, gp68, originates from a spliced mRNA that spans the *UL119–UL118* gene region[151–153]. All four of these proteins are heavily glycosylated type I integral membrane proteins that possess an amino-terminal extracellular immunoglobulin-like domain that confers a particular pattern of Fcγ binding[154]. For example, gp34 and gp68 are specific for IgG, but do not appear to distinguish between subtypes[151], whereas gpRL13 and gp95 recognize only IgG1 and IgG2 (REF.[152]). Studies on gp34 and gp68 indicate that the manner in which these molecules recognize Fcγ is likely to be distinct from the host FcγR–Fcγ interaction. In particular, gp68 is considered to bind to Fc at the interface between the C_H2 and C_H3 domains in a 2:1 stoichiometry, whereas host FcγRIII engages the C_H1–C_H2 hinge and C_H2 domain in a 1:1 binding mode[155]. Moreover, unlike host FcRs, the interaction of gp34 and gp68 with Fcγ is independent of the N-linked glycosylation status of IgG[155]. Thus, the viral FcRs may function via a mechanism that does not involve direct competition for Fcγ binding with their host counterparts. Consistent with this proposition, binding of gp68 to Fcγ results in endocytosis of the entire antigen–gp68–Fcγ complex and targeting of all the components to lysosomes, presumably for degradation[156]. In this regard, it is noteworthy that the different viral FcRs possess distinct endosomal trafficking motifs within their cytoplasmic tails, including a putative dileucine consensus motif in gp34 and a YxxL motif in gpRL13 (REF.[151]), while gp69 instead harbours a potential immunoreceptor tyrosine-based inhibition motif.

In contrast to HCMV, only one immunoevasin that targets IgG-mediated immune protection has been identified in MCMV. Here, the early expressed cell surface glycoprotein m138 binds to Fcγ at the cell surface[157], although the attenuated replication of mutant MCMV lacking m138 may instead be due to its capacity to downregulate NKG2D ligands (described earlier), since these effects were also evident in mice lacking antibodies[158]. Recently, in rhesus CMV, an additional RL11 family member, *Rh05*, was identified that encodes a unique type I transmembrane glycoprotein that antagonizes host FcγR activation[159], although its mechanism of action remains to be determined.

Countermeasures for host protection

Due to their extended lifespan, relatively slow mutational rate and limited capacity to acquire new genes, it is incredibly difficult for individual hosts to respond to the ever-increasing repertoire of immunoevasins they will encounter. Nevertheless, some limited examples (discussed below) highlight the intrinsic adaptability of the host in the face of this assault.

The evolutionary response of the host to immunoevasins is perhaps best understood in the context of surrogate ligands for inhibitory receptors, which appears to have triggered the emergence of activating receptors that typically do not recognize any host ligand but are specific for their cognate viral target[160] (FIG. 2b). From an evolutionary standpoint, this strategy is rather elegant, requiring only minor modifications to existing receptors, namely loss/ablation of inhibitory signalling motifs combined with the acquisition of a single transmembrane charged residue to mediate association with a signalling adaptor, thereby providing a likely explanation as to why it has been adopted in different species (mice and humans) and in distinct receptor systems, spanning Ly49, NKR-P1 and CD94–NKG2.

Within the mouse NKR-P1 receptor axis, the stimulatory NKR-P1A and NKR-P1C (NK1.1) receptors directly recognize the MCMV-encoded molecule m12, and this interaction can counteract the immunoevasin function of m12 both in vitro and in vivo[52]. Similarly, in resistant C57BL/6 mice, the activating Ly49H receptor targets the m157 immunoevasin and confers dominant resistance to MCMV even in the presence of inhibitory signals derived from co-engagement of the Ly49C receptor[49,161,162]. Here, Ly49H–m157 engagement results in the induction of IFNγ and other activating cytokines and chemokines[163,164], leading to efficient MCMV control in C57BL/6 mice[49] and the formation of memory-like NK cells (reviewed in REF.[16]). While Ly49H expression is restricted to just a single mouse strain, a variety of activating Ly49 receptors have emerged in other strains of mice (Ly49P in MA/My mice, Ly49L in BALB mice and Ly49D2 in PWK/Pas mice), where they trigger NK cell activation in a manner dependent on the precise MHC-I haplotype and the presence of the MCMV-encoded m04 glycoprotein[165,166]. Since m04 escorts newly assembled MHC-I molecules to the cell surface, where they serve as ligands for inhibitory Ly49 receptors[53], this mechanism presumably arose to allow detection of infected cells that would otherwise bypass missing-self recognition. Importantly, these activating Ly49 receptors do not normally bind to self MHC-I, and the mechanism by which m04 confers this capacity is still unclear[166], although it does require another viral protein, MAT1p[108]. A related mechanism is also evident in the human CD94–NKG2–HLA-E system, where a subset of adaptive-like NK cells expressing the stimulatory CD94–NKG2C receptor are rapidly expanded on HCMV infection to permit recognition of UL40–HLA-E complexes[168]. The expansion and differentiation of these cells requires an inflammatory milieu[169,170] and is driven in part by the UL40 signal peptide, being exquisitely sensitive to even single-residue substitutions in the HLA-E binding sequence[169].

An overarching theme in each of these systems is that the viral ligand is subjected to intense immune pressure that drives the selection of polymorphisms that reduce/abolish binding to activating receptors while maintaining the interaction with their inhibitory counterparts. For example, sequencing of UL40 isolated from haematopoietic stem cell transplant recipients experiencing HCMV reactivation revealed UL40-encoded peptides harbouring polymorphisms that retained the capacity to inhibit target cell lysis via CD94–NKG2A, but had a diminished ability to activate NK cells via CD94–NKG2C[102]. Moreover, passage of MCMV through Ly49H$^+$ mice results in rapid emergence of m157 escape mutants that no longer bind the Ly49H receptor[161,171,172]. Likewise, in mixed infections, viral strains expressing m157 are dominated by strains that escape Ly49H-mediated NK cell control[173]. Similarly to m157, the sequences of m04 and m12 are also highly variable among wild-derived isolates[52,107].

Although this cut and thrust exchange of evolution and counterevolution appears to have occurred within the context of CMV and multiple independent receptor systems, it is curious that no immunoevasin identified to date has been reported to target the killer cell immunoglobulin-like receptors (KIRs), which constitute a major class of MHC-I-binding receptors in humans. This is particularly surprising given that the KIRs display all the hallmarks that indicate such an evolutionary history: namely (1) they are a paired receptor family comprising inhibitory and activating receptors; (2) the ligands for inhibitory KIR (HLA) are downregulated on CMV infection; (3) both activating and inhibitory KIRs are highly polymorphic and many of the polymorphisms lie outside of the HLA-binding site[174]; and (4) many activating KIRs do not bind host ligands and remain orphans[175]. Indeed, epidemiological studies indicate that some KIR alleles influence the outcome of certain viral infections, including HCMV infection[176,177], hepatitis C virus infection[178] and HIV infection[179].

In addition to the emergence of activating receptors, other simple genetic changes may assist the host in countering immunoevasin function. For example, the 008 allele of *MICA* has acquired a frameshift mutation within its transmembrane domain that results in a shortened, glycosylphosphatidylinositol-anchored form of the protein that is able to escape UL142-mediated downregulation[113,114,180,181]. Notably, this truncated *MICA*008* allele has become highly prevalent in the human population[182–184], suggesting that this escape variant has been positively selected. However, a recent report indicates that these unique features of *MICA*008* allow it to be specifically targeted for proteasomal degradation by the US9 protein of HCMV[116], highlighting the adaptability and versatility of the virus in responding to modifications in the host immune recognition apparatus.

Conclusions and future directions

Over the last 20 years, the impressive array of immunoevasins identified in CMV species has firmly established these viruses as a paradigm for immune evasion. Indeed, it appears that CMV in particular may have played a significant role in shaping the mammalian immune system, as evidenced by the increasing repertoire of CMV-encoded molecules that have been identified to constitute ligands for previously orphan immune receptors[49,52,166]. However, the capacity to evade host immunity is a property associated with many diverse viruses, particularly among the herpesvirus family, which includes Epstein–Barr virus, varicella zoster virus, Kaposi sarcoma-associated herpesvirus, herpes simplex virus 1 and herpes simplex virus 2. Comparison of the molecules and pathways targeted by these herpesviruses and the strategies that they use reveals many similarities to those described herein for CMV. For example, all classes of herpesviruses downregulate MHC-I[185] and NKG2D[186] ligands, further highlighting the important role that T cell-mediated and NK cell-mediated immunity have in controlling persistent herpesvirus infections.

Although the strategies used to subvert host immunity are often conserved with those identified in CMV, some interesting mechanistic differences have also become apparent. For example, whereas the US6 protein of CMV binds to the ER luminal portion of TAP,

herpes simplex virus 1 encodes a small protein ICP47 that binds with high affinity to the inside of the TAP pore on the cytosolic side, thereby precluding peptide binding and freezing TAP in an inactive inward-facing conformation[187,188]. Similarly, like US3, the adenoviral protein E3-19K interferes with the capacity of tapasin to link TAP to MHC-I, but it does so by binding to TAP rather than tapasin[189]. In the future it will be important to further probe the biology surrounding other herpesvirus immunoevasins alongside those of CMV.

The potential benefits of studying viral immunoevasins extend beyond understanding a key viral–host interface. For example, the capacity to bypass the immune system is now considered a defining hallmark of cancer. Accordingly, understanding how viruses dampen immunity could provide insights into future strategies for cancer immunotherapy. Indeed, several established immunoevasin targets have recently been identified to play a role in antitumour immunity[190–192]. On a more basic level, investigations into viral immune escape strategies have already yielded important mechanistic insights into several fundamental biological processes and immunoevasins have proved invaluable research tools to interrogate immune cell function. For example, E3-19K played a vital role in the discovery that the association of MHC-I with peptide occurs within the ER[193], while investigations focused on US2 and US11 identified key components of the machinery involved in the translocation of misfolded proteins from the ER to the cytosol[61,64]. More recently, ICP47 was pivotal in stabilizing TAP, thereby allowing structural determination of this peptide transporter and subsequently the entire peptide loading complex by cryogenic electron microscopy[72,188], while studies using m12 have demonstrated that innate lymphoid cells can exhibit antigen-specific memory features[194].

There is also a growing appreciation that viral immunoevasins might be a potentially lucrative source of selective and potent 'ready-made' immunomodulatory molecules that could have therapeutic applications in situations where it is desirable to dampen the immune system, namely excessive inflammation, autoimmunity or transplantation. At this stage the validity of this approach has been tested in a range of animal models and has primarily been focused on cytokine inhibitors and/or chemokine mimetics derived from herpesviruses, myxoma or cowpox[195]. It will be interesting to see whether such strategies can be expanded to include the CMV-encoded immunoevasins described herein. The use of viral immunoevasins has also attracted interest in the field of oncolytic viruses, which are emerging as a promising strategy for the treatment of cancer due to their ability to selectively replicate in and kill cancer cells. Here, genetic incorporation of HCMV-encoded UL141, which downregulates DNAM1 ligands, into a recombinant vesicular stomatitis virus vector reduced clearance by the immune system, resulting in enhanced tumour killing and increased survival in a mouse model of hepatocellular carcinoma[196]. Viral immunoevasins could also impact the field of xenotransplantation, where robust human immune responses to animal donor organs are a major obstacle limiting clinical applicability. In this context, retroviral expression of the LIR1 decoy ligand UL18 in swine endothelial cells has been shown to significantly reduce their lysis and IFNγ production by human NK cells[197]. While clear challenges remain, particularly in regard to potential immunogenicity of any viral-based reagents, translating our knowledge regarding mechanisms of viral immune escape should be a major focus of future research.

Published online 30 October 2019

1. Murphy, E. & Shenk, T. Human cytomegalovirus genome. *Curr. Top Microbiol. Immunol.* **325**, 1–19 (2008).
2. Rawlinson, W. D., Farrell, H. E. & Barrell, B. G. Analysis of the complete DNA sequence of murine cytomegalovirus. *J. Virol.* **70**, 8833–8849 (1996).
3. Vink, C., Beuken, E. & Bruggeman, C. A. Complete DNA sequence of the rat cytomegalovirus genome. *J. Virol.* **74**, 7656–7665 (2000).
4. Swinkels, B. W., Geelen, J. L., Wertheim-van Dillen, P., van Es, A. A. & van der Noordaa, J. Initial characterization of four cytomegalovirus strains isolated from chimpanzees. Brief report. *Arch. Virol.* **82**, 125–128 (1984).
5. Powers, C. & Fruh, K. Rhesus CMV: an emerging animal model for human CMV. *Med. Microbiol. Immunol.* **197**, 109–115 (2008).
6. Babic, M., Krmpotic, A. & Jonjic, S. All is fair in virus-host interactions: NK cells and cytomegalovirus. *Trends Mol. Med.* **17**, 677–685 (2011).
7. Cannon, M. J., Schmid, D. S. & Hyde, T. B. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev. Med. Virol.* **20**, 202–213 (2010).
8. Boppana, S. B. & Britt, W. J. in *Cytomegaloviruses: from Molecular Pathogenesis to Intervention* Vol. 2 (ed. Reddehase M. J.) (Caister Academic Press, 2013).
9. Manicklal, S., Emery, V. C., Lazzarotto, T., Boppana, S. B. & Gupta, R. K. The "silent" global burden of congenital cytomegalovirus. *Clin. Microbiol. Rev.* 26, 86–102 (2013).
10. Scalzo, A. A., Corbett, A. J., Rawlinson, W. D., Scott, G. M. & Degli-Esposti, M. A. The interplay between host and viral factors in shaping the outcome of cytomegalovirus infection. *Immunol. Cell Biol.* **85**, 46–54 (2007).
11. Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P. & Salazar-Mather, T. P. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* **17**, 189–220 (1999).
12. Orange, J. S. Natural killer cell deficiency. *J. Allergy Clin. Immunol.* **132**, 515–525 (2013).
13. Stetson, D. B. et al. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* **198**, 1069–1076 (2003).
14. Fehniger, T. A. et al. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. *Immunity* **26**, 798–811 (2007).
15. Sun, J. C., Beilke, J. N. & Lanier, L. L. Adaptive immune features of natural killer cells. *Nature* **457**, 557–561 (2009).
16. Cerwenka, A. & Lanier, L. L. Natural killer cell memory in infection, inflammation and cancer. *Nat. Rev. Immunol.* **16**, 112–123 (2016).
17. Reddehase, M. J. Antigens and immunoevasins: opponents in cytomegalovirus immune surveillance. *Nat. Rev. Immunol.* **2**, 831–844 (2002).
18. Jonjic, S., Mutter, W., Weiland, F., Reddehase, M. J. & Koszinowski, U. H. Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. *J. Exp. Med.* **169**, 1199–1212 (1989).
19. Jonjic, S., Pavic, I., Lucin, P., Rukavina, D. & Koszinowski, U. H. Efficacious control of cytomegalovirus infection after long-term depletion of CD8+ T lymphocytes. *J. Virol.* **64**, 5457–5464 (1990).
20. Verma, S. et al. Cytomegalovirus-specific CD4 T cells are cytolytic and mediate vaccine protection. *J. Virol.* **90**, 650–658 (2016).
21. Jeitziner, S. M., Walton, S. M., Torti, N. & Oxenius, A. Adoptive transfer of cytomegalovirus-specific effector CD4+ T cells provides antiviral protection from murine CMV infection. *Eur. J. Immunol.* **43** (2013).
22. Walton, S. M. et al. Absence of cross-presenting cells in the salivary gland and viral immune evasion confine cytomegalovirus immune control to effector CD4 T cells. *PLOS Pathog.* **7**, e1002214 (2011).
23. Blyth, E. et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. *Blood* **121**, 3745–3758 (2013).
24. Lilleri, D. et al. Human cytomegalovirus-specific CD4+ and CD8+ T-cell reconstitution in adult allogeneic hematopoietic stem cell transplant recipients and immune control of viral infection. *Haematologica* **93**, 248–256 (2008).
25. Quinnan, G. V. et al. Cytotoxic t cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow-transplant recipients. *N. Engl. J. Med.* **307**, 7–13 (1982).
26. Reusser, P., Riddell, S. R., Meyers, J. D. & Greenberg, P. D. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. *Blood* **78**, 1373–1380 (1991).
27. Walter, E. A. et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N. Engl. J. Med.* **333**, 1038–1044 (1995).
28. Gabanti, E. et al. Human cytomegalovirus (HCMV)-specific CD4+ and CD8+ T cells are both required for prevention of HCMV disease in seropositive solid-organ transplant recipients. *PLOS ONE* **9**, e106044 (2014).

29. Gabanti, E. et al. Reconstitution of human cytomegalovirus-specific CD4+ T cells is critical for control of virus reactivation in hematopoietic stem cell transplant recipients but does not prevent organ infection. *Biol. Blood Marrow Transplant.* **21**, 2192–2202 (2015).
30. Gamadia, L. E. et al. Primary immune responses to human CMV: a critical role for IFN-gamma-producing CD4+ T cells in protection against CMV disease. *Blood* **101**, 2686–2692 (2003).
31. Andoniou, C. E. et al. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. *Nat. Immunol.* **6**, 1011–1019 (2005).
32. Andrews, D. M., Scalzo, A. A., Yokoyama, W. M., Smyth, M. J. & Degli-Esposti, M. A. Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* **4**, 175–181 (2003).
33. Andrews, D. M. et al. Innate immunity defines the capacity of antiviral T cells to limit persistent infection. *J. Exp. Med.* **207**, 1333–1343 (2010).
34. Dunn, W. et al. Functional profiling of a human cytomegalovirus genome. *Proc. Natl Acad. Sci. USA* **100**, 14223–14228 (2003).
35. Davison, A. J. et al. Homology between the human cytomegalovirus RL11 gene family and human adenovirus E3 genes. *J. Gen. Virol.* **84**, 657–663 (2003).
36. Fielding, C. A. *et al*. Control of immune ligands by members of a cytomegalovirus gene expansion suppresses natural killer cell activation. *eLife* **6**, e22206 (2017).
This study identifies the US12 family to be a major new hub of immune regulation.
37. Llano, M., Guma, M., Ortega, M., Angulo, A. & Lopez-Botet, M. Differential effects of US2, US6 and US11 human cytomegalovirus proteins on HLA class Ia and HLA-E expression: impact on target susceptibility to NK cell subsets. *Eur. J. Immunol.* **33**, 2744–2754 (2003).
38. Jones, T. R. et al. Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *J. Virol.* **69**, 4830–4841 (1995).
39. Gewurz, B. E. et al. Antigen presentation subverted: Structure of the human cytomegalovirus protein US2 bound to the class I molecule HLA-A2. *Proc. Natl Acad. Sci. USA* **98**, 6794–6799 (2001).
40. Sekulin, K., Gorzer, I., Heiss-Czedik, D. & Puchhammer-Stockl, E. Analysis of the variability of CMV strains in the RL11D domain of the RL11 multigene family. *Virus Genes* **35**, 577–583 (2007).
41. Fielding, C. A. et al. Two novel human cytomegalovirus NK cell evasion functions target MICA for lysosomal degradation. *PLOS Pathog.* **10**, e1004058 (2014).
42. Pande, N. T., Powers, C., Ahn, K. & Fruh, K. Rhesus cytomegalovirus contains functional homologues of US2, US3, US6, and US11. *J. Virol.* **79**, 5786–5798 (2005).
43. Powers, C. J. & Fruh, K. Signal peptide-dependent inhibition of MHC class I heavy chain translation by rhesus cytomegalovirus. *PLOS Pathog.* **4**, e1000150 (2008).
44. Revilleza, M. J. et al. How the virus outsmarts the host: function and structure of cytomegalovirus MHC-I-like molecules in the evasion of natural killer cell surveillance. *J. Biomed. Biotechnol.* **2011**, 724607 (2011).
45. Ziegler, H. et al. A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/cis-Golgi compartments. *Immunity* **6**, 57–66 (1997).
46. Hasan, M. et al. Selective down-regulation of the NKG2D ligand H60 by mouse cytomegalovirus m155 glycoprotein. *J. Virol.* **79**, 2920–2930 (2005).
47. Krmpotic, A. et al. NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145. *J. Exp. Med.* **201**, 211–220 (2005).
48. Lodoen, M. B. et al. The cytomegalovirus m155 gene product subverts natural killer cell antiviral protection by disruption of H60-NKG2D interactions. *J. Exp. Med.* **200**, 1075–1081 (2004).
49. Arase, H., Mocarski, E. S., Campbell, A. E., Hill, A. B. & Lanier, L. L. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296**, 1323–1326 (2002).
This study (and also reference 164) reports the first direct interaction between a viral ligand and an activating NK cell receptor.
50. Berry, R. et al. The structure of the cytomegalovirus-encoded m04 glycoprotein, a prototypical member of the m02 family of immunoevasins. *J. Biol. Chem.* **289**, 23753–23763 (2014).
51. Sgourakis, N. G. et al. The structure of mouse cytomegalovirus m04 protein obtained from sparse NMR data reveals a conserved fold of the m02-m06 viral immune modulator family. *Structure* **22**, 1263–1273 (2014).
52. Aguilar, O. A. et al. A viral immunoevasin controls innate immunity by targeting the prototypical natural killer cell receptor family. *Cell* **169**, 58–71 (2017).
This study describes the identification of m12 as a ligand for inhibitory and activating NKR-P1 receptors (including NK1.1).
53. Babic, M. et al. Cytomegalovirus immunoevasin reveals the physiological role of "missing self" recognition in natural killer cell dependent virus control in vivo. *J. Exp. Med.* **207**, 2663–2673 (2010).
54. Reusch, U. et al. A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation. *EMBO J.* **18**, 1081–1091 (1999).
55. Goodwin, C. M., Ciesla, J. H. & Munger, J. Who's driving? human cytomegalovirus, interferon, and NFkappaB signaling. *Viruses* **10**, E447 (2018).
56. Rossjohn, J. et al. T cell antigen receptor recognition of antigen-presenting molecules. *Annu. Rev. Immunol.* **33**, 169–200 (2015).
57. Ameres, S., Besold, K., Plachter, B. & Moosmann, A. CD8 T cell-evasive functions of human cytomegalovirus display pervasive MHC allele specificity, complementarity, and cooperativity. *J. Immunol.* **192**, 5894–5905 (2014).
58. Barel, M. T. et al. Amino acid composition of alpha1/alpha2 domains and cytoplasmic tail of MHC class I molecules determine their susceptibility to human cytomegalovirus US11-mediated down-regulation. *Eur. J. Immunol.* **33** (2003).
59. Barel, M. T. et al. Human cytomegalovirus-encoded US2 differentially affects surface expression of MHC class I locus products and targets membrane-bound, but not soluble HLA-G1 for degradation. *J. Immunol.* **171**, 6757–6765 (2003).
60. Wiertz, E. J. et al. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* **84**, 769–779 (1996).
61. Wiertz, E. J. et al. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. *Nature* **384**, 432–438 (1996).
This study and reference 60 provide the first mechanistic insight into how HCMV downregulates MHC-I surface expression.
62. Furman, M. H., Ploegh, H. L. & Tortorella, D. Membrane-specific, host-derived factors are required for US2- and US11-mediated degradation of major histocompatibility complex class I molecules. *J. Biol. Chem.* **277**, 3258–3267 (2002).
63. Lee, S. O. et al. Functional dissection of HCMV US11 in mediating the degradation of MHC class I molecules. *Biochem. Biophys. Res. Commun.* **330**, 1262–1267 (2005).
64. Lilley, B. N. & Ploegh, H. L. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* **429**, 834–840 (2004).
65. Hsu, J. L. et al. Plasma membrane profiling defines an expanded class of cell surface proteins selectively targeted for degradation by HCMV US2 in cooperation with UL141. *PLOS Pathog.* **11**, e1004811 (2015).
This study uses proteomics to reveal the full breadth of molecules that can be targeted by US2 and highlights how a single immunoevasin can modulate multiple immune-related pathways.
66. Vahdati-Ben Arieh, S. et al. A single viral protein HCMV US2 affects antigen presentation and intracellular iron homeostasis by degradation of classical HLA class I and HFE molecules. *Blood* **101**, 2858–2864 (2003).
67. Ben-Arieh, S. V. et al. Human cytomegalovirus protein US2 interferes with the expression of human HFE, a nonclassical class I major histocompatibility complex molecule that regulates iron homeostasis. *J. Virol.* **75**, 10557–10562 (2001).
68. Han, J. et al. Human cytomegalovirus (HCMV) US2 protein interacts with human CD1d (hCD1d) and down-regulates invariant NKT (iNKT) cell activity. *Mol. Cells* **36**, 455–464 (2013).
69. Tomazin, R. et al. Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by CD4+ T cells. *Nat. Med.* **5**, 1039–1043 (1999).
70. Park, B., Spooner, E., Houser, B. L., Strominger, J. L. & Ploegh, H. L. The HCMV membrane glycoprotein US10 selectively targets HLA-G for degradation. *J. Exp. Med.* **207**, 2033–2041 (2010).
71. Furman, M. H., Dey, N., Tortorella, D. & Ploegh, H. L. The human cytomegalovirus US10 gene product delays trafficking of major histocompatibility complex class I molecules. *J. Virol.* **76**, 11753–11756 (2002).
72. Blees, A. et al. Structure of the human MHC-I peptide-loading complex. *Nature* **551**, 525–528 (2017).
73. Park, B. et al. Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. *Immunity* **20**, 71–85 (2004).
74. Jones, T. R. et al. Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proc. Natl Acad. Sci. USA* **93**, 11327–11333 (1996).
75. Huard, B. & Fruh, K. A role for MHC class I down-regulation in NK cell lysis of herpes virus-infected cells. *Eur. J. Immunol.* **30**, 509–515 (2000).
76. Ahn, K. et al. The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. *Immunity* **6**, 613–621 (1997).
77. Lehner, P. J., Karttunen, J. T., Wilkinson, G. W. & Cresswell, P. The human cytomegalovirus US6 glycoprotein inhibits transporter associated with antigen processing-dependent peptide translocation. *Proc. Natl Acad. Sci. USA* **94**, 6904–6909 (1997).
78. Hewitt, E. W., Gupta, S. S. & Lehner, P. J. The human cytomegalovirus gene product US6 inhibits ATP binding by TAP. *EMBO J.* **20**, 387–396 (2001).
79. Ziegler, H., Muranyi, W., Burgert, H. G., Kremmer, E. & Koszinowski, U. H. The luminal part of the murine cytomegalovirus glycoprotein gp40 catalyzes the retention of MHC class I molecules. *EMBO J.* **19**, 870–881 (2000).
80. Ramnarayan, V. R. et al. Cytomegalovirus gp40/m152 uses TMED10 as ER anchor to retain MHC class I. *Cell Rep.* **23**, 3068–3077 (2018).
81. Karre, K., Ljunggren, H. G., Piontek, G. & Kiessling, R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* **319** (1986).
82. Beck, S. & Barrell, B. G. Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. *Nature* **331**, 269–272 (1988).
83. Browne, H., Smith, G., Beck, S. & Minson, T. A complex between the MHC class I homologue encoded by human cytomegalovirus and beta 2 microglobulin. *Nature* **347**, 770–772 (1990).
84. Fahnestock, M. L. et al. The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides. *Immunity* **3**, 583–590 (1995).
This study and references 82 and 83 describe the first identification of an HCMV-encoded MHC-I homologue that was subsequently shown to inhibit NK cell activation (Reyburn et al., 1997).
85. Chapman, T. L., Heikeman, A. P. & Bjorkman, P. J. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* **11**, 603–613 (1999).
86. Prod'homme, V. et al. The human cytomegalovirus MHC class I homolog UL18 inhibits LIR-1+but activates LIR-1- NK cells. *J. Immunol.* **178**, 4473–4481 (2007).
87. Reyburn, H. T. et al. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells. *Nature* **386**, 514–517 (1997).
This is the first description of an HCMV-encoded immunoevasin that subverts NK cell function.
88. Leong, C. C. et al. Modulation of natural killer cell cytotoxicity in human cytomegalovirus infection: the role of endogenous class I major histocompatibility complex and a viral class I homolog. *J. Exp. Med.* **187**, 1681–1687 (1998).
89. Yang, Z. & Bjorkman, P. J. Structure of UL18, a peptide-binding viral MHC mimic, bound to a host inhibitory receptor. *Proc. Natl Acad. Sci. USA* **105**, 10095–10100 (2008).
90. Kim, Y. et al. Human cytomegalovirus UL18 utilizes US6 for evading the NK and T-cell responses. *PLoS Pathog.* **4**, e1000123 (2008).
91. Park, B. et al. The MHC class I homolog of human cytomegalovirus is resistant to down-regulation mediated by the unique short region protein (US)2, US3, US6, and US11 gene products. *J. Immunol.* **168**, 3464–3469 (2002).
92. Adams, E. J. et al. Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors. *Proc. Natl Acad. Sci. USA* **104**, 10128–10133 (2007).

93. Aguilar, O. A. et al. Modulation of Clr ligand expression and NKR-P1 receptor function during murine cytomegalovirus infection. *J. Innate Immun.* **7**, 584–600 (2015).
94. Kirkham, C. L. et al. Interferon-dependent induction of Clr-b during mouse cytomegalovirus infection protects bystander cells from natural killer cells via nkr-p1b-mediated inhibition. *J. Innate Immun.* **9**, 343–358 (2017).
95. Voigt, S. et al. Cytomegalovirus evasion of innate immunity by subversion of the NKR-P1B:Clr-b missing-self axis. *Immunity* **26**, 617–627 (2007).
96. Voigt, S., Sandford, G. R., Ding, L. & Burns, W. H. Identification and characterization of a spliced C-type lectin-like gene encoded by rat cytomegalovirus. *J. Virol.* **75**, 603–611 (2001).
97. Braud, V. M. et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795–799 (1998).
98. Braud, V., Jones, E. Y. & McMichael, A. The human major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. *Eur. J. Immunol.* **27**, 1164–1169 (1997).
99. Tomasec, P. et al. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science* **287**, 1031 (2000). **This study finds that the leader sequence of gpUL40 binds HLA-E and upregulates its surface expression, thereby protecting infected cells from NK cell attack**.
100. Ulbrecht, M. et al. Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. *J. Immunol.* **164**, 5019–5022 (2000).
101. Cerboni, C. et al. Synergistic effect of IFN-gamma and human cytomegalovirus protein UL40 in the HLA-E-dependent protection from NK cell-mediated cytotoxicity. *Eur. J. Immunol.* **31**, 2926–2935 (2001).
102. Heatley, S. L. et al. Polymorphism in human cytomegalovirus UL40 impacts on recognition of human leukocyte antigen-E (HLA-E) by natural killer cells. *J. Biol. Chem.* **288**, 8679–8690 (2013).
103. Wang, E. C. et al. UL40-mediated NK evasion during productive infection with human cytomegalovirus. *Proc. Natl Acad. Sci. USA* **99**, 7570–7575 (2002).
104. Hoare, H. L. et al. Structural basis for a major histocompatibility complex class Ib-restricted T cell response. *Nat. Immunol.* **7**, 256–264 (2006).
105. Sullivan, L. C. et al. A conserved energetic footprint underpins recognition of human leukocyte antigen-E by two distinct alphabeta T cell receptors. *J. Biol. Chem.* **292**, 21149–21158 (2017).
106. Kleijnen, M. F. et al. A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER which is not retained but is transported to the cell surface. *EMBO J.* **16**, 685–694 (1997).
107. Corbett, A. J., Forbes, C. A., Moro, D. & Scalzo, A. A. Extensive sequence variation exists among isolates of murine cytomegalovirus within members of the m02 family of genes. *J. Gen. Virol.* **88**, 758–769 (2007).
108. Zeleznjak, J. et al. The complex of MCMV proteins and MHC class I evades NK cell control and drives the evolution of virus-specific activating Ly49 receptors. *J. Exp. Med.* **216**, 1809–1827 (2019).
109. Orr, M. T., Murphy, W. J. & Lanier, L. L. 'Unlicensed' natural killer cells dominate the response to cytomegalovirus infection. *Nat. Immunol.* **11**, 321–327 (2010).
110. Slavuljica, I., Krmpotic, A. & Jonjic, S. Manipulation of NKG2D ligands by cytomegaloviruses: impact on innate and adaptive immune response. *Front. Immunol.* **2**, 85 (2011).
111. Jonjic, S., Babic, M., Polic, B. & Krmpotic, A. Immune evasion of natural killer cells by viruses. *Curr. Opin. Immunol.* **20**, 30–38 (2008).
112. Lanier, L. L. NKG2D Receptor and its ligands in host defense. *Cancer Immunol. Res.* **3**, 575–582 (2015).
113. Ashiru, O. et al. NKG2D ligand MICA is retained in the cis-Golgi apparatus by human cytomegalovirus protein UL142. *J. Virol.* **83**, 12345–12354 (2009).
114. Chalupny, N. J., Rein-Weston, A., Dosch, S. & Cosman, D. Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142. *Biochem. Biophys. Res. Commun.* **346**, 175–181 (2006).
115. Dassa, L. et al. The human cytomegalovirus protein UL148a downregulates the nk cell-activating ligand mica to avoid NK cell attack. *J. Virol.* **92** (2018).
116. Seidel, E. et al. Dynamic co-evolution of host and pathogen: HCMV downregulates the prevalent allele MICA *008 to escape elimination by NK cells. *Cell Rep.* **10**, 968–982 (2015).
117. Dunn, C. et al. Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity. *J. Exp. Med.* **197**, 1427–1439 (2003). **Collectively, this study and references 120 and 123 highlight that both HCMV and MCMV have evolved molecules that interfere with surface expression of NKG2D ligands**.
118. Stern-Ginossar, N. et al. Host immune system gene targeting by a viral miRNA. *Science* **317**, 376–381 (2007).
119. Raulet, D. H., Gasser, S., Gowen, B. G., Deng, W. & Jung, H. Regulation of ligands for the NKG2D activating receptor. *Annu. Rev. Immunol.* **31**, 413–441 (2013).
120. Lodoen, M. et al. NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. *J. Exp. Med.* **197**, 1245–1253 (2003).
121. Arapovic, J. et al. Differential susceptibility of RAE-1 isoforms to mouse cytomegalovirus. *J. Virol.* **83**, 8198–8207 (2009).
122. Zhi, L. et al. Direct interaction of the mouse cytomegalovirus m152/gp40 immunoevasin with RAE-1 isoforms. *Biochemistry* **49**, 2443–2453 (2010).
123. Lenac, T. et al. The herpesviral Fc receptor fcr-1 down-regulates the NKG2D ligands MULT-1 and H60. *J. Exp. Med.* **203**, 1843–1850 (2006).
124. Shibuya, A. et al. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity* **4**, 573–581 (1996).
125. Bottino, C. et al. Identification of PVR (CD155) and nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J. Exp. Med.* **198**, 557–567 (2003).
126. Tahara-Hanaoka, S. et al. Functional characterization of DNAM-1 (CD226) interaction with its ligands PVR (CD155) and nectin-2 (PRR-2/CD112). *Int. Immunol.* **16**, 533–538 (2004).
127. Lenac Rovis, T. et al. Inflammatory monocytes and NK cells play a crucial role in DNAM-1-dependent control of cytomegalovirus infection. *J. Exp. Med.* **213**, 1835–1850 (2016).
128. Pignoloni, B. et al. Distinct roles for human cytomegalovirus immediate early proteins IE1 and IE2 in the transcriptional regulation of MICA and PVR/CD155 expression. *J. Immunol.* **197**, 4066–4078 (2016).
129. Prod'homme, V. et al. Human cytomegalovirus UL141 promotes efficient downregulation of the natural killer cell activating ligand CD112. *J. Gen. Virol.* **91**, 2034–2039 (2010).
130. Tomasec, P. et al. Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus UL141. *Nat. Immunol.* **6**, 181–188 (2005).
131. Nemcovicova, I., Benedict, C. A. & Zajonc, D. M. Structure of human cytomegalovirus UL141 binding to TRAIL-R2 reveals novel, non-canonical death receptor interactions. *PLOS Pathog.* **9**, e1003224 (2013).
132. Smith, W. et al. Human cytomegalovirus glycoprotein UL141 targets the TRAIL death receptors to thwart host innate antiviral defenses. *Cell Host Microbe* **13**, 324–335 (2013).
133. Chan, C. J. et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. *Nat. Immunol.* **15**, 431–438 (2014).
134. Deuss, F. A., Gully, B. S., Rossjohn, J. & Berry, R. Recognition of nectin-2 by the natural killer cell receptor T cell immunoglobulin and ITIM domain (TIGIT). *J. Biol. Chem.* **292**, 11413–11422 (2017).
135. Deuss, F. A., Watson, G. M., Fu, Z., Rossjohn, J. & Berry, R. Structural basis for CD96 immune receptor recognition of nectin-like protein-5, CD155. *Structure* **27**, 219–228 e213 (2019).
136. Stengel, K. F. et al. Structure of TIGIT immunoreceptor bound to poliovirus receptor reveals a cell-cell adhesion and signaling mechanism that requires cis-trans receptor clustering. *Proc. Natl Acad. Sci. USA* **109**, 5399–5404 (2012).
137. Deuss, F. A. et al. Structural basis for the recognition of nectin-like protein-5 by the human activating immune receptor, DNAM-1. *J. Biol. Chem.* **294**, 12534–12546 (2019).
138. Valiante, N. M. & Trinchieri, G. Identification of a novel signal transduction surface molecule on human cytotoxic lymphocytes. *J. Exp. Med.* **178**, 1397–1406 (1993).
139. Garni-Wagner, B. A., Purohit, A., Mathew, P. A., Bennett, M. & Kumar, V. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J. Immunol.* **151**, 60–70 (1993).
140. Lee, K. M. et al. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J. Exp. Med.* **199**, 1245–1254 (2004).
141. Zarama, A. et al. Cytomegalovirus m154 hinders CD48 cell-surface expression and promotes viral escape from host natural killer cell control. *PLOS Pathog.* **10**, e1004000 (2014).
142. Romo, N. et al. Natural killer cell-mediated response to human cytomegalovirus-infected macrophages is modulated by their functional polarization. *J. Leukoc. Biol.* **90**, 717–726 (2011).
143. Martinez-Vicente, P. et al. Subversion of natural killer cell responses by a cytomegalovirus-encoded soluble CD48 decoy receptor. *PLOS Pathog.* **15**, e1007658 (2019).
144. Kruse, P. H., Matta, J., Ugolini, S. & Vivier, E. Natural cytotoxicity receptors and their ligands. *Immunol. Cell Biol.* **92**, 221–229 (2014).
145. Arnon, T. I. et al. Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. *Nat. Immunol.* **6**, 515–523 (2005).
146. Charpak-Amikam, Y. et al. Human cytomegalovirus escapes immune recognition by NK cells through the downregulation of B7-H6 by the viral genes US18 and US20. *Sci. Rep.* **7**, 8661 (2017).
147. Miletic, A., Krmpotic, A. & Jonjic, S. The evolutionary arms race between NK cells and viruses: who gets the short end of the stick? *Eur. J. Immunol.* **43**, 867–877 (2013).
148. Hogarth, P. M. & Pietersz, G. A. Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. *Nat. Rev. Drug Discov.* **11**, 311–331 (2012).
149. Ross, S. A. et al. Cytomegalovirus reinfections in healthy seroimmune women. *J. Infect. Dis.* **201**, 386–389 (2010).
150. Furukawa, T., Hornberger, E., Sakuma, S. & Plotkin, S. A. Demonstration of immunoglobulin G receptors induced by human cytomegalovirus. *J. Clin. Microbiol.* **2**, 332–336 (1975).
151. Atalay, R. et al. Identification and expression of human cytomegalovirus transcription units coding for two distinct Fcgamma receptor homologs. *J. Virol.* **76**, 8596–8608 (2002).
152. Cortese, M. et al. Recombinant human cytomegalovirus (HCMV) RL13 binds human immunoglobulin G Fc. *PLOS ONE* **7**, e50166 (2012).
153. Lilley, B. N., Ploegh, H. L. & Tirabassi, R. S. Human cytomegalovirus open reading frame TRL11/IRL11 encodes an immunoglobulin G Fc-binding protein. *J. Virol.* **75**, 11218–11221 (2001).
154. Corrales-Aguilar, E., Hoffmann, K. & Hengel, H. CMV-encoded Fcgamma receptors: modulators at the interface of innate and adaptive immunity. *Semin. Immunopathol.* **36**, 627–640 (2014).
155. Sprague, E. R. et al. The human cytomegalovirus Fc receptor gp68 binds the Fc CH2-CH3 interface of immunoglobulin G. *J. Virol.* **82**, 3490–3499 (2008).
156. Ndjamen, B., Joshi, D. S., Fraser, S. E. & Bjorkman, P. J. Characterization of antibody bipolar bridging mediated by the human cytomegalovirus Fc receptor gp68. *J. Virol.* **90**, 3262–3267 (2016).
157. Thale, R., Lucin, P., Schneider, K., Eggers, M. & Koszinowski, U. H. Identification and expression of a murine cytomegalovirus early gene coding for an Fc receptor. *J. Virol.* **68**, 7757–7765 (1994).
158. Crnkovic-Mertens, I. et al. Virus attenuation after deletion of the cytomegalovirus Fc receptor gene is not due to antibody control. *J. Virol.* **72**, 1377–1382 (1998).
159. Kolb, P. et al. Identification and functional characterization of a novel fc gamma-binding glycoprotein in rhesus cytomegalovirus. *J. Virol.* **93**, e02077–18 (2019).
160. Abi-Rached, L. & Parham, P. Natural selection drives recurrent formation of activating killer cell immunoglobulin-like receptor and Ly49 from inhibitory homologues. *J. Exp. Med.* **201**, 1319–1332 (2005). **This study investigates the evolutionary history of KIRs and Ly49 receptors and proposes a model in which the activating receptors evolved more recently from their inhibitory counterparts in response to selective pressure induced by pathogens**.
161. Corbett, A. J., Coudert, J. D., Forbes, C. A. & Scalzo, A. A. Functional consequences of natural sequence variation of murine cytomegalovirus m157

for Ly49 receptor specificity and NK cell activation. *J. Immunol.* **186**, 1713–1722 (2011).
162. Berry, R. et al. Targeting of a natural killer cell receptor family by a viral immunoevasin. *Nat. Immunol.* **14**, 699–705 (2013).
This study reports the structure of the Ly49H–m157 complex and demonstrates that this immunoevasin targets the membrane proximal stalk region of the receptor.
163. Dorner, B. G. et al. Coordinate expression of cytokines and chemokines by NK cells during murine cytomegalovirus infection. *J. Immunol.* **172**, 3119–3131 (2004).
164. Smith, H. R. et al. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl Acad. Sci. USA* **99**, 8826–8831 (2002).
165. Desrosiers, M. P. et al. Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat. Genet.* **37**, 593–599 (2005).
166. Pyzik, M. et al. Distinct MHC class I-dependent NK cell-activating receptors control cytomegalovirus infection in different mouse strains. *J. Exp. Med.* **208**, 1105–1117 (2011).
167. Kielczewska, A. et al. Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response. *J. Exp. Med.* **206**, 515–523 (2009).
This study and references 165 and 166 show that activating Ly49 receptors can recognize infected cells via a novel mechanism that is dependent on certain MHC-I allotypes.
168. Guma, M. et al. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* **107**, 3624–3631 (2006).
169. Hammer, Q. et al. Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nat. Immunol.* **19**, 453–463 (2018).
170. Rolle, A. et al. IL-12-producing monocytes and HLA-E control HCMV-driven NKG2C+ NK cell expansion. *J. Clin. Invest.* **124**, 5305–5316 (2014).
171. Voigt, V. et al. Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells. *Proc. Natl Acad. Sci. USA* **100**, 13483–13488 (2003).
172. French, A. R. et al. Escape of mutant double-stranded DNA virus from innate immune control. *Immunity* **20**, 747–756 (2004).
173. McWhorter, A. R. et al. Natural killer cell dependent within-host competition arises during multiple MCMV infection: consequences for viral transmission and evolution. *PLOS Pathog.* **9**, e1003111 (2013).
174. Vivian, J. P. et al. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature* **479**, 401–405 (2011).
175. Saunders, P. M. et al. A bird's eye view of NK cell receptor interactions with their MHC class I ligands. *Immunol. Rev.* **267**, 148–166 (2015).
176. Cook, M. et al. Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation. *Blood* **107**, 1230–1232 (2006).
177. van Duin, D. et al. KIR and HLA interactions are associated with control of primary CMV infection in solid organ transplant recipients. *Am. J. Transpl.* **14**, 156–162 (2014).
178. Khakoo, S. I. et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* **305**, 872–874 (2004).
179. Martin, M. P. et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* **39**, 733–740 (2007).
180. Ashiru, O. et al. A GPI anchor explains the unique biological features of the common NKG2D-ligand allele MICA*008. *Biochem. J.* **454**, 295–302 (2013).
181. Mizuki, N. et al. Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behcet disease. *Proc. Natl Acad. Sci. USA* **94**, 1298–1303 (1997).
182. Romphruk, A. V. et al. Diversity of MICA (PERB11.1) and HLA haplotypes in northeastern Thais. *Tissue Antigens* **58**, 83–89 (2001).
183. Tian, W., Boggs, D. A., Ding, W. Z., Chen, D. F. & Fraser, P. A. MICA genetic polymorphism and linkage disequilibrium with HLA-B in 29 African-American families. *Immunogenetics* **53**, 724–728 (2001).
184. Zhang, Y. et al. MICA polymorphism in South American Indians. *Immunogenetics* **53**, 900–906 (2002).
185. van de Weijer, M. L., Luteijn, R. D. & Wiertz, E. J. Viral immune evasion: Lessons in MHC class I antigen presentation. *Semin. Immunol.* **27**, 125–137 (2015).
186. De Pelsmaeker, S., Romero, N., Vitale, M. & Favoreel, H. W. Herpesvirus evasion of natural killer cells. *J. Virol.* **92**, e02105–e02117 (2018).
187. Matschulla, T. et al. A highly conserved sequence of the viral TAP inhibitor ICP47 is required for freezing of the peptide transport cycle. *Sci. Rep.* **7**, 2933 (2017).
188. Oldham, M. L., Grigorieff, N. & Chen, J. Structure of the transporter associated with antigen processing trapped by herpes simplex virus. *eLife* **5**, e21829 (2016).
189. Bennett, E. M., Bennink, J. R., Yewdell, J. W. & Brodsky, F. M. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. *J. Immunol.* **162**, 5049–5052 (1999).
190. Barkal, A. A. et al. Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. *Nat. Immunol.* **19**, 76–84 (2018).
191. Dougall, W. C., Kurtulus, S., Smyth, M. J. & Anderson, A. C. TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy. *Immunol. Rev.* **276**, 112–120 (2017).
192. Tanaka, M. et al. The Inhibitory NKR-P1B:Clr-b recognition axis facilitates detection of oncogenic transformation and cancer immunosurveillance. *Cancer Res.* **78**, 3589–3603 (2018).
193. Cox, J. H., Yewdell, J. W., Eisenlohr, L. C., Johnson, P. R. & Bennink, J. R. Antigen presentation requires transport of MHC class I molecules from the endoplasmic reticulum. *Science* **247**, 715–718 (1990).
194. Weizman, O. E. et al. Mouse cytomegalovirus-experienced ILC1s acquire a memory response dependent on the viral glycoprotein m12. *Nat. Immunol.* **20**, 1004–1011 (2019).
195. Lucas, A. & McFadden, G. Secreted immunomodulatory viral proteins as novel biotherapeutics. *J. Immunol.* **173**, 4765–4774 (2004).
196. Altomonte, J. et al. Enhanced oncolytic potency of vesicular stomatitis virus through vector-mediated inhibition of NK and NKT cells. *Cancer Gene Ther.* **16**, 266–278 (2009).
197. Kim, J. S. et al. Human cytomegalovirus UL18 alleviated human NK-mediated swine endothelial cell lysis. *Biochem. Biophys. Res. Commun.* **315**, 144–150 (2004).
198. Wilkinson, G. W. et al. Human cytomegalovirus: taking the strain. *Med. Microbiol. Immunol.* **204**, 273–284 (2015).
199. Cha, T. A. et al. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *J. Virol.* **70**, 78–83 (1996).
200. Cerboni, C. et al. Human cytomegalovirus strain-dependent changes in NK cell recognition of infected fibroblasts. *J. Immunol.* **164**, 4775–4782 (2000).
201. Stanton, R. J. et al. Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. *J. Clin. Invest.* **120**, 3191–3208 (2010).
202. Murrell, I. et al. Genetic stability of bacterial artificial chromosome-derived human cytomegalovirus during culture in vitro. *J. Virol.* **90**, 3929–3943 (2016).
203. Coaquette, A. et al. Mixed cytomegalovirus glycoprotein B genotypes in immunocompromised patients. *Clin. Infect. Dis.* **39**, 155–161 (2004).
204. Cudini, J. et al. Human cytomegalovirus haplotype reconstruction reveals high diversity due to superinfection and evidence of within-host recombination. *Proc. Natl Acad. Sci. USA* **116**, 5693–5698 (2019).
205. Smith, C. et al. Coinfection with human cytomegalovirus genetic variants in transplant recipients and its impact on antiviral t cell immune reconstitution. *J. Virol.* **90**, 7497–7507 (2016).
206. Suarez, N. M. et al. Human cytomegalovirus genomes sequenced directly from clinical material: variation, multiple-strain infection, recombination and gene loss. *J. Infect. Dis.* **220**, 781–791 (2019).
207. Smith, L. M., McWhorter, A. R., Masters, L. L., Shellam, G. R. & Redwood, A. J. Laboratory strains of murine cytomegalovirus are genetically similar to but phenotypically distinct from wild strains of virus. *J. Virol.* **82**, 6689–6696 (2008).
208. Martins, J. P. et al. Strain-specific antibody therapy prevents cytomegalovirus reactivation after transplantation. *Science* **363**, 288–293 (2019).
209. Reddehase, M. J. & Lemmermann, N. A. W. Mouse model of cytomegalovirus disease and immunotherapy in the immunocompromised host: predictions for medical translation that survived the "test of time". *Viruses* **10**, E693 (2018).
210. Petrie, E. J. et al. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J. Exp. Med.* **205**, 725–735 (2008).
211. Li, P., McDermott, G. & Strong, R. K. Crystal structures of RAE-1beta and its complex with the activating immunoreceptor NKG2D. *Immunity* **16**, 77–86 (2002).
212. Li, P. et al. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nat. Immunol.* **2**, 443–451 (2001).
213. Radaev, S., Rostro, B., Brooks, A. G., Colonna, M. & Sun, P. D. Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3. *Immunity* **15**, 1039–1049 (2001).
214. Zuo, J. et al. A disease-linked ULBP6 polymorphism inhibits NKG2D-mediated target cell killing by enhancing the stability of NKG2D ligand binding. *Sci. Signal* **10**, eaai8904 (2017).
215. Wang, R. et al. Structural basis of mouse cytomegalovirus m152/gp40 interaction with RAE1gamma reveals a paradigm for MHC/MHC interaction in immune evasion. *Proc. Natl Acad. Sci. USA* **109**, E3578–E3587 (2012).
216. Muller, S., Zocher, G., Steinle, A. & Stehle, T. Structure of the HCMV UL16-MICB complex elucidates select binding of a viral immunoevasin to diverse NKG2D ligands. *PLOS Pathog.* **6**, e1000723 (2010).
217. Balaji, G. R. et al. Recognition of host Clr-b by the inhibitory NKR-P1B receptor provides a basis for missing-self recognition. *Nat. Commun.* **9**, 4623 (2018).
218. Gao, G. F. et al. Crystal structure of the complex between human CD8alpha(alpha) and HLA-A2. *Nature* **387**, 630–634 (1997).

Acknowledgements
The authors acknowledge funding from the National Health and Medical Research Council of Australia to R.B. (APP1109901) and M.A.D-E. (GNT1119298), the Deutsche Forschungsgemeinschaft-funded research unit Advanced Concepts in Cellular Immune Control of Cytomegalovirus (FOR 2830) project 'Solving the m04 paradox: evasion of missing-self recognition and CD8 T cell killing by MAT uORF' to S.J. (JO 1634/1-1), the grant KK.01.1.1.01.0006, awarded to the Scientific Centre of Excellence for Virus Immunology and Vaccines and co-financed by the European Regional Development Fund, to S.J. and the Australian Research Council to J.R. (FL160100049).

Author contributions
R.B. and G.M.W. researched data for the article, R.B. and J.R. substantially contributed to the discussion of the content, R.B., G.M.W., S.J. and M.A.D.-E. wrote the article and R.B. and J.R. were responsible for the review and editing of the article before submission.

Competing interests
The authors declare no competing interests.

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

Reviewer information
Nature Reviews Immunology thanks A. Cerwenka and C. Biron for their contribution to the peer review of this work.