Virus subversion of immunity: a structural perspective

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Over the past year, we have witnessed the discovery of further virus immuno-evasins – proteins that alter the host immune response. Although many of these factors have been described over the past decade, the structural basis underlying their biology has lagged behind. Structural data have now been obtained for several such proteins. Major advances of the past year include the structures of a viral chemokine-binding protein, of an intact viral regulator of complement activation and of an immuno-evasin with its cellular target.

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Abbreviations

CCP	complement-control protein
EBV	Epstein–Barr virus
ER	endoplasmic reticulum
HCMV	human cytomegalovirus
HHV-7	human herpesvirus 7
Ig	immunoglobulin
IL-18BP	IL-18-binding protein
NMR	nuclear magnetic resonance
TAP	transporter associated with antigen presentation
US	unique short
vCCI	viral chemokine inhibitor
VCP	vaccinia complement-control protein

Introduction

The immune response presents a challenge to viruses, which must survive and multiply within a host for a sufficient period of time to allow successful transmission to susceptible individuals. In response, many viruses are equipped with multiple 'immuno-evasins' — proteins that modulate the host immune response — and many different immuno-modulatory mechanisms have been described (see recent reviews [1–4]). Virus immuno-evasins can be divided into two classes. First, viruses carry genes encoding immunomodulatory proteins that have been directly captured from the host, as evidenced by significant sequence and structural similarity with proteins of the host immune system. In these instances, viruses either exploit the regulatory capacity of the captured factors or utilize them as competitive inhibitors of the host immune receptors, as will be summarized below. As immunoglobulin (Ig) folds are found amongst many immune system receptors, viruses frequently capture Ig superfamily proteins. The second category of immuno-evasins has no obvious mammalian counterparts in either amino acid sequence or function and this enables activities that are not utilized by the immune system. Here, too, virus immuno-evasins include members of the Ig superfamily, as well as altogether novel folds.

In this review, we consider various strategies used by viruses to evade host responses and focus on where themes are beginning to emerge about virus immuno-evasins, as informed by structural biology.

Interference with cytokines

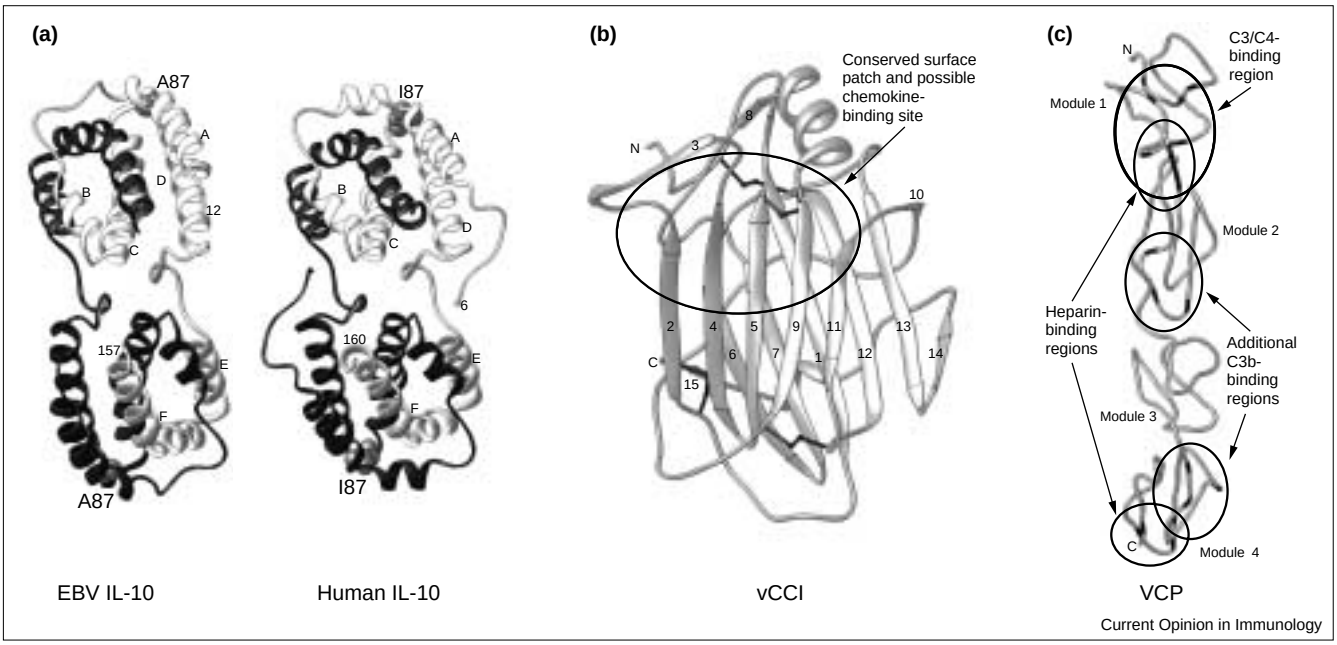
Poxviruses utilize soluble cytokine-receptor homologues to neutralize proinflammatory cytokines [2]. This principle is illustrated by a family of recently identified IL-18-binding proteins (IL-18BPs), which demonstrate significant sequence similarity to the Ig superfamily domain of human IL-18BP [5••]. IL-18 is a potent cytokine that promotes Th1 cell polarization, IFN-γ synthesis, and NK cell and cytotoxic T lymphocyte activation [5••]. Poxvirus-encoded IL-18BPs bind IL-18 with high affinity and slow off-rates, allowing them to inhibit IL-18 signaling in the vicinity of the infected cell [6,7,8•].

Cells infected with vaccinia virus secrete additional soluble cytokine-binding proteins — B8R, B15R and B18R — that are each composed of multiple Ig-like domains. Again, these soluble molecules compete with membrane-bound cellular cytokine-receptors. B8R binds IFN-γ [9] and recent studies indicate that B8R may bind additional cytokines *in vivo* [10,11]. B18R binds both to type I interferons and to cell surfaces, perhaps allowing the virus to protect neighboring uninfected cells from the antiviral effects of type I interferons [12•]. Similarly, Orf poxvirus encodes a factor, GIF, which binds to both GM-CSF and IL-2 [13•].

In addition to blocking cytokine signals, viruses encode cytokine homologues. The Epstein–Barr virus (EBV) IL-10 homologue has been studied in detail biochemically and structurally. Human IL-10 is a pleiotropic cytokine, stimulating several immune pathways while downregulating others. IL-10 inhibits macrophage activation, antigen presentation and Th1 cell polarization [14]. Despite 87% sequence identity with human IL-10, EBV IL-10 exerts only IL-10's immunosuppressive effects [15••,16].

The EBV IL-10 structure is highly similar to that of human IL-10 [17]. Both human and EBV IL-10 are

Figure 1



Structures of human IL-10 and three of the viral immuno-evasins mentioned in this review. (**a**) Ribbon diagrams of human IL-10 [51] (residues 6–160) and EBV IL-10 [17] (residues 12–157). Both cytokines are homodimers (subunit 1, white; subunit 2, gray), where each subunit contains six α-helices, labeled A–F. Space-filling models are shown for alanine 87 (EBV IL-10) and isoleucine 87 (human IL-10) side-chains. (**b**) Ribbon diagram of vCCI [20••], a cowpox CC-chemokine-binding protein. β-strands are numbered. A region implicated in chemokine binding is indicated (oval). (**c**) Ribbon diagram of VCP, an inhibitor of complement activation coded by vaccinia virus. VCP contains four CCP modules. Regions implicated in complement C3/C4- and heparin-binding are indicated. Residues implicated in substrate binding are colored black. Amino (N) and carboxyl (C) termini are indicated.

homodimers, each subunit containing six α-helices (Figure 1a) [15••]. Human IL-10 binds IL-10 receptor 1 (IL-10R1) with 1000-fold higher affinity than EBV IL-10. The structural and sequence similarity between the IL-10 homologues raises the question of how EBV IL-10 differentially interacts with cellular receptors. Most residues predicted to interact with IL-10R1 are well conserved between cellular and viral IL-10s. However, a conservative isoleucine→alanine replacement (I87A) present in EBV IL-10 dramatically alters receptor-binding properties. Mutation A87I in EBV IL-10 partially restores the viral cytokine-homologue's immunostimulatory properties, whereas mutation I87A abrogates immunostimulatory properties of human IL-10 [15••]. Thus, isoleucine 87 may interact with a component of the IL-10-receptor complex. When mapped onto the crystal structures, residue 87 is in the loop between helices C and D, at the tip of each arm of the IL-10 dimer (Figure 1a). This position lies close to the putative receptor-binding site of human IL-10, which includes helix A, loop AB and helices B and F′ [17].

Human cytomegalovirus (HCMV) also encodes an IL-10 homologue [18•]. Interestingly, a leucine residue is present at the position corresponding to IL-10 residue 87. It will be interesting to learn whether this conservative substitution has biological consequences.

Antagonism to chemokines

Most poxviruses encode a secreted chemokine antagonist that binds to CC chemokines with high affinity. Whereas cellular receptors for CC chemokines belong to the G-protein-coupled receptor family, the viral chemokine inhibitor (vCCI; also known as p35) is a soluble protein. Further, vCCI binds to all CC chemokines tested, whereas cellular receptors bind smaller subgroups of chemokines [19]. vCCI binds CC chemokines with a subnanomolar dissociation constant, an affinity for CC chemokines 10-fold greater than that exhibited by host receptors. In contrast, vCCI binds weakly to C and CXC chemokines [19].

The recently published cowpox vCCI structure shows a novel fold containing two parallel β-sheets and two short α-helices (Figure 1b) [20••]. Though the structure of vCCI distantly resembles that of a *Staphylococcus aureus* adhesin that binds collagen [20••], the evolutionary origin of p35 remains a mystery. Since vCCI competes with cellular receptors for chemokines, mammalian and poxvirus receptors may bind overlapping chemokine regions. It is not yet known whether vCCI and cellular chemokine-receptors share similar features at their chemokine-binding surfaces. A patch of residues on β-sheet II is well conserved amongst poxvirus vCCI homologues. This patch includes several acidic side-chains that could play an important role in binding chemokines, which are positively charged ([20••]; Figure 1b).

Table 1

Virus modulation of MHC class I antigen presentation.

Virus	Gene/ protein	Structure features	Function	References
HCMV	US2	Type I glycoprotein, Ig-like fold	Targets class I to proteasome	[3,32••]
	US3	Type I glycoprotein, predicted Ig-like fold	Retention of folded class I complexes	[3]
	US6	Type I glycoprotein, predicted Ig-like fold	Blocks TAP from ER-lumenal side	[3]
	US8	Type I glycoprotein, predicted Ig-like fold	Binds class I, function unknown	(a)
	US10	Type I glycoprotein, predicted Ig-like fold	Binds class I, function unknown	(b)
	US11	Type I glycoprotein, predicted Ig-like fold	Targets class I to proteasome	[3]
	UL40	Leader sequence mimics that of HLA-C	Provides signal peptide ligand for HLA-E	[60,61]
	UL18	Virus MHC class I homologue	Binds to ILT-2 (LIR-1) inhibitory receptor	[62•]
Murine CMV	*m04*/gp34	Type I glycoprotein	Travels in complex with class I to cell surface, significance unknown	[63]
	m06/ gp48	Type I glycoprotein, dileucine motif in cytoplasmic tail	Targets class I molecules to lysosome	[64]
	m152/gp40	Type I glycoprotein	Retains class I molecules in ERGIC by transient or indirect mechanism	[65•,66•]
	m144	Virus MHC class I homologue	Evasion of NK lysis	[67]
Herpes simplex virus	ICP47	Cytosolic protein, active site contains helix–linker–helix motif	Blocks TAP from cytosolic side	[46•,47,48,49•]
Varicella zoster virus	Unknown		Retains class I in Golgi complex	[68•]
HHV-7	U21	Type 1 glycoprotein	Binds class I, targets for degradation	(c)
KSHV (HHV-8)	K3	Membrane protein with putative LAP/PHD zinc finger	Induces rapid internalization of cell surface class I	[39•,40•]
	K5	Membrane protein with putative LAP/PHD zinc finger	Induces rapid internalization of cell surface class I	[39•,40•, 41••,69•]
Murine γ-herpesvirus 68	K3	Membrane protein with putative LAP/PHD zinc finger	Induces rapid internalization of cell surface class I	[70]
Adenovirus	E3-19K	Type 1 glycoprotein, predicted Ig-like fold	Retains class I molecules in ER	[35–37,71•]
HIV	Nef	Myristoylated cytoplasmic protein, polyproline type II helix allows SH3 association	Induces PACS-1-mediated internalization and lysosomal degradation of class I	[72–74]
	Vpu	Small integral membrane phosphoprotein	Destabilization of newly synthesized class I molecules	[75]
Myxoma	Unknown		Directs class I to late endosomes or lysosome	[76]

(a) RS Tirabassi, HL Ploegh, unpublished data; (b) MH Furman, N Dey, HL Ploegh, unpublished data; (c) A Hudson, HL Ploegh, PM Howley, unpublished data. ERGIC, ER-Golgi intermediate compartment; KSHV, Kaposi's-sarcoma-associated virus.

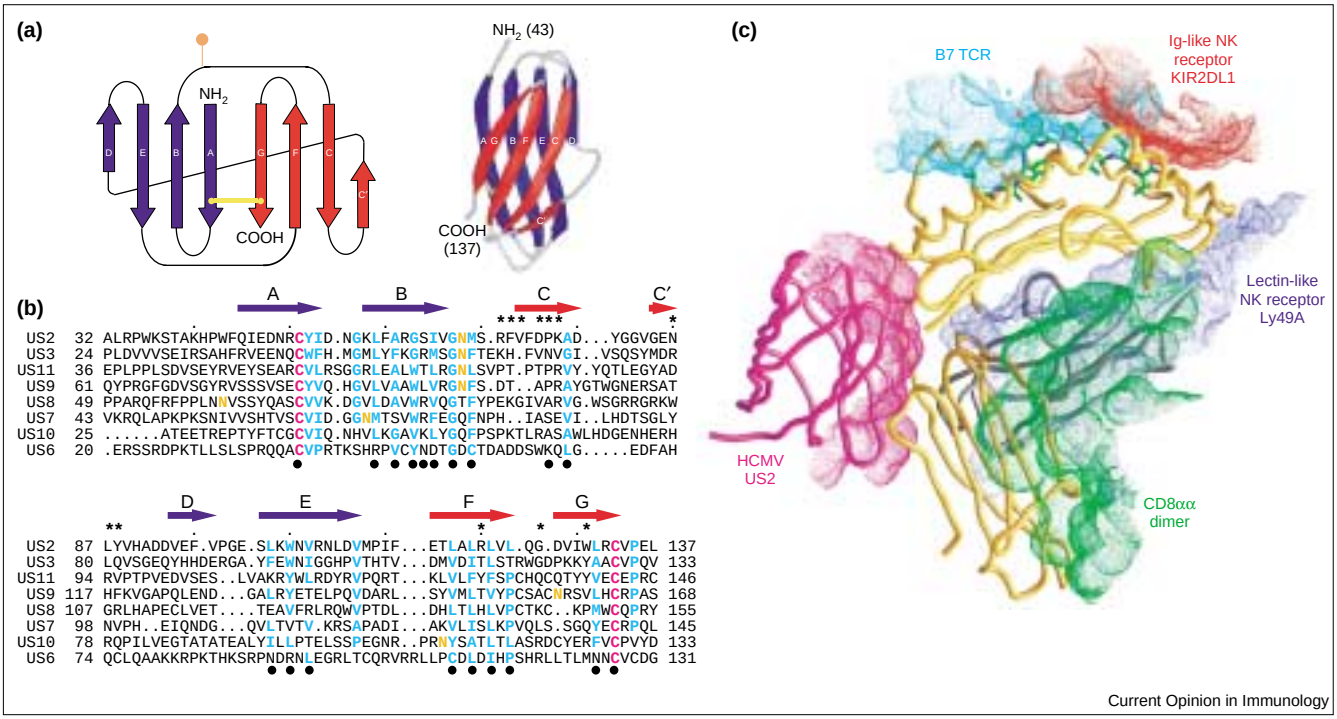
A broad-spectrum chemokine antagonist, M3, which is coded by murine γ-herpesvirus 68, was recently identified [21•,22•]. Reminiscent of vCCI, M3 is a soluble chemokine-receptor that displays no sequence similarity to known proteins and binds chemokines from all four subfamilies with high affinity. As structural information becomes available, it will be interesting to compare features of M3 with those of vCCI.

Inhibition of complement activation

Vaccinia virus encodes a potent complement inhibitor, vaccinia complement-control protein (VCP). This inhibitor promotes Factor I cleavage of C3b and C4b, which disrupts both the classical [23] and alternative [24] complement pathways. Akin to host regulators of complement activation (RCAs), VCP contains four complement-control protein (CCP) modules (also known as short consensus repeats), composed of six β-strands.

VCP assumes an extended three-dimensional structure, in which there is little interaction between the individual CCP modules ([25••]; Figure 1c). Positively charged patches are positioned at both ends of VCP, whereas the middle two modules have a net negative charge. Mutagenesis studies suggest that RCA proteins interact with negatively charged residues on C3 [26]. Studies with truncated versions of VCP, together with the VCP structure, highlight a positively charged patch between CCP modules 1 and 2 as a likely C3/C4-binding domain [25••,27•]. Additional residues important for C3b-binding map to modules 2 and 4. VCP tethers itself to the extracellular matrix via a putative heparin-recognition surface. Comparison of VCP with other heparin-binding domains of fibroblast growth factors suggests that VCP binds heparin via its carboxy-terminal module. Several lysine residues form a patch at the tip of CCP module 4 that could bind heparin [25••].

Figure 2



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The HCMV US2 Ig-like fold binds class I uniquely. (**a**) Folding diagram of the US2 ER-lumenal Ig-like fold. β-strands are lettered A–G and colored red (β-sheet I) or blue (β-sheet II). The disulfide bond (yellow) and single N-linked glycan attachment sites (orange circle) are indicated (see text for details). (**b**) Alignment of the predicted Ig-like fold domains of the US2, US6 and US11 families (adapted from [32••]). US2 residues that contact HLA-A2 are indicated (*). Conserved hydrophobic residues are blue, predicted N-linked glycosylation sites are orange, cysteines predicted to form the A-to-G strand disulfide bond are magenta. US2 core residues (>90% buried) are indicated by filled circles (•). (**c**) Superposition of five class-I–ligand complexes demonstrates distinct binding surfaces recognized by each ligand. Equivalent Cα atoms for the class I heavy chain were used to generate pairwise superpositions for this hypothetical representation. HLA-A2 heavy chain (yellow), β_2-microglobulin (gray), US2 (magenta), B7 TCR (cyan) [52], KIR2DL1 NK cell receptor (red) [53,54], Ly49A murine NK cell receptor (dark blue) [55,56] and CD8αα (green) [57] are shown. The class I heavy chain and β_2-microglobulin are shown in worms, the human T cell leukemia virus Tax peptide (LLFGYPVYV) that is presented by HLA-A2 is shown in atomic representation and the interaction surfaces of the class I binding partners are represented as dot surfaces.

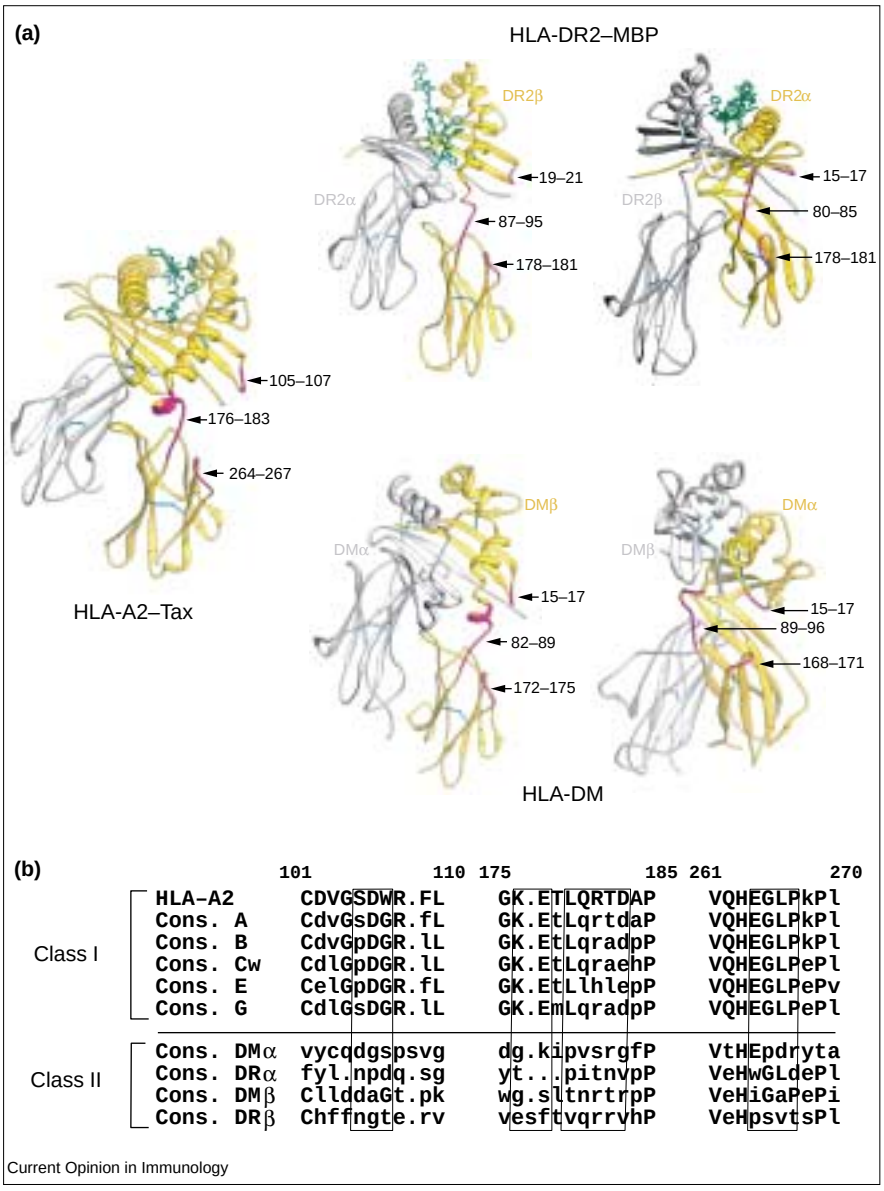
Modulation of MHC class I

Ig-like domains contain two β-sheets, composed of 7–10 characteristically connected β-strands, packed face-to-face in a β-sandwich [28]. A small number of structurally important residues dictate the folding of Ig-like domains, including those forming the internal hydrophobic core. In contrast, loops connecting the β-strands vary in sequence and length, at times accommodating insertions of whole domains. In addition, many residues — especially those with exposed side-chains — are likewise free to vary in sequence. As a result, Ig-like fold structures are far more conserved than their sequences [28]. Such plasticity makes the Ig-like fold a useful scaffold on which to tailor surfaces that recognize host structures.

The products of a gene cluster in the unique short (US) region of the HCMV genome exemplifies the utility of Ig-like domains in immune evasion. Eight US genes appear to have arisen from duplication and divergence of an ancestral US gene (Table 1, Figure 2b). The US2, US6 and US11 families [29] consist of 20–28 kDa membrane glycoproteins with similar overall architecture: a body that is found in the endoplasmic reticulum (ER) lumen, a single transmembrane segment and a short cytoplasmic tail. The chief function of the products of this gene cluster appears to be modulation of MHC class I cell surface expression through a surprisingly diverse number of mechanisms. US3 retains newly synthesized class I molecules within the ER [30], whereas US2 and US11 each bind and target newly synthesized MHC class I complexes for dislocation to the cytosol, via the Sec61 translocon. Class I heavy chains are subsequently degraded by the proteasome [31].

Although the US2, US6 and US11 protein families do not possess any significant sequence similarity to other known proteins, the crystal structure of the ER-lumenal domain of US2 that was bound to the class I molecule HLA-A2 revealed that this series of proteins also possess Ig-like folds [32••] (Figure 2a,b). Importantly, US2 binding does not significantly alter the conformation of HLA-A2 [32••]. Furthermore, a recombinant TCR and US2 can simultaneously recognize class I molecules *in vitro* [33]. Ig-like

Figure 3



Poor HLA-DM/DR conservation of the US2 class-I-binding site. **(a)** Ribbon diagrams of HLA-A2–Tax-peptide [58], HLA-DR2 with a peptide derived from myelin basic protein (MBP) [59] and HLA-DM [57]. The chains are colored as follows: class I heavy chain is yellow, β_2-microglobulin is gray, peptides are green and disulfide bonds are cyan. HLA-A2 residues contacted by US2 – and homologous residues on HLA-DR2 and HLA-DM α and β chains – are colored magenta and numbered. **(b)** Alignment of consensus (Cons.) class I, HLA-DR and HLA-DM residues near the US2-binding site of HLA-A2. Residues that contact US2 are enclosed in boxes. Residues that match the consensus class I sequence are capitalized. Whereas the US2-binding site on class I is relatively well conserved amongst class I loci, HLA-DR and HLA-DM are markedly different.

domains generally mediate adherence between structures, rather than possessing direct enzymatic function. Thus, US2 may act as an adaptor protein, recruiting unidentified components of the ER-dislocation machinery to the US2–class-I complex. Tight binding of the US2 ER-lumenal domain to class I molecules raises the question of whether the US2–class-I complex transits through the translocon or whether disassembly of the complex precedes dislocation.

It is notable that HCMV evolved novel class-I-binding proteins, rather than capturing and modifying existing cellular class-I-binding proteins. US2 binds to a novel surface on class I that may arise late in class I biosynthesis at the junction of the peptide-binding cleft and the α3 domain (Figure 2c). US2 associates with folded, peptide-loaded class I in a peptide-sequence-independent fashion [32••,33], although the US2 class-I-binding surface is likely to also be available prior to peptide loading. Late in class I biogenesis, empty class I molecules associate with a multiprotein assembly, the peptide-loading complex, that includes calreticulin, ERp57, tapasin and the transporter associated with antigen presentation (TAP) [34]. Virus proteins that bind class I in the ER must compete with the peptide-loading complex for access to class I. Indeed, the US2-interaction surface maps to a face of class I opposite to where mutagenesis studies have localized the docking site of the peptide-loading complex [32••]. Thus, US2 evolved a binding site that should allow free access to class I complexes independently of whether the peptide-loading complex is present [32••]. Importantly, the region on the class I molecule to which US2 binds shows rather limited polymorphism, allowing US2 to broadly down-regulate class I complexes (Figure 3b).

The binding site for adenovirus E3-19K, which retains class I complexes in the ER, is thought to include residues on the amino terminus of the α1 helix and carboxyl terminus of the α2 helix [35–37]. Thus, like US2, E3-19K appears to have evolved a class-I-binding surface that would not be occluded by the peptide-loading complex. It is likely, based on inspection of the primary sequence, that adenovirus E3-19K will fold into an Ig-like domain.

Virus proteins can also recognize the transmembrane domain and cytoplasmic tails of the class I product in question. Several virus proteins, such as HIV Nef, circumvent interference by the loading-complex by interacting with the class I cytoplasmic tail [38••]. Kaposi's-sarcoma-associated herpesvirus (KSHV) encodes two transmembrane proteins, K3 and K5, that possess RING-finger-like domains and target class I complexes on the cell surface for lysosomal degradation [39•,40•,41••]. K3 and K5 interact with the transmembrane domain and/or tail of class I molecules. A K3 homologue has recently been described in murine γ-herpesvirus 68 (Table 1). The product of the human herpesvirus 7 (HHV-7) U21 gene binds MHC class I molecules and reduces their cell surface expression mainly by redirecting them to an endocytic compartment. The U21 protein shows no similarity to any immuno-evasins described so far, or to any cellular protein (A Hudson, HL Ploegh, PM Howley, unpublished data). The U21-binding site on class I has not yet been determined.

Although US2, US3 and US11 share sequence and structural similarity, the US2 residues that bind class I are not conserved in US3 and US11 (Figure 2b). Therefore, these immuno-evasins are likely to interact with unique interaction surfaces on class I molecules. Whereas US2 and US11 fail to interact with HLA-C and HLA-E locus products *in vivo* [42], US3 can retain molecules from each class I locus [43]. Recent studies indicate that US3 requires both its transmembrane and ER-lumenal domains to retain class I molecules in the ER [44] (Table 1). This interaction appears specific for the US3 transmembrane domain, since US3 chimeras possessing a CD4 transmembrane domain were not able to retain class I molecules *in vivo* [44]. In agreement with these results, recombinant soluble US3 fails to interact tightly with class I molecules *in vitro* (BE Gewurz, HL Ploegh, unpublished data). Further, mutation of a single HLA-A2 residue present at the US2/HLA-A2 interface, arginine 181, diminishes degradation of class I heavy chains by US2 [32••], but not by US11 (D Tortorella, BE Gewurz, J Mitra, HL Ploegh, unpublished data). Recent unpublished data from our laboratory suggest that several additional HCMV US proteins associate with class I molecules (see Table 1).

It has been reported that US2 also recognizes HLA-DR and HLA-DM complexes [45•]. Interestingly, the class-I-binding site recognized by US2 is poorly conserved on HLA-DM and HLA-DR (Figure 3). Whereas soluble US2 readily forms stable complexes *in vitro* with multiple recombinant HLA-A locus products, no complex can be detected between US2 and soluble HLA-DR or HLA-DM *in vitro* [33]. We have likewise been unable to detect complex formation between US2 and soluble recombinant HLA-B locus products *in vitro* even though, in living cells, US2 apparently fails to differentiate between HLA-A and HLA-B locus products. These observations raise the possibility of additional US2 interaction sites, maybe within the transmembrane or cytosolic regions.

Inhibition of TAP

The ER-lumenal domain of US6, which may also be a derivative of the Ig-like fold present in US2, inhibits TAP peptide transport. US6 binding stabilizes a TAP conformation that prevents subsequent ATP binding [46•] In contrast, a 34-residue peptide of Herpes simplex virus ICP47 inhibits TAP from the cytosolic face [47]. ICP47 competitively inhibits peptide binding [48] and therefore most probably binds to the TAP substrate-binding site [47]. Preliminary nuclear magnetic resonance (NMR) structural characterization of this ICP47 peptide reveals a helix–linker–helix motif, induced by a lipid environment [49•]. Interestingly, other peptide or polypeptide transporters that are homologous to TAP recognize helix–linker–helix substrate-recognition motifs [50]. Further understanding of US6 and ICP47 function awaits structural analysis of the complexes between TAP and its viral inhibitors.

Conclusions

The study of immune evasion has shown a remarkable diversity in targets and mechanisms employed, as displayed by large DNA viruses. A great deal of structural information is available on host targets, but for the viral proteins this analysis is only now beginning. The ability to inspect the structures of immuno-evasins is likely to shed light on their mode of action and future experiments may now be designed with the benefit of more detailed structural knowledge. Similarly, knowledge of structural motifs may allow a more directed approach to testing the presumed function of some immuno-evasins. As more structures become available for virus immuno-evasins with little homology to host structures, Ig-like folds will probably emerge as a frequently exploited scaffold for recognition of host structures.

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