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Viral mechanisms of immune evasion

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During the millions of years they have coexisted with their hosts, viruses have learned how to manipulate host immune control mechanisms. Viral gene functions provide an overview of many relevant principles in cell biology and immunology. Our knowledge of viral gene functions must be integrated into virus–host interaction networks to understand viral pathogenesis, and could lead to new anti-viral strategies and the ability to exploit viral functions as tools in medicine.

Viruses must be extremely successful predators as they depend on living cells for replication. Almost all living species represent prey for a viral invader. Viruses have coevolved with their hosts and therefore have limited pathogenicity in an immunocompetent natural host. In turn, probably as a result of the constant evolutionary pressure from viral invaders, higher vertebrates have developed a complex immune system. Only in the last decade have we have caught a glimpse of what viruses do beyond invading cells for replication. For millions of years viruses have studied cell biology and immunology the hard way, to acquire and defend an ecological niche. It is remarkable that, in the process, individual virus families have targeted many common immunological principles.

Viruses that belong to different families are subject to different constraints. Owing to the low fidelity of RNA polymerase, the genome size of RNA viruses is limited. Although this confers the advantage of being able to use mutation to escape immune control, there is little room in the genome to allow immune defenses to be encoded by individual genes. The proteins encoded by RNA viruses are therefore multifunctional. This particular constraint is less rigid for DNA viruses as their genome size allows a larger number of genes to be devoted to host control. In the case of herpesviruses and poxviruses, these genes probably account for >50% of the total genome.

Viruses can exist in two forms: extracellular virion particles and intracellular genomes. Virions are more resistant to physical stress than genomes but are susceptible to humoral immune control. Virus genomes can be maintained in host cells by limited gene expression and can evade the host immune response. Nevertheless, to exist as a species, virus replication and transfer to a new host are essential. These processes are associated with the production of antigenic proteins that make the virus vulnerable to immune control mechanisms 'warning' the host of the presence of an invader. However, viruses have evolved strategies to evade such immune control mechanisms, and the list of these strategies forms the 'Who's who' of today's immunology.

There are two classes of viral immunoregulatory proteins: those encoded by genes with, and those encoded by genes without, sequence homology to cellular genes. Viral homologs of host genes involved in the immune system are mainly found in large DNA viruses (herpesviruses and poxviruses) and their existence suggests that viruses have 'stolen' genes from the host that were subsequently modified for the benefit of the virus. Viral genes without sequence similarity to cellular genes might represent a paradigm for co-evolution or could simply be examples of proteins for which the host homologs have not yet been identified. These proteins might possess specific motifs or particular folding properties required for interaction with the host cellular machineries.

In this review, and the accompanying poster, we provide an overview of the different mechanisms that viruses use to evade host immune responses. The basic concepts of virus immune evasion will be discussed, with some examples to illustrate particular points; however, space constraints have not allowed a comprehensive review of all immune-evasion strategies. The strategies are listed in the accompanying tables and are discussed in more detail in the references given throughout the text.

Inhibition of humoral responses

Antigenic variability was one of the first viral immune-evasion strategies to be identified. Because of the low fidelity of RNA polymerases, viral RNA genomes comprise a collection of RNA species (quasispecies) with random mutations. Therefore, in RNA viruses, the generation and selection of variants with different antigenic properties that can evade recognition by neutralizing antibodies is common. Genetic variability can also generate variant peptide sequences that are either new antigens or that do not bind to major histocompatibility complex (MHC) molecules at all.

The complement system is a major non-specific host defense mechanism[1–3]. Viruses encode homologs of complement regulatory proteins that are secreted and block complement activation and neutralization of virus particles (Table 1; Box 1). The cowpox virus (CPV) complement inhibitor, termed inflammation modulatory protein (IMP), blocks immunopathological tissue damage at the site of infection, presumably by inhibiting production of the macrophage chemoattractant factors C3a and C5a (Ref. 3). Viruses protect the membranes of infected cells and the lipid envelopes of virus particles from complement lysis by encoding homologs of inhibitors of the membrane-attack complex. Viruses such as HIV, human cytomegalovirus (HCMV) and vaccinia virus (VV) utilize a clever strategy, 'borrowing' host cellular factors, including CD59, which normally protects cells from complement lysis, and incorporating them into the viral envelope.

Lastly, some viruses encode Fc receptors[1] (Table 1). Antibodies bound to infected cells or virus particles might therefore be bound at the Fc region, thereby inhibiting Fc-dependent immune activation of

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Table 1. Viral inhibition of humoral immunity (complement and antibodies)

Function/activity	Gene/protein	Virus	Mechanism	Refs
Inhibition of soluble complement factors	vCP/C21L, IMP, SPICE, gC, ORF4, CCPH, gp120-gp41	VV, CPV, VaV, HSV-1, HSV-2 HVS, HHV-8, MHV-68	Viral homologs of C4BP, CR1, CD46 or CD55	1–3
		HIV	Recruitment of factor H	1
Blockade of formation of membrane-attack complex	ORF15	HVS	Viral CD59 homolog	1, 3
	Host proteins CD59, CD55 or CD46	VV, HIV, HTLV, HCMV	Host proteins incorporated into virion envelope	1, 3, 34
Viral IgG Fc receptors	gE-gI, gE, Fcr1, S peplomer	HSV-1, HSV-2, MCMV, coronavirus	Binding of IgG and inhibition of Fc-dependent immune activation	1, 4, 34

complement and phagocytes. Fc receptors probably have additional functions *in vivo*[4].

Interference with interferons

Interferons (IFNs) were discovered because of their ability to protect cells from viral infection. The key role of both type I (α and β) and type II (γ) IFNs as one of the first anti-viral defense mechanisms is highlighted by the fact that anti-IFN strategies are present in most viruses[5–7] (Table 2). Viruses block IFN-induced transcriptional responses and the janus kinase (JAK)/signal transducers and activators of transcription (STAT) signal transduction pathways, and also inhibit the activation of IFN effector pathways that induce an anti-viral state in the cell and limit virus replication. This is mainly achieved by inhibiting double-stranded (ds)-RNA-dependent protein kinase (PKR) activation, the phosphorylation of eukaryotic translation initiation factor 2α (eIF-2α) and the RNase L system, which might degrade viral RNA and arrest translation in the host cell.

Poxviruses encode soluble versions of receptors for IFN-α and -β (IFN-α/βR) and IFN-γ (IFN-γR), which also block the immune functions of IFNs[6]. The VV-secreted IFN-α/βR is also localized at the cell surface to protect cells from IFN (Table 3). Additionally, several viruses inhibit the activity of IFN-γ, a key activator of cellular immunity, by blocking the synthesis or activity of factors required for its production, such as interleukin (IL)-18 or IL-12 (Table 4): CPV cytokine response modifier (Crm) A inhibits caspase-1, which processes the mature forms of IL-1β and IL-18 (Refs 2, 6); various poxviruses encode soluble IL-18-binding proteins (IL-18BPs)[8–10]; measles virus (MeV) binds CD46 in macrophages and inhibits IL-12 production[1]; and herpesviruses and poxviruses express IL-10 homologs that diminish the Th1 response by downregulating the production of IL-12 (Refs 1, 11, 12).

Box 1. Abbreviations in Tables

2′5′OA, 2′5′ oligoadenylate; 2′5′OS, 2′5′ OA synthetase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; AHV, alcelaphine herpesvirus; ASFV, African swine fever virus; BHV, bovine herpesvirus; BP, binding protein; BPV, bovine papilloma virus; CaPV, capripox virus; CCI, chemokine inhibitor; CCPH, complement control protein homolog; CK, chemokine; CKBP, chemokine-binding protein; CP, complement control protein CPV, cowpox virus; Crm, cytokine response modifier; CSF, colony-stimulating factor; dsRNA, double stranded RNA; EBER, EBV-encoded small RNA; EBV, Epstein–Barr virus; EGF, epidermal growth factor; EHV, equine herpesvirus; eIF-2α, eukaryotic translation initiation factor 2α; EMCV, encephalomyocarditis virus; ER, endoplasmic reticulum; ESPR, virus-encoded semaphorin protein receptor; EV, ectromelia (mousepox) virus; FLIP, FLICE inhibitory protein; GF growth factor; GM-CSF, granulocyte–macrophage CSF; gp, glycoprotein; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; HHV, human herpesvirus; HHV-8, human herpesvirus 8 or Kaposi's-sarcoma-associated herpesvirus; HPIV, human parainfluenza virus; HPV, human papilloma virus; HTLV, human T cell leukemia virus; HSV, herpes simplex virus; HVS, herpesvirus saimiri; IκB, inhibitor of κB; IAP, inhibitor of apoptosis; ICP, infected cell protein; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IRF, interferon regulatory factor; JAK janus kinase; LMP, latent membrane protein; LT, lymphotoxin; MCK, murine cytomegalovirus chemokine; MCMV, murine cytomeglovirus; MCV, molluscum contagiosum virus; MeV, measles virus; MGF, myxoma growth factor; MHC, major histocompatibility complex; MHV-68, murine gammaherpesvirus 68; MIP, macrophage inflammatory protein; MPV, murine polyoma virus; MV, myxoma virus; NF-κB, nuclear factor κB; NFAT, nuclear factor activated T cell; NK, natural killer; ORF, open reading frame; OV, Orf virus; PKR, dsRNA-dependent protein kinase; R, receptor; RANTES, regulated upon activation normal T cell expressed and secreted; RCMV, rat cytomegalovirus; RID, receptor internalization and degradation complex; SEMA, semaphorin; serpin, serine protease inhibitor; SeV, Sendai virus; SFV, Shope fibroma virus; SPI, serine protein inhibitor; SPV, swinepox virus; STAT, signal transducers and activators of transcription; SV, simian virus; TAP, transporters associated with antigen processing; TAR, trans-acting response element; TNF, tumor necrosis factor; TPV, Tanapox virus; v, viral; VaV, variola (smallpox) virus; VEGF, vascular endothelial growth factor; VV, vaccinia virus.

Table 2. Viral interference with IFN

Function/activity	Gene/protein	Virus	Mechanism	Refs
Inhibition of JAK/STAT pathway	EIA	Adenovirus	Decreases the levels of STAT1 and p48	1, 7, 42
	EBNA-2	EBV	Downregulates IFN-induced transcription	1, 7
	Unknown	HCMV	Reduces levels of JAK1 and p48; involvement of proteasome	7
	Unknown	HPIV-2	Targets STAT2 for degradation	7
	Unknown	HPIV-3, SeV	Block STAT1 phosphorylation	7
	E7	HPV-16	Binds to p48	7
	T antigen	MPV	Binds to and inactivates JAK1	7
	V protein	SV5	Targets STAT1 for proteasome-mediated degradation	7
IFN-induced transcription	IRF homolog	HHV-8	Represses transcriptional responses to IFNs	1, 7
	Capsid protein	HBV	Inhibits *MxA* gene expression	7
Inhibition of PKR activity	σ3, NSP3, E3L, OV20.0L, NS1	Reovirus, rotavirus, VV, OV, influenza virus	Bind dsRNA and prevent PKR activation	2, 5–7
	VAI RNA, EBER RNA, TAR RNA	Adenovirus, EBV, HIV	RNA that binds to, but fails to activate, PKR	5, 7, 42
	PK2, NS5A and E2, US11, Tat	Baculovirus, HCV, HSV, HIV	Bind to and inhibit PKR	5, 7
	Unknown	Poliovirus	Induced degradation of PKR	5, 7
	Unknown	Influenza virus	Induction of p58IPK, a cellular inhibitor of PKR	5, 7
Inhibition of eIF-2α phosphorylation and translational arrest	K3L	VV	eIF-2α homolog, prevents eIF-2α phosphorylation, also inhibits PKR	2, 5–7
	ICP34.5	HSV	Redirects protein phosphatase 1 to dephosphorylate and re-activate eIF-2α	7
Inhibition of 2′5′OS/RNase L system	σ3,NSP3, E3L, OV20.0L, NS1	Reovirus, rotavirus, VV, OV, influenza virus	Bind dsRNA and prevent activation of 2′5′OS/RNase L	2, 6, 7
	Unknown	EMCV, HIV	Induce RNase L inhibitor, which antagonizes 2′5′OA binding to RNase L	7
	Unknown	HSV	Synthesis of 2′5′OA antagonists	7

Inhibition and modulation of cytokines and chemokines

Cytokines play a key role in the initiation and regulation of the innate and adaptive immune responses, and viruses have learned how to block cytokine production, activity and signal transduction (Tables 3 and 4). African swine fever virus (ASFV) replicates in macrophages and encodes an IκB homolog that blocks cytokine expression mediated by nuclear factor (NF)-κB and the nuclear factor activated T cell (NFAT) transcription factors[13]. Many viruses block signal tranduction by ligands of the tumor necrosis factor (TNF) family, whereas others deliberately induce some cytokine pathways; for example, the Epstein–Barr virus (EBV) latent membrane protein 1 (LMP1) recruits components of the TNF receptor (TNFR) and CD40 transduction machinery to mimic cytokine responses that could be beneficial for the virus, such as cell proliferation[14] (Table 4).

One of the most interesting mechanisms identified in recent years is the mimicry of cytokines (virokines) and cytokine receptors (viroceptors) by large DNA viruses (herpesviruses and poxviruses)[1,2,11,15,16] (Table 3). The functions of these molecules in the animal host are diverse. Soluble viral cytokine receptors might neutralize cytokine activity and cytokine homologs might redirect the immune response for the benefit of the virus. Alternatively, viruses that infect immune cells might use these homologs to induce signaling pathways in the infected cell that promote virus replication.

The herpesvirus cytokine homologs vIL-6 and vIL-17 might have immunomodulatory activity but might also increase proliferation of cells that are targets for viral replication[1,11]. Viral semaphorin homologs have uncovered a role for the semaphorin family – previously known as chemoattractants or chemorepellents involved in axonal guidance during development – in the immune system, and have identified a semaphorin receptor in macrophages that mediates cytokine production[17].

Secreted cytokine receptors or binding proteins are mainly encoded by poxviruses[2,6,11,15,18]. These proteins were originally

Table 3. Viral cytokines and cytokine receptors

Function/activity	Gene/protein	Virus	Mechanism	Refs
vTNFR	M-T2	MV, SFV	Secreted, binds rabbit TNF	2, 11, 15, 18
	CrmB	CPV, VaV	Secreted, binds TNF and LT-α	2, 11, 18
	CrmC	CPV, VV	Secreted, binds TNF	2, 18
	CrmD	CPV, EV	Secreted, binds TNF and LT-α	19
	CrmE	CPV	Secreted, binds TNF	
	Unknown	VV	TNFR at the surface of VV-infected cells	20
	UL144	HCMV	TNFR homolog, unknown function	34
vIL-1βR	B15R	VV	Secreted, binds IL-1β, blocks febrile response	2, 11, 18
vIFN-γR	M-T7, B8R	MV, VV, CPV	Secreted, binds IFN-γ from various species	2, 6, 11, 15
vIFN-α/βR	B18R	VV	Secreted and cell surface, binds type I IFN from various species	2, 6, 11
vCSF-1R	BARF-1	EBV	Secreted, binds CSF-1	1
vGM-CSF/IL-2BP	GIF	OV	Secreted, binds GM-CSF and IL-2	21
vIL-18BP	MC54	MCV	Secreted, binds IL-18, inhibits IL-18 induced IFN-γ production	8, 9
	MC53	MCV	Secreted, binds IL-18, inhibits IL-18-induced IFN-γ production	8
	D7L	EV, VV, CPV, VaV	Secreted, binds IL-18, inhibits IL-18-induced IFN-γ production and NK response	9, 10
vIFN-γ/IL-2/IL-5BP	Unknown	TPV	35 kDa, secreted, binds IFN-γ, IL-2 and IL-5	2, 18
vCKBP	vCKBP-I, M-T7	MV	Secreted, binds C, CC and CXC CKs through heparin-binding site	2, 15, 16, 18
	vCKBP-II, B29R, G3R, CCI, H5R, M-T1, S-T1	VV, EV, VaV, CPV, MV, SFV	Secreted, binds CC CKs	2, 15, 16, 18, 28
	vCKBP-III, M3	MHV-68	Secreted, binds CC, CXC, C and CX3C CKs	29
vCKR	ORF74	HHV-8, HVS, MHV-68, EHV-2	HVS ORF74 is a functional CXCR, HHV-8 ORF74 binds CC and CXC CKs, is constitutively activated and induces cell proliferation *in vitro* and tumors in transgenic mice	16, 22, 27
	US28, E1	HCMV, EHV-2	HCMV US28 binds CC CKs, mediates cell migration and decreases local concentration of RANTES; EHV-2 E1 binds eotaxin	16, 22, 26, 43
	US27, E6	HCMV, EHV-2	Unknown	16, 22
	U12, UL33, M33, R33	HHV-6, HHV-7, HCMV, MCMV, RCMV	HHV-6 U12 binds CC CKs, required for *in vivo* replication of MCMV and RCMV	16, 22
	U51, UL78, M78	HHV-6, HHV-7, HCMV, MCMV	HHV-6 U51 binds CC and CXC CKs and induces downregulation of RANTES transcription	16, 22, 25
	K2R	SPV	IL-8 CKR homolog	16, 18
	Q2/3L	CaPV	CC CKR homolog	16, 18
vCK	vMIP-I	HHV-8	CCR8 agonist, Th2 chemoattractant, angiogenic activity	16, 22
	vMIP-II	HHV-8	C, CC, CXC and CX3C CK antagonist	16, 22, 44
	vMIP-III	HHV-8	Unknown	16, 22
	U83	HHV-6	CC CK agonist	16, 22
	MCK-1/2, m131	MCMV	CC CK agonist, chemoattraction of monocytes, promotes monocyte-associated viremia *in vivo*	16, 22, 23
	vCXC-1/UL146	HCMV	CXC CK agonist, chemoattraction of neutrophils	16
	vCXC-2/UL147	HCMV	Unknown	16
	MCC-1/MC148	MCV	Specific CCR8 antagonist, interference with monocyte function	16, 44
	Tat	HIV	Partial CK similarity, chemoattractant for monocytes	24
vGF	C11R, MGF	VV, MV	EGF, a TGF-α homolog, stimulates cell growth, virulence factor	11, 15
vVEGF	A2R	OV	Angiogenic factor	11, 45
vIL-10	BCRF-1, IL-10 gene	EBV, EHV, OV	IL-10 activity, downregulates Th1 response	1, 11
	UL111a	HCMV	IL-10 activity, low sequence similarity to other vIL-10	12
vIL-17	ORF13	HVS	T cell mitogen	1, 11
vIL-6	K2	HHV-8	Angiogenic factor, B cell growth factor	1, 5
vSEMA	A39R	VV, EV	Semaphorin homolog, binds semaphorin receptor vESPR	17
	AHV-SEMA	AHV	Semaphorin homolog	17

[a]M. Saraiva and A. Alcami, unpublished.

Table 4. Viral inhibitors and modulators of cytokine activity

Function/activity	Gene/protein	Virus	Mechanism	Refs
Inhibition of TNF signaling	E3 14.7K, E3 10.4/15.4K, E1B 19K	Adenovirus	Prevent TNF cytolysis and block phopholipase A2 activation	42
Mimicry of TNFR/CD40 signaling	LMP-1	EBV	Recruits death-domain-containing proteins and induces signals of the TNFR/CD40 pathway	14
IκB homolog	A238L	ASFV	Inhibition of NFκB/NFAT signaling	13
Inhibition of maturation of cytokines	CrmA, SPI-2, B13R, SERP-2	CPV, VV, MV	Inhibition of IL-1β converting enzyme (ICE, caspase-1), inhibition of IL-1β, and possibly IL-18, cleavage	2, 15, 18
Inhibition of IL-12 production	Hemagglutinin	MeV	Binds to CD46 and blocks induction of IL-12 by macrophages	1

identified as homologs of host TNFRs, IL-1Rs and IFN-γRs. The discovery of four distinct soluble poxvirus TNFRs, and a membrane TNF-binding activity in VV infections, is remarkable and suggests that viral TNFRs might have additional functions[18–20] (M. Saraiva and A. Alcami, unpublished). Binding and activity assays have identified secreted proteins that bind IFN-α and -β, chemokines (CKs) or granuloctye–macrophage colony-stimulating factor (GM-CSF) and IL-2, and that have no sequence similarity to cellular counterparts[6,18,21]. In poxviruses, three distinct secreted IL-18BPs that have recently been identified are homologs of human and mouse secreted IL-18BPs but not of membrane IL-18Rs[8–10]. Inactivation of poxvirus cytokine receptor genes results in virus attenuation *in vivo* but, interestingly, deletion of the VV IL-1βR enhances virus virulence and the onset of fever, suggesting that the purpose of some immune-evasion mechanisms is to reduce the immunopathology caused by viral infection[18].

Herpesviruses and poxviruses modulate the activity of chemoattractant cytokines or CKs that regulate leukocyte trafficking to sites of infection[16,18,22]. Virus-encoded CKs are either antagonists that block leukocyte recruitment to sites of infection, or agonists that could enhance the recruitment of cells that support viral replication or prevent Th1 anti-viral responses. Murine cytomegalovirus (MCMV) chemokine 1 (MCK-1) activates monocytes *in vitro* and increases monocyte-associated viremia *in vivo*[23]. HIV Tat is partially homologous to CKs and is a potent monocyte chemoattractant[24]. Herpesviruses encode many CK receptors (vCKRs) but their function is not clear. Kaposi's-sarcoma-associated virus [human herpesvirus 8 (HHV-8)] open reading frame (ORF) 74 is constitutively activated and induces cell proliferation, which might favor virus replication. vCKRs encoded by HCMV and HHV-6 reduce the amount of the factor regulated upon activation normal T-cell expressed and secreted (RANTES) in tissue culture and/or its transcription and might inhibit CK activity locally[16,25]. A role for vCKRs *in vivo* has been shown for MCMV. vCKs and vCKRs might contribute directly to pathology. The angiogenic properties of HHV-8 macrophage inflammatory protein I (vMIP-I) could account for the increased vascularization found in HHV-8-associated tumors, human cytomegalovirus (HCMV) US28 mediates vascular smooth muscle cell migration and perhaps vascular disease[26], and expression of HHV-8 ORF74 in transgenic mice results in Kaposi's-sarcoma-like lesions[27].

Three soluble vCKBPs have been identified that have no sequence similarity to cellular CKRs[2,15,16,18]. vCKBP-I is a soluble IFN-γR encoded by MV, but not VV, which binds the heparin-binding domain of a wide range of CKs and might prevent the correct localization of CKs *in vivo* by blocking their interaction with proteoglycans. The poxvirus-secreted vCKBP-II, which has a novel protein structure[28], binds CC CKs with high affinity and blocks their activity. Murine gamma-herpesvirus 68 (MHV-68) has recently been shown to encode a distinct secreted protein (vCKBP-III) that sequesters C, CC, CXC and CX_3C CKs[29].

Inhibitors of apoptosis

Apoptosis, or programmed cell death, can be triggered by a variety of inducers, including ligands of the TNF family, irradiation, cell-cycle inhibitors or infectious agents such as viruses. Apoptosis can be considered an innate cellular response to limit viral propagation, and viruses express proteins that block the death response (Table 5); however, apoptosis might also facilitate virus dissemination, and viral pro-apoptotic mechanisms have been described[30]. In addition, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells kill virus-infected cells by inducing apoptosis via secretion of cytokines such as TNF, the release of perforin and granzymes, or the activation of Fas in the target cell.

The cellular proteins implicated in the control of apoptosis are targeted by viral anti-apoptotic mechanisms[1,5,30,31]. Viruses inhibit activation of caspases, encode homologs of the anti-apoptotic protein Bcl-2, block apoptotic signals triggered by activation of TNFR family members by encoding death-effector-domain-containing proteins, and inactivate IFN-induced PKR and the tumor suppressor p53, both of which promote apoptosis. An alternative mechanism is provided by the glutathione peroxidase of molluscum contagiosum virus (MCV), which provides protection from peroxide- or UV-induced apoptosis, and perhaps from peroxides induced by TNF, macrophages or neutrophils.

Table 5. Viral inhibitors of apoptosis

Function/activity	Gene/protein	Virus	Mechanism	Refs
vFLIP	K13, ORF71, E8, BORFE2, MC159, MC160	HHV-8, HVS, EHV-2, BHV-4, MCV	Inhibition of activation of caspases and apoptosis induced by death receptors	1, 30, 31
vBcl-2	ORF16, M11, BHRF1, BALF1, 5HL/A179L, E1B-19K, A9, BORF-B2	HHV-8, HVS, EHV, MHV-68, EBV, ASFV, adenovirus, AHV, BHV-4	Homologs of the anti-apoptotic protein Bcl-2	1, 5, 30
Anti-oxidant selenoprotein	MC66	MCV	Selenocysteine-containing glutathione peroxidase, scavenger of reactive oxygen metabolites	1, 30, 31
Caspase inhibitor	CrmA, SPI-2, B13R, SERP-2	CPV, VV, MV	Serpin, inhibits caspase-1/8 and granzyme B	1, 5, 15, 30, 31
	p35, IAP	Baculovirus	Inhibit multiple caspases	1, 5, 30
	4CL/A224L	ASFV	IAP homolog	1, 5, 30
	14.7K	Adenovirus	Inhibits caspases; interacts with caspase-8	1, 5, 30, 42
Inactivation of p53	E6	HPV	Targets p53 for proteolytic degradation	1, 30
	T antigen, E1B-55K, E4 orf6	SV40, adenovirus	Bind and inactivate p53	1, 30, 42
GADD34 homology	g1, 23NL	HSV, ASFV	Homology to cellular growth arrest and cellular damage gene GADD34	5
Others	E3 10.4/14.5-RID complex	Adenovirus	Targets Fas for lysosomal degradation	1, 30, 42
	M11L	MV	Targets to mitochondria, inhibits apoptosis of monocytes	15, 30, 31, 46
	US3	HSV-1	Unknown; Ser/Thr kinase	1, 5
	IE-1, IE-2	HCMV	Inhibits TNF-induced apoptosis	1, 5, 34
	SPI-1, B22R	VV, CPV	Serpin	31
	M-T4	MV	Unknown, retained in ER	15, 30
	p28, N1R	EV, SFV	RING finger motif, prevents UV-induced apoptosis	47
	UL37	HCMV	Blocks apoptosis mediated by death receptors, localizes in mitochondria, not Bcl-2 homolog	48

Evading CTLs and NKs, and modulating MHC function

How to achieve persistence in the face of a vigorous host immune response is a problem that must be solved by viruses that establish lifelong infections. Cellular proteins are degraded by the proteasome, the complex major intracellular protease, and the resulting peptides are translocated by transporters associated with antigen processing (TAP) molecules into the endoplasmic reticulum (ER), where they contribute to the assembly of MHC class I molecules[1,11,32–34]. MHC class I molecules indicate the composition of cellular proteins to cells of the immune system. The presentation of foreign peptides activates and attracts cytolytic $CD8^+$ T cells. Interference with antigen processing [e.g. Epstein–Barr nuclear antigen A1 (EBNA1)] or TAP function [e.g. herpes simplex virus (HSV) infected cell protein 47 (ICP47) and HCMV US6 and pp65] prevents peptide generation and transport either specifically or generally (Table 6). Viruses use various mechanisms to modify the maturation, assembly and export of MHC class I molecules. To date, no cellular homologs have been found for the proteins and functions that target peptide processing, transport and MHC maturation. With few exceptions[35], the viral proteins bind their target molecule directly. There is only limited functional homology and no sequence homology among the different viral effectors. Nevertheless, the general outcome of these functions is the same: downregulation of MHC class I molecules or of some MHC class I alleles. The study of MHC class I regulation has revealed additional genes in herpesviruses of different species[36–38], which might affect many cell types or only those tissues relevant for virus maintenance.

Although the downregulation of MHC class I expression prevents $CD8^+$ T-cell recognition, cells that downregulate these molecules become targets for NK cells[1,11,32–34]. NK cells, the first line of cellular defense against viruses, have receptors for certain MHC molecules. Some of these receptors silence the cytolytic machinery of NK cells and act as killer cell inhibitory receptors (KIR). Other receptors, designated leukocyte immunoglobulin-like receptors (LIR), are expressed mainly on monocytes and B cells. Engagement of an NK receptor can alternatively result in NK activation as not all receptors have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their intracellular domains. The HCMV protein UL18 and the MCMV m144 protein, which are homologous to MHC class I, could be associated with NK killing, and UL18 is instrumental in the identification of LIR-1. In addition, the HCMV UL40 protein provides a peptide selectively required for the maturation of the HLA-E molecule, an NK target[39,40]. However, clinical isolates of HCMV confer a much stronger NK resistance than the laboratory strains sequenced and tested so far, and this resistance is unrelated to MHC

Table 6. Viral interference with MHC functions

Function/activity	Gene/protein	Virus	Mechanism	Refs
Effect on MHC class I	E3/19K	Adenovirus	Binding and retention of class I in ER	1, 11, 32, 33, 42
	US3	HCMV	Binding and retention of class I in ER	1, 11, 32–34
	US2, US11	HCMV	Relocation of heavy chain into ER for degradation	1, 11, 32–34, 49
	m4	MCMV	Binds class I molecules	1, 11, 32–34
	m6	MCMV	Binding of class I molecules and transport to lysosomes for degradation	1, 11, 32–34
	m152	MCMV	Retains class I in ER–Golgi intermediate compartment	1, 11, 32–35
	K3, K5	HHV-8, MHV-68	Downregulation of class I molecules	36–38
	Nef	HIV	Endocytosis of surface class I and CD4	1, 11, 32, 33, 49
	Vpu	HIV	Destabilization of class I, targets CD4 to proteasome	1, 11, 32, 33, 49
Effect on MHC class II	E1A	Adenovirus	Interferes with class II upregulation (IFN-γ signal transduction cascade)	1, 11, 32, 33
	Unknown	HSV	Interference with class II function	1, 11, 32, 33
	Unknown	HCMV, MCMV	Interference with class II upregulation (IFN-γ signal transduction cascade)	1, 11, 32–34
	US2	HCMV	Targets class II DR, DM α chain for degradation	1, 11, 32–34
	ORF14	HSV	Class II binding	1, 11, 32, 33
	E5, E6	HPV, BPV	Interference with class II processing, E5 acidification of endosomes, E6 interaction with AP complex	1, 11, 32, 33
	Nef	HIV	Interference with class II processing	1, 11, 32, 33
Effect on TAP	ICP-47	HSV	Prevents peptide binding to TAP in cytosol	1, 11, 32, 33
	US6	HCMV	Prevents peptide transport through TAP pore	1, 11, 32–34
Effect on antigen processing	EBNA-1	EBV	A Gly–Ala repeat motif prevents proteasomal degradation	1, 11, 32, 33
	pp65	HCMV	Modulates processing of another HCMV protein	1, 11, 32–34
Effect on NK cells	UL18, m144, r144	HCMV, MCMV, RCMV	Class I homolog, inhibits NK cell lysis	1, 11, 32–34
	MC80	MCV	Class I homolog, function unknown	1, 11, 32, 33
	UL40	HCMV	UL40 peptide causes HLA-E upregulation	39, 40

class I expression and LIR-1 (Ref. 41). Clinical isolates carry additional genes, and *in vitro* propagation has probably led to a loss of certain NK-specific gene functions.

Effects on MHC class II expression fall into two classes, namely effects on transcription and post-translational effects[1,11,32–34]. Adenovirus, MCMV and HCMV affect MHC class II transcription but the target in the signal cascade, although known to be different for these viruses, has not been defined and the viral gene or genes responsible are unknown. At the post-translational level, the HCMV US2 protein, which affects MHC class I, apparently also translocates the DRα and the DMα chain into the cytosol for degradation by the proteasome. Another target involved in interference with MHC class II function is the shuttling between endosomal peptide loading and surface expression. Human papilloma virus (HPV) and HIV Nef affect vesicle traffic as well as the function of the endocytic machinery. Accordingly, in addition to MHC class II, other proteins that use this pathway, for example the CD4 molecule, are also affected.

Future perspectives

An understanding of the functions of the viral immunoregulatory genes isolated to date is now emerging. However, we do not yet know whether the list is complete (Table 7). Additionally, it is unclear when and why a virus deploys one specific function rather than another. Many questions therefore remain unanswered, including which genes are needed during primary infection to 'conquer the territory'; which genes are required to support active replication; and which genes are required to ensure transmission to a new host in the face of a vigorous host immune response? Moreover, why is there such complexity and functional redundancy? Is there a hierarchy in terms of general importance or do some functions operate only in certain tissues? Is complexity and redundancy a viral strategy that enables viruses to infect individuals resistant to some functions? Are the functions of an individual viral gene modulated by its genetic context, and is there any evidence for cooperativity? To date, we only have limited information because the construction of virus mutants and the *in vivo* testing of the predicted gene function is still in its infancy and, additionally, owing to the species specificity of many viruses, this information can only be gathered from some models.

The identification of novel immune-evasion strategies and the analysis of their functions in the context of a viral infection should lead to a better understanding of the immune system and the interaction of viruses with their hosts. This will help us to treat virus-induced pathology, to design safer and more immunogenic virus

Table 7. Other viral immune evasion mechanisms

Function/activity	Gene/protein	Virus	Mechanism	Ref.
Inhibition of inflammation	SERP-1	MV	Secreted serpin, potent anti-inflammatory properties	15
	3β-HSD	VV	Synthesis of steroids with immunosuppressive properties	2
vCD2	EP402R, 8DR	ASFV	Adhesion molecules responsible for erythrocyte hemadsorption; immunosuppressive; might modulate T-cell activation	50

vectors as vaccines or gene delivery systems, and to identify new strategies for immune modulation.

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Antigen-processing machinery breakdown and tumor growth

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Defects in the major histocompatibility complex (MHC) class I antigen-processing machinery (APM) have been described in tumors of different histology. Murine data suggest that defects in the MHC class II APM might also be associated with malignant transformation of human cells. This article describes the pathophysiology of the MHC class I and II APM, reviews APM abnormalities in tumor cells and discusses their role in the escape of tumor cells from in vitro *recognition by T cells.*

For many years, $CD8^+$ cytotoxic T lymphocytes (CTLs) have been used as the major effector cells to control tumor growth both in mice and in humans. However, abnormalities in the major histocompatibility complex (MHC) class I antigens are frequently found in human malignant cells (reviewed in Refs 1–3), which represents a major obstacle to the successful implementation of T-cell-based immunotherapy. This has rekindled interest in the control of tumor growth by tumor-associated antigen (TAA)-specific $CD4^+$ T cells[4]. Indeed, $CD4^+$ and $CD8^+$ T-cell-defined epitopes might have to be incorporated in a tumor vaccine in order to induce an effective TAA-specific cellular immune response[5,6]. In addition, appropriate TAA processing and presentation is a prerequisite for the successful outcome of T-cell-based immunotherapy of malignant diseases[7]. Optimization of the design of the T-cell epitope greatly benefits from detailed knowledge of the pathophysiology of the MHC class I and class II antigen-processing machinery (APM) and of the molecular defects used by tumor cells to escape from T-cell recognition. In this article, the terms class I/II are used to refer to HLA class I and class II molecules.

Class I and class II APM

Class I APM

Over the past decade, the molecular steps of the classical class I-restricted antigen-processing pathway have been well characterized[7] (Fig. 1). The class I molecules assemble in the endoplasmic reticulum (ER) with peptides generated from cytosolic proteins by the multicatalytic proteasome complex and by additional, recently described, cytosolic proteases[7]. The expression of some of the proteasome subunits, such as low-molecular-weight proteins 2 (LMP2), LMP7 and LMP10, and the proteasome activators PA28α and β, can be modulated by cytokines such as interferon γ (IFN-γ) (Ref. 5). Changes in subunit composition sharpen the quantitative and qualitative ability

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