

# Viral interference with the host immune response

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## INTRODUCTION

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P027.1

The clearance of viruses from infected tissues requires a coordinated scenario of multiple effector functions. The immune response with its two arms of innate and adaptive immune responses represents the system by which the organism controls, preserves, and restores integrity. Clearing of the infection can often cause a substantial loss of cells with little functional impairment as a result of cellular regeneration. In the absence of cellular regeneration, e.g. in the central nervous system (CNS), the same loss may already result in considerable functional defects. Whether or not the immunological clearing of a virus infection protects tissues or causes pathology is defined by the speed and amount of virus replication, the strength of the immune response, and the properties of the infected tissues. Therefore, the outcome of either immunoprotection or immunopathology is defined by properties of the virus, the strength of the immune response and by the regenerative activity of the tissues (Levine 2002). Remarkably, viral immune evasion mechanisms target and modulate all aspects of the innate and the adaptive immune response. To discuss the functional relevance of the different viral function it should be recollected that host defense against viruses occurs in a series of waves.

P027.2

Cells monitor their normal state and many of the effects of viruses on the intracellular milieu tend to

activate the process of apoptosis or programmed cell death. This 'suicide' response can be considered a form of host defense, and most viruses seem to need to interfere with this response in order to be able to replicate. The innate immune response starts with the release of soluble mediators of inflammation. These mediators comprise, among others, the interferons, cytokines, chemokines, complement fragments, prostaglandins, and nitric oxide. These key regulators control cell trafficking and cell recruitment to inflamed sites, cell adhesion, and cell activation, but also induce apoptosis. Different cells produce different mediators. Usually, the organism seeks to eliminate the virus completely and the antiviral activities of some soluble mediators are very potent. However, the life cycle of some viruses, in particular those that establish persistent infections, is more complex and for them the evasion of innate immunity is important to reach the target tissues from which the virus cannot be cleared. Among other viruses the well-known hepatitis viruses B and C, human immunodeficiency virus (HIV), and herpesviruses share this property.

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In the temporal scenario of the immune response natural killer (NK) cells form the second line of defense. NK cells play a major role in the early defense to viruses. They do not require prior sensitization to recognize and to kill virus-infected cells. The response is induced within hours of infection and usually peaks around 3 days. Herpesviruses, for example, are very

sensitive to the control by NK cells and the molecules by which these viruses defend themselves against this strong effector mechanism of innate immunity today are the subject of active research.

P027.4 The third wave includes adaptive immune responses which comprise cellular and humoral effector arms. Important cellular effectors are CD8 and CD4 T cells: these are first detectable at days 3–4, and usually peak at about 7 days. CD8 T cells play the critical role and in most cases are able to eliminate the virus. It was noticed first that viruses manage to escape the control of T cells by various molecular principles and this area of research has brought several interesting findings that reshape our understanding of the virus–host balance.

P027.5 In the antibody response initially immunoglobulin IgM appears about 10 days postinfection, followed by IgG. Antibody plays some role in controlling primary infection but its main role is to prevent reinfection (by neutralization). Antibody effects can be mediated by complement activation. Even the antibody response and complement are diverted by some viruses to their profit.

## EFFECTS ON MOLECULES THAT SIGNAL BETWEEN CELLS

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### Modulation of interferon functions

Interferons (IFN) were discovered because of their ability to protect cells from viral infection. The key role of both type I ( $\alpha$  and  $\beta$ ) and type II ( $\gamma$ ) IFN as one of the first antiviral defense mechanisms is highlighted by the fact that anti-IFN strategies are present in most viruses (Figure 31.1).

P027.6

Interferons are produced in response to viral infection, secreted from cells, and bind specific receptors that trigger intracellular events through Janus kinase (JAK) and signal transducers and activators of transcription (STAT) pathways (Goodbourn et al. 2000). These signaling events result in the upregulation of cellular proteins that limit viral replication in several ways. Some of the genes upregulated by IFN are the low-molecular-weight protein (LMP)-2 and LMP-7 subunits of the proteasome, involved in the generation of viral peptides

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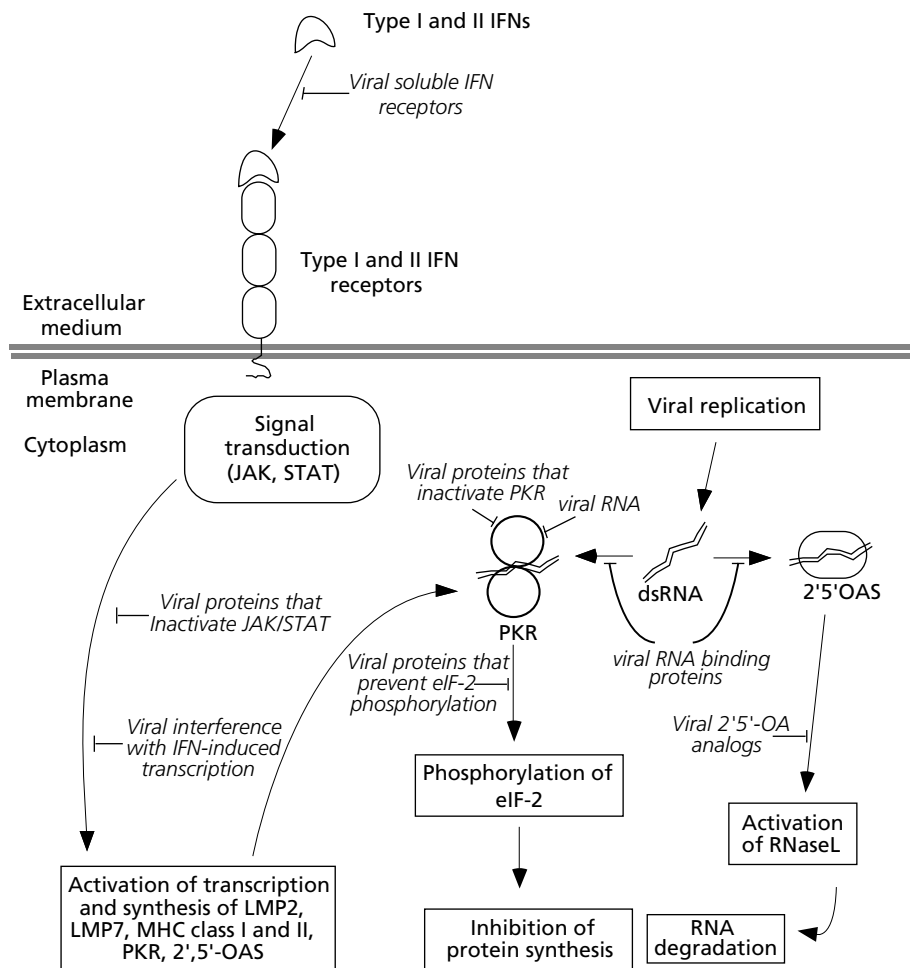


FIG027.1 **Figure 31.1** Viral inhibition of interferon activity. See text for abbreviations.

for the major histocompatibility complex (MHC) class I and class II molecules, the molecules that present viral peptides to T cells. Two major pathways that induce an antiviral state in cells are also upregulated by IFN: the double-stranded ribonucleic acid (RNA) (dsRNA)-dependent protein kinase (PKR) and the 2',5'-oligoadenylate system (2',5'-OAS) that activates RNase L. In addition, IFNs have immunomodulatory activity that can influence the type of immune response mounted as a reaction to infection.

P027.8 Viruses block the activity of IFNs at three different levels: (1) blockade of IFN binding to specific receptors; (2) inhibition of signal transduction pathways induced by IFNs; and (3) inhibition of antiviral effector functions induced by IFNs (see Figure 31.1).

### SECRETED VIRAL IFN RECEPTORS AND IFN-BINDING PROTEINS

P027.9 Poxviruses encode proteins that are secreted from infected cells and bind with high affinity type I or II IFN (McFadden and Murphy 2000; Alcami 2003). These proteins prevent the interaction of IFNs with their receptors and the initiation of biological effects that lead to an antiviral state in the cell. Soluble cytokine receptors or binding proteins for other cytokines have been identified, mainly in the poxvirus family, and examples are discussed below.

P027.10 The viral IFN- $\gamma$  receptor (vIFN- $\gamma$ R) was first identified in myxoma virus (MV) and later in other members of the poxvirus family including vaccinia virus (VV) and cowpox virus (CV) (Upton et al. 1992; Alcami and Smith 1995; Mossman et al. 1995). The vIFN- $\gamma$ R has sequence similarity to the IFN-binding domain of the cellular IFN- $\gamma$  receptor. In addition, VV and other poxviruses also encode a secreted IFN- $\alpha/\beta$  receptor or binding protein (vIFN- $\alpha/\beta$ BP) that has very limited sequence similarity to the cellular counterparts (Colamonici et al. 1995; Symons et al. 1995). The vIFN- $\alpha/\beta$ R has the unique property of binding to the cell surface after secretion (Alcami et al. 2000). This property allows the vIFN- $\alpha/\beta$ R to cover infected tissues with decoy receptors that prevent in a very efficient way the activation of IFN-mediated pathways. The role of vIFN- $\gamma$ R and vIFN- $\alpha/\beta$ BP during viral infection has been demonstrated by the attenuated phenotype of VV and MV mutants lacking these IFN inhibitors (Symons et al. 1995; Mossman et al. 1996).

### INTERFERENCE WITH IFN SIGNALING

P027.11 Some viral proteins interfere with the signaling cascade initiated in cells by the interaction of IFN with their receptors, and prevent the activation of antiviral mechanisms that limit viral replication (Goodbourn et al. 2000), e.g. the T antigen of murine polyoma virus binds to and inactivates JAK1 (Weihua et al. 1998), whereas protein V from simian virus 5 interacts with STAT1 and

induces its degradation by the proteasome (Didcock et al. 1999). A different mechanism is used by Kaposi's sarcoma-associated herpesvirus (KSHV), a virus that encodes a homolog of the IFN-regulatory factor (IRF) which represses the IFN-mediated transcriptional activation of host genes (Zimring et al. 1998).

### VIRAL MODULATION OF IFN EFFECTOR FUNCTIONS

P027.12 One of the major antiviral effects of IFNs is the induction of the dsRNA-dependent PKR and 2',5'-OAS pathways which lead to the blockade of gene expression in the cell and prevent the formation of infectious virus particles (Goodbourn et al. 2000). The replication and transcription of viral genomes can lead to the formation of double-stranded RNA (dsRNA) that activates PKR, which phosphorylates the translation initiation factor 2 $\alpha$  (eIF-2 $\alpha$ ), causing its inactivation and the subsequent arrest of protein synthesis in infected cells. Viruses use several strategies to inhibit the activity of PKR (Gale and Katze 1998; Katze et al. 2002). Some viruses encode proteins that bind dsRNA and prevent activation of PKR, such as the VV E3L and reovirus  $\sigma$ 3 proteins. Adenovirus and HIV encode RNAs that bind PKR but do not activate the enzyme, whereas herpes simplex virus (HSV) US11 and HIV Tat bind directly to PKR and inactivate the enzyme. An alternate strategy to block the PKR pathway is to prevent the phosphorylation of eIF-2 $\alpha$ . This can be achieved by the expression of an eIF-2 $\alpha$  homolog, the K3L protein of VV, which acts as a substrate of PKR, or by HSV IC34.5 protein-activating protein phosphatase 1 $\alpha$  which removes the phosphate groups from eIF-2 $\alpha$ . The formation of dsRNA during viral replication also activates the enzyme 2',5'-OAS, producing 2',5'-OA which in turn activates RNase L and the degradation of RNA within the infected cell, causing the blockade of viral replication. The synthesis of analogs of 2',5'-OA by HSV, which bind RNase L, inhibits this pathway (Goodbourn et al. 2000). The proteins  $\sigma$ 3 from reovirus and E3L from VV bind dsRNA and thus inhibit the activation of 2',5'-OAS as well as PKR (see above).

### Modulation of cytokine networks

P027.13 Cytokines constitute a numerous family of molecules that play an important role in the initiation and regulation of the immune response. Cytokines are normally secreted from cells, although some are expressed at the cell surface, and bind to specific receptors on other cells. In this way, cytokines are used by the immune system to communicate among cells and to send signals that drive the immune response in a particular direction. In addition, some cytokines such as IFN and tumor necrosis factor (TNF) may act directly on infected cells and restrict virus replication.

P027.14 The importance of cytokines in antiviral defense is highlighted by the number of mechanisms that viruses encode to block the activity of cytokines. The viral anticytokine strategies will normally result in down-regulation of immune responses. However, there are examples in which these viral mechanisms increase viral replication. Large DNA viruses such as poxviruses and herpesviruses have captured in their genomes genes that encode host cytokines and cytokine receptors (McFadden and Murphy 2000; Alcami 2003). This strategy enables these viruses to interfere with or to exploit cytokine pathways for their own benefit (Figure 31.2).

## VIRAL CYTOKINES

P027.15 The first viral cytokine identified was the VV epidermal growth factor homologue (VGF). This viral protein activates cell growth and favors viral replication, but an immune-related function has not been ascribed to it (McFadden and Murphy 2000). The first cytokine with immunomodulatory activity found in viruses is the IL-10 homolog (vIL-10) encoded by Epstein–Barr virus (EBV) (Hsu et al. 1990). Genes encoding IL-10 homologs have also been identified in other viruses. It has been shown that the EBV vIL-10 suppresses cellular immunity by inhibiting the production of IFN- $\gamma$ , but has lost the immunostimulatory properties of the host counterpart. Other viral cytokines are the IL-6 homologue (vIL-6) encoded by KSHV (Moore et al. 1996) and the IL-17 homologue (vIL-17) encoded by herpesvirus Saimiri (HVS) (Yao et al. 1995). These cytokines may have immunomodulatory activity but vIL-6 and vIL-17 may also promote the proliferation of B and T cells, respectively, which represent the major host cell types for these viruses.

P027.16 The identification of semaphorin homologs (vSEMA) in poxvirus and herpesvirus genomes was somehow surprising because semaphorins were described as chemoattractants and chemorepellents involved in

axonal guidance during nervous system development, and only CD100 had been identified as a semaphorin involved in the immune system (Spriggs 1999). The characterization of vSEMA encoded by VV and ectromelia virus (EV) uncovered a specific receptor in macrophages and its proinflammatory activity, which was observed in mice infected with a VV expressing an active vSEMA protein. The precise role of vSEMA in immune modulation during viral infection remains to be determined.

## SECRETED VIRAL CYTOKINE RECEPTORS

P027.17 Viral genes encoding soluble cytokine receptors have been identified mainly in the poxvirus family. These cytokine receptors lack the transmembrane and cytoplasmic domains of the host counterparts, are secreted in large quantities from infected cells, and have the ability to bind with high affinity the cognate cytokine and block its activity. The poxvirus soluble cytokine receptors were initially identified because of their sequence similarity to the extracellular binding domain of their host counterparts. A second class of cytokine receptors, known as binding proteins, have very limited sequence similarity to the host cytokine receptors and were identified in cytokine binding or activity assays.

P027.18 The first viral cytokine receptor identified was the viral TNF receptor (vTNFR) M-T2 encoded by MV (Smith et al. 1991). Subsequent studies identified four different genes encoding vTNFR in poxviruses such as CPV, VV, and EV, and were designated cytokine response modifier B (CrmB), CrmC, CrmD, and CrmE (Hu et al. 1994; Smith et al. 1996; Loparev et al. 1998; Saraiva and Alcami 2001). CrmB is equivalent to MV M-T2 but the other proteins are distinct with respect to binding properties and molecular size, and are produced at different times during viral replication. The reasons for such a variety of proteins apparently targeting the

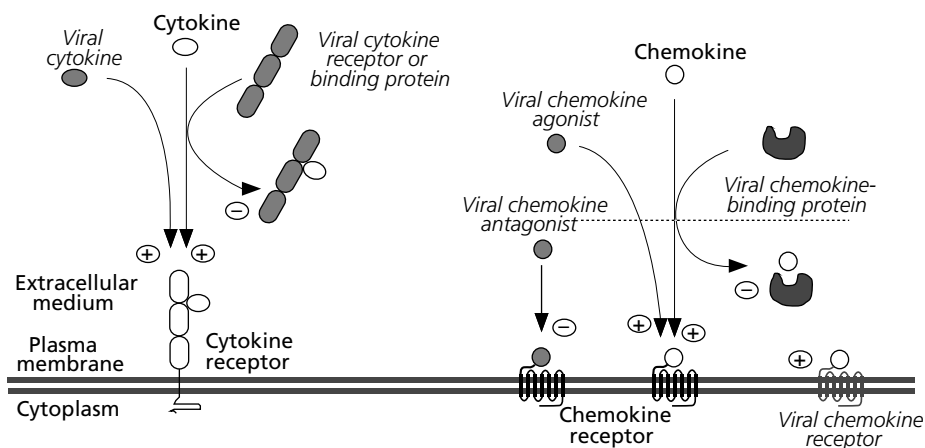


FIG027.2 **Figure 31.2** Viral mimicry of cytokines, chemokines, and their receptors.

same ligand are unknown. Clinical isolates of human cytomegalovirus (HCMV), but not laboratory strains, encode a membrane TNFR homolog related to herpesvirus entry mediator (HVEM) which is retained intracellularly (Benedict et al. 1999). No ligand has been identified for this protein and its function remains to be determined. More recently, another member of the TNFR superfamily has been identified in CPV and EV. In this case, the viral protein is a secreted and shorter version of human and mouse CD30 (Panus et al. 2002; Saraiva et al. 2002). Viral cytokine receptors function as decoy receptors neutralizing the activity of cytokines and, accordingly, vCD30 blocks the interaction of CD30 to CD30 ligand (CD30L). However, in this case the ligand is expressed only at the cell surface and vCD30 induces reverse signaling in CD30L-expressing cells. The function of vCD30 in the context of infection has not been elucidated, but administration of vCD30 to mice has been shown to downregulate type 1 cytokine-mediated inflammatory responses, a property that may help the virus to escape an efficient antiviral response (Saraiva et al. 2002).

P027.19 The viral IL-1 $\beta$  receptor (vIL-1 $\beta$ R) is related to the human type II IL-1R and also acts as a decoy receptor (Alcami and Smith 1992; Spriggs et al. 1992). In contrast to the membrane IL-1Rs encoded by the host, the viral protein is specific for IL-1 $\beta$  and does not bind IL-1 $\alpha$  or IL-1 receptor antagonist. Deletion of this gene in VV causes attenuation when the virus is administered intracranially (Spriggs et al. 1992), but leads to enhanced virulence associated with fever and accelerated death in mice infected through the respiratory route, a natural route of poxvirus transmission (Alcami and Smith 1992, 1996). The enhanced virulence observed after deletion of the vIL-1 $\beta$ R from VV illustrates that the role of some viral immunomodulatory proteins may be to reduce pathology induced by excess production of cytokines, in this case IL-1 $\beta$ , during infection rather than to cause immunosuppression.

### VIRAL CYTOKINE-BINDING PROTEINS

P027.20 IL-18 is a proinflammatory cytokine that is required, together with IL-12, for induction of IFN- $\gamma$  and the generation of an efficient cellular response against viral infection. The poxvirus-encoded IL-18 binding protein (vIL-18BP) is a homolog of the human IL-18BP but has no sequence similarity to membrane IL-18 receptors. The vIL-18BP is an effective scavenger of IL-18, and downregulates IFN- $\gamma$  production and NK responses in EV-infected mice (Born et al. 2000; Smith et al. 2000). Interestingly, the vIL-18BP is also expressed by molluscum contagiosum virus (MCV), a poxvirus that causes benign skin tumors and persists in the skin for months without inducing an inflammatory response (Xiang and Moss 1999; Smith et al. 2000). The vIL-18BP is the only cytokine receptor encoded by MCV and may

be responsible in part for the lack of inflammation in MCV lesions.

The only soluble cytokine-binding protein identified in herpesvirus is encoded by EBV and binds colony-stimulating factor (vCSF-1BP) (Strockbine et al. 1998). This protein has limited sequence similarity to the cellular receptor and may modulate the response of macrophages during infection. Another example of a viral cytokine-binding protein that binds ligands using different host receptors is a secreted protein that binds granulocyte-macrophage-CSF and IL-2 encoded by orf virus, a virus that causes skin lesions in sheep, goats, and humans (Deane et al. 2000).

### Modulation of chemokine networks

Chemokines are a family of small-molecular-size cytokines that induce cell migration and other biological effects, such as cell differentiation and angiogenesis, and play a critical role in the regulation of cells of the immune system throughout the body. There are more than 40 chemokines identified that can be structurally classified as CC, CXC, C, or CX3C, and mediate their biological response by binding and signaling through G-protein-coupled receptors, proteins that have seven-transmembrane domains. More than 20 chemokine receptors have been identified and their distribution in immune cells determines which type of cell migrates in response to infection or inflammation. There are three structural concepts used by viruses, mainly herpesviruses and poxviruses, to modulate chemokine activity: expression of chemokine homologs, chemokine receptor homologs, and secreted chemokine-binding proteins unrelated to the sequence of known host receptor proteins (McFadden and Murphy 2000; Murphy 2001; Alcami 2003)

### VIRAL CHEMOKINE HOMOLOGS

The virus-encoded chemokine homologs are secreted from infected cells and function as agonists or antagonists. Chemokine antagonists such as vMIP-II from KSHV and MC148 from MCV, bind to chemokine receptors but do not transduce signals (Boshoff et al. 1997; Kledal et al. 1997; Krathwohl et al. 1997; Damon et al. 1998). These antagonists function as inhibitors of specific chemokine pathways and influence the migration of immune cells into infected tissues. By contrast, the viral chemokine agonists induce cell migration into areas of infection, and thus their function may be unrelated to immune evasion. The current thinking is that viral chemokine agonists recruit to areas of infection immune cells that represent good targets for viral replication and enhance viral dissemination. The chemokine encoded by the gene *UL146* from HCMV, known as vCXC-1, induces neutrophil chemotaxis by binding to CXCR2

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and may explain the association of neutrophils with HCMV infections (Penfold et al. 1999). Human herpesvirus 6 (HHV-6) U83 induces monocyte migration that may favor the establishment of viral latency and persistence in the infected host (Zou et al. 1999). Another example of chemokine mimetics is the protein Tat from HIV which shows limited sequence similarity to chemokines. HIV Tat is a chemoattractant for monocytes and may enhance replication and spread of HIV in infected individuals (Albini et al. 1998). Direct evidence for a role of viral chemokine agonists in vivo comes from studies with murine cytomegalovirus (MCMV) mutants lacking the chemokine genes *M131/I29*. The MCMV chemokine is encoded by the *m131orf* and is expressed as a fusion protein including the *m129orf*. The isolated synthetic *m131* product attracts activated macrophages by engaging a receptor with structural properties related to human CCR3 (Saederup et al. 1999). The mutant MCMV lacking the function shows reduced spread to salivary glands, indicating that monocyte attraction serves for virus trafficking.

P027.24 The role of the viral chemokines encoded by KSHV in viral dissemination has not been defined but they may play a role in pathogenesis as a result of their angiogenic properties which may cause the enhanced vascularization observed in Kaposi's sarcoma lesions (Boshoff et al. 1997). In addition, the KSHV chemokines are chemoattractants for Th2-polarized T cells and may influence the type of immune response initiated after viral infection (Sozzani et al. 1998; Dairaghi et al. 1999; Endres et al. 1999; Stine et al. 2000).

P027.25 An interesting and recent addition to chemokine mimicry by viruses is the finding that glycoprotein G (gG) of respiratory syncytial virus has partial sequence similarity to fractalkine and has chemokine-like activity (Tripp et al. 2001). This property of gG may be used to facilitate virus attachment to infected cells and to modulate the host immune response.

## VIRAL CHEMOKINE RECEPTOR HOMOLOGS

P027.26 There are several examples of chemokine receptor homologs in members of the herpesvirus family and some in the poxvirus family. The function of these receptors in the viral infectious cycle is varied and difficult to demonstrate. Some viral chemokine receptors bind chemokines and transduce signals inside the cell. The ORF74 protein from KSHV is constitutively active and binding of chemokines can modulate the activation status of the receptor (Arvanitakis et al. 1997; Rosenkilde et al. 1999). The receptor has been shown to induce cell proliferation when expressed in cells in culture and its expression in transgenic mice causes the development of Kaposi's sarcoma-like lesions (Arvanitakis et al. 1997; Yang et al. 2000). The possibility that expression of KSHV ORF74 may contribute to neoplasia through a paracrine effect has been proposed,

but may be difficult to demonstrate in KSHV-infected individuals. In any case, ORF74 illustrates how such proteins encoded by viruses may have a direct and profound effect on the pathology caused by viral infection. In vivo studies on the homologous ORF 74 encoded by murine  $\gamma$ -herpesvirus 68 (MHV-68) have revealed that the virus uses this receptor for virus reactivation from latently infected mouse splenocytes (Lee et al. 2003). Of the four chemokine receptor homologs encoded by HCMV, UL78, UL33, US27, and US28, US28 is most extensively studied. MCMV encodes the viral chemokine receptor (vCKR) M33 and M78, which are homologous to their counterparts in HCMV. HCMV US28 can modify the chemokine environment of infected cells by sequestering and internalizing chemokines, and depleting them from the medium (Bodaghi et al. 1998). Similarly, HHV-6 UL51 reduces extracellular accumulation of chemokines by sequestration, but also by a novel mechanism of downregulation of CCL5 (RANTES) transcription (Milne et al. 2000). The potential contribution of viral chemokine receptors to pathology is also illustrated by the fact that HCMV US28 expression on the cell surface mediates cell adhesion and vascular smooth muscle cell migration, and it is thought to be related to vascular disease (Streblov et al. 1999). Evidence for a role for viral chemokine receptors in virus replication in vivo was demonstrated first for MCMV M33 (Davis-Poynter et al. 1997).

## VIRAL CHEMOKINE-BINDING PROTEINS

The third class of viral chemokine inhibitors are the viral chemokine-binding proteins (vCKBP) that sequester chemokines in solution without apparent sequence similarity to host chemokine receptors. These proteins were initially described in poxviruses, but an example has been identified recently in the herpesvirus family.

P027.27 The MV M-T7 (vCKBP-1) is a soluble vIFN- $\gamma$ R with sequence similarity to human IFN receptors but was later found to interact with chemokines. MV M-T7 binds a broad spectrum of chemokines and it has been proposed that it prevents the interaction of chemokines with glycosaminoglycans (GAG) and the correct presentation of chemokines to leukocytes (Lalani et al. 1997). There is evidence that the interaction of chemokines with cell-surface GAGs is important for their function in vivo. The protein vCKBP-2 was identified in the medium from cultures infected with MV, VV, or CPV (Graham et al. 1997; Smith et al. 1997; Alcamì et al. 1998). In this case, the protein binds CC chemokines with high affinity and neutralizes their effect by preventing the binding of chemokines to specific receptors in leukocytes. The protein M3 (vCKBP-3) encoded by murine MHV-68 was found to bind a broad range of chemokines, including C, CC, CXC, and CX3C chemokines, and to

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block their interaction with cellular receptors (Parry et al. 2000; van Berkel et al. 2000).

P027.29 The generation of relevant virus mutants lacking specific vCKBPs and the infection of rabbits or mice suggest that these proteins may block chemokine-mediated leukocyte infiltration into infected areas in vivo (Mossman et al. 1996; Graham et al. 1997; Lalani et al. 1997). In addition, MHV-68 M3 has been proposed to be required by the virus to establish virus latency and to enable persistence of the virus in the host (Bridgeman et al. 2001). Sequestration of chemokines can also prevent the process of dendritic cell (DC) maturation and trafficking (see below).

### VIRAL MODULATION OF CYTOKINE EXPRESSION AND ACTIVATION

P027.30 Other mechanisms that modulate the synthesis of cytokines and the signaling cascade triggered by them have been described. The expression of cytokines mediated by the nuclear factor  $\kappa$ B (NF- $\kappa$ B) and nuclear factor-activated T cell (NFAT) transcription factors in macrophages is blocked by an I $\kappa$ B homolog encoded by African swine fever virus (Miskin et al. 1998). The CPV protein CrmA was found to inhibit the IL-1 $\beta$ -converting enzyme (ICE) (or caspase-1), which proteolytically cleaves the precursor form of proinflammatory cytokine IL-1 $\beta$  (pro-IL-1 $\beta$ ) to generate mature, active IL-1 $\beta$  (Ray et al. 1992). CPV CrmA may also prevent the maturation of IL-18, which is also proteolytically cleaved by ICE, and is an inhibitor of apoptosis (see below under Viral inhibitors of caspases).

P027.31 There are several examples of viruses that inhibit cytokine-mediated signaling. Adenoviruses interfere with signaling induced by TNF at different levels (Mahr and Gooding 1999), and some herpesviruses and poxviruses inhibit signaling through death domains that lead to apoptosis (see below under Viral inhibitors of death receptor-mediated apoptosis). The poxvirus VV encodes two intracellular proteins that block signaling mediated by Toll-like and IL-1 receptors by mimicking intracellular regulatory domains of these receptors (Bowie et al. 2000). The Toll-like receptor pathway is important in NF- $\kappa$ B activation and amplification of inflammatory signals.

P027.32 Lastly, some viruses may subvert cytokine-mediated signaling for their own benefit. The latent membrane protein 1 (LMP1) of EBV recruits components of the TNFR and CD40 transduction machinery and induce biological responses such as cell proliferation that may enhance virus replication (Farrell 1998).

### H027.3 APOPTOSIS

P027.33 Programmed cell death (apoptosis) is a ubiquitous and evolutionarily conserved mode of cell death. Individual cells are eliminated or removed from the organism in a

temporal manner or in response to specific signals without affecting neighboring cells. Apoptosis plays a central role in tissue homeostasis throughout life (Rathmell and Thompson 2002). Apoptosis is characterized by loss of cell volume or cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation, phosphatidylserine exposure, membrane blebbing, and the formation of apoptotic bodies. These stereotypical morphological changes, associated with natural death, are based on a highly regulated genetic program. The activation of a class of cysteine proteases, termed 'caspases', as the central effectors plays an essential role. Caspases are synthesized initially as single polypeptide chains representing latent precursors (zymogens) which undergo proteolytic processing at specific aspartic acid residues to produce two distinct subunits that assemble to the active heterotetrameric protease. In mammalian cells, activation of the caspase zymogens has been reported to occur through at least three independent mechanisms: cleavage by upstream active caspases; cleavage by granzyme B, an aspartate-specific serine protease found in the granules of cytolytic T cells; and autoprocessing of zymogens with assistance from other caspase-interacting proteins that can occur in either a *cis*- or *trans*-acting manner (for reviews, see Salvesen and Dixit 1997; Benedict et al. 2002; Bortner and Cidlowski 2002; Trapani and Smyth 2002)

There are several caspases that are functionally redundant. Once activated, one caspase can activate other caspases in the proteolytic cascade (Stennicke et al. 1998). The trigger of the proteolytic cascade involves the physical association of zymogens via adaptor proteins with protein interaction modules that assemble the receptor signaling complex. The apoptotic program in cells includes mechanisms that activate and those that inhibit apoptosis. There are two main pathways of apoptosis.

### Death receptor-initiated activation of caspases

Cells can receive extrinsic signals that instruct them to die. The primary receptor through which death signals are signaled are members of the TNFR family, such as Fas and TNFR-1(19,24). Binding of receptor and ligand on the cell surface results in the formation of the hetero-oligomeric death-inducing signal complex (DISC). The apoptosis-inducing quality of a death receptor ligand system is defined by the death domain (DD) located in the intracellular portion of the death receptor. The DISC is assembled by complex protein interactions. The DDs and death effector domains (DED) carried by different proteins, e.g. caspases, are essential components. DISC proteins interact with other proteins through homotypic interactions of DD and DED contacts. The basis of the interaction is structural motifs

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of the DD which form six antiparallel helical bundles. Adaptor proteins such as FADD contain both a DD and a DED motif in one polypeptide and provide the bridging between DD domains of receptors with a DED-containing caspase. Among the caspases, mainly the initiator caspases-8 and -10 are recruited to the DISC. It is thought that the conformational properties and local concentrations of different proteins in the DISC complex govern caspase activation by autocatalytic processing (Schmitz et al. 2000; Walczak and Sprick 2001).

## Mitochondrial pathway of caspase activation

P027.36 Independent of the extrinsic death receptor pathway, apoptosis is also controlled intrinsically at the level of mitochondria. Mitochondrial death can result from the loss of extracellular vital signals (death by neglect), and by signals affecting the cell cycle, through activation of p53 and the proapoptotic p53 target genes (Rathmell and Thompson 2002). Changes in nutrient uptake, serum withdrawal, the deprivation of cytokines, and other metabolic events that affect the cellular redox potential, or the ability properly to regulate active oxygen species, lead to apoptosis. An important molecule in mitochondrial apoptosis is cytochrome *c*. Cytochrome *c* resides in the space between the inner and outer membranes of the mitochondria. The release of cytochrome *c* initiates mitochondrial apoptosis at the apoptosome, a protein complex called Apaf-1. It consists of the caspase-activating molecules, which can bind to caspase-9 through homotypic interaction via a domain defined as caspase-recruitment domain (CARD). For activation of caspases, Apaf-1 requires cytochrome *c*. A number of signals control the oligomerization of Apaf-1–caspase-9 complexes. Additional proteins that have CARD domains can have opposing activities on apoptosome formation, either inhibition or enhancement.

P027.37 Procaspase-8 activation can also occur at sites other than the cell surface. BAP-31, an integral protein of the endoplasmic reticulum, can recruit an isoform of procaspase-8, procaspase-8L, containing an N-terminal extension in response to apoptotic signals. This suggests an additional organelle-specific pathway of cell death (Ng et al. 1997; Nguyen et al. 2000; Breckenridge et al. 2002). This pathway is also regulated by the Bcl-2 proteins discussed below (Figure 31.3).

## Cellular regulation of extrinsic apoptosis

P027.38 The cellular homolog of the viral caspase-8 (FLICE) inhibitory protein (cFLIP) consists of two DED domains and a caspase domain that lacks the enzymatic active site. Therefore, cFLIP can bind but not cleave cellular

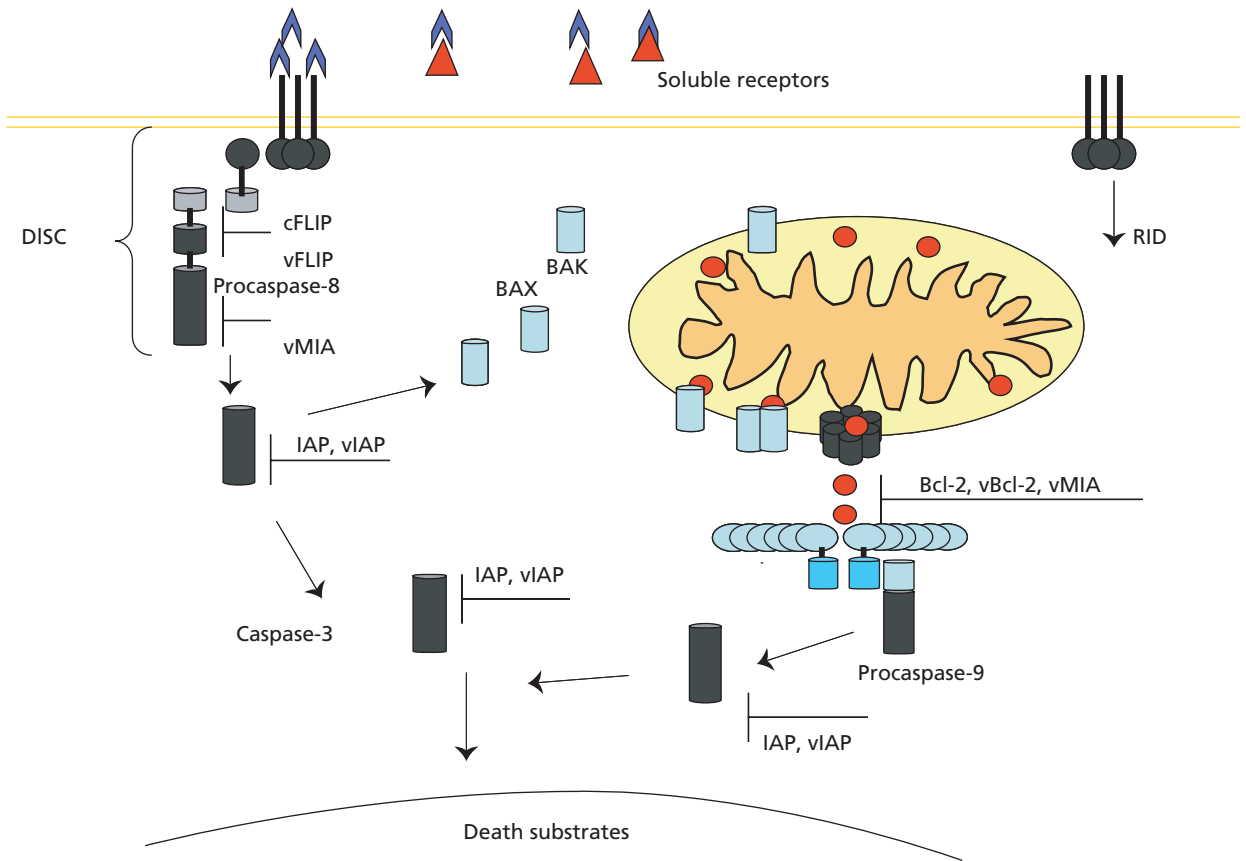
zymogen substrates. It is spliced in two forms; one form contains only the two DED domains; the other, long form includes the caspase-like domain. In addition, cFLIP may block caspase-8 recruitment to the DISC or may inhibit proteolytic processing of the proenzyme. However, cFLIP can also promote recruitment of caspase-8, which can stimulate autoprocessing to the active form.

## REGULATION OF CASPASE ACTIVITY

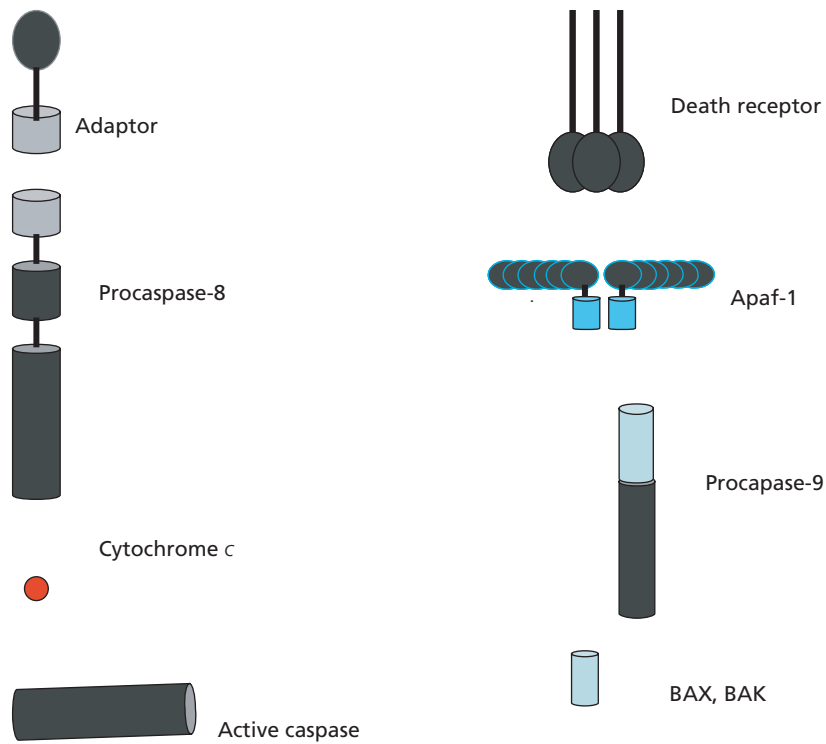
Regulation of caspase activity is central to the death process (Bortner and Cidlowski 2002). The inhibitor of apoptosis (IAP) family of proteins regulates caspase activation and activity. The first member of the IAP family was found in baculovirus. The cellular proteins with antiapoptotic function share, with the baculovirus protein, the baculoviral IAP repeat (BIR), a domain that consists of about 70 amino acids with conserved spacing of cysteine and histidine residues, which fold into a zinc-binding structure. Through the BIR domain, IAP molecules bind and directly inhibit caspase activation. Several IAPs also contain a ring-finger domain located near the C termini. Through the activity of the ring-finger domains, IAPs can target caspases for ubiquitinylation and degradation. IAPs themselves are again subject to inhibition by other proteins that activate caspases. IAPs can bind to caspases in either the active configuration or the inactive proform state.

## REGULATION OF MITOCHONDRIAL APOPTOSIS

The Bcl-2 family of proteins controls the release of proapoptotic proteins from mitochondria. Bcl-2 is present in the outer mitochondrial membrane and Bcl-2-like proteins have pore-forming activities and prevent the release of cytochrome *c*. The Bcl-2 family proteins are characterized by protein domains defined as Bcl-2 homology (BH). Four such domains, BH1–4, have been identified. BCL-2 proteins have up to four BH domains. The  $\alpha$ -helical BH3 domain of one protein interacts with a receptor formed by a hydrophobic pocket created by BH1–3 of the other protein. Through BH domains BH2 family members interact with each other and form homo- and heterodimers, which results in the promotion of abrogation of the function of the binding partner. Bcl-2 family members have either proapoptotic or antiapoptotic properties. Many antiapoptotic Bcl-2 family members contain domains BH1, -2, and -4. Proteins that carry only the BH3 domain define a family of proapoptotic proteins. Many death-signaling pathways require one BH3-only protein to activate the Bcl-2 family members BAX and BAK in order to activate the caspase. BAX and BAK are functionally redundant and their overexpression leads to mitochondrial accumulation of the protein and initiation of apoptosis. BAK-type proteins make the mitochondrial membrane permeable so that it releases intermembrane apoptogenic factors.



(a)



(b)

FIG027.3 **Figure 31.3** Viral regulation of apoptosis. See text for abbreviations.

BAX is primarily cytoplasmic but translocates to mitochondria on apoptotic stimuli. Specific conformational alterations of BAX allow mitochondrial docking. Like BAX, BAK has a transmembrane domain and is targeted to mitochondria where it exists as a monomer or in a complex with other Bcl-2 family members. Anti-apoptotic Bcl-2 family proteins bind and inhibit the proapoptotic proteins.

## CELLS DIFFER IN APOPTOSIS REGULATION

P027.41 Cell lines differ with respect to the preferential usage of the two signaling pathways leading to apoptosis. Type 1 cells constitutively contain a large amount of caspases at the DISC. Fas triggering results in caspase-8 activation, followed by cleavage of caspase-3 and loss of the mitochondrial transmembrane potential. In type 2 cells, there is little DISC formation and the intrinsic mitochondrial pathway is required as an amplifier to initiate the full apoptosis cascade after Fas activation. Only in type 2 cells can apoptosis triggered by extrinsic signals be blocked by BCL-2 overexpression. Apoptosis in type 2 cells is dependent on the activities of mitochondria, whereas apoptosis in type 1 cells is not. T cells have type 1 and liver cells have type 2 characteristics (Scaffidi et al. 1998).

P027.42 Chaperones and chaperone-like proteins that support the folding of newly synthesized proteins can also have pro- and antiapoptotic functions. Heat shock protein (HSP) 70-like chaperones inhibit apoptosis, whereas HSP-60 and HSP-10 have proapoptotic functions. Likewise, protein kinases modulate apoptosis. Mitogen-activated kinases (MAPK) control apoptosis through transcription, e.g. the upregulation of cFLIP, and also through transcription-independent mechanisms.

## Viral control of apoptosis

P027.43 Replication for viruses, as obligatory intracellular parasites, is dependent on cellular function. Therefore, for virus replication it is important that apoptosis triggered by soluble mediators of innate immunity via death receptors and caspase cascades is inhibited. The viral replication machinery diverts and exhausts cellular functions. Thus, viral protein synthesis, viral morphogenesis, and viral release must destabilize the homeostasis and integrity of the cell. This results in the activation of the intrinsic pathway of apoptosis. For effective viral progeny generation, it is important to delay this process. Some viruses also have proapoptotic properties, which may contribute to the release of virus particles from the cell that is already depleted from its resources. The execution of a proapoptotic function may also be advantageous, if apoptosis of an infected cell causes phagocytosis by another cell and enables the virus transfer to cells that the virus cannot infect directly. Therefore, it is

not surprising that viruses may contain both anti-apoptotic and proapoptotic functions. Some of the viral genes controlling apoptosis are derived from the host cell genome; for others there are no known homologs. NK and T-effector cells have two important functions: they kill infected cells through the perforin/granzyme pathway or by engaging fas, and they secrete several cytokines, among which IFN- $\gamma$  is known to be essential for control of virus in some situations. Both pathways of cytolysis induce apoptosis of targets. The viral anti-apoptotic functions may play a role in diminishing effector cell efficacy, but caution is warranted before making this assumption. Virus-infected cells are generally readily lysed by T cells, so long as the T-cell receptor (TCR) is triggered, in spite of the presence of antiapoptotic mechanisms. It is likely that the strength of the death signal imposed, particularly by the perforin/granzyme pathway, is able to overcome most anti-apoptotic mechanisms.

## VIRAL INHIBITORS OF DEATH RECEPTOR-MEDIATED APOPTOSIS

P027.44 Poxviruses secrete proteins that bind to cytokines, which in turn activate death receptor-mediated apoptosis. This probably represents the earliest possible defense against apoptosis signals. The expression of the death receptor CD95 is inhibited by viral IFN regulatory factors from HHV-8 that inhibit the binding of IFN- $\gamma$  induced IFN regulatory factor 1 (IRF-1) to specific domains in the CD95L promoter (Kirchhoff et al. 2002). The functions of adenovirus interfere with the apoptosis mediated by the TNF receptor superfamily at a very early stage and at various levels (Burgert et al. 2002; McNeese and Gooding 2002). Post-translational downregulation of FAS is carried out by the E3/10.4K–E3/14.5K protein complex, also named receptor internalization (RID) and degradation, together with the E3/6.7K protein. The RID complex contains transport motifs in the cytoplasmic tail of the proteins which are recognized by the intracellular transport machinery that directs the CD 95/RID complex to lysosomes. Apart from CD95, the TNF-related apoptosis-inducing ligand (TRAIL) receptor can form a death-inducing signaling complex. In addition TRAIL receptors are downregulated by the E3 proteins of adenovirus.

P027.45 The DISC is the next target for modulatory viral proteins. It is assembled by protein interaction between DDs and DEDs. Search for proteins carrying homology to DEDs led to the identification of viral proteins with two DEDs (Derfuss and Meisl 2002). These viral proteins block the activation of caspase-8 (FLICE) and were therefore named viral FLICE inhibitor proteins (vFLIPs). These vFLIPs have been detected in poxvirus and several herpesvirus genomes. In addition, vFLIPs regulate the expression of transcription factors for proteins involved in apoptosis regulation. The product of

the HCMV gene, *UL36 vICA*, binds procaspase-8 but lacks homology to cFLIP (Goldmacher 2002).

## VIRAL INHIBITORS OF CASPASES

P027.46 The archetypal member of the cellular IAP family was found in baculovirus. Through the BIR, a motif that can be present up to three times, IAP molecules bind and directly inhibit caspase activation. Other caspase inhibitors are the poxvirus serine protease inhibitor CrmA (which targets a number of caspases) and related proteins from other poxviruses and African swine fever virus, and p35 of baculovirus. Interestingly, viral proteins can combine IAP properties with properties of BCL-2 protein functions (Wang et al. 2002b).

## VIRAL INHIBITORS OF MITOCHONDRIAL APOPTOSIS

P027.47 Prevention of mitochondrial permeabilization is essential for the replication of many viruses. Many  $\gamma$ -herpesviruses encode one or more molecular homologs of BCL-2, the cellular antiapoptotic protein family (Cuconati and White 2002). Viral BCL-2 homologs are also found in adenovirus and poxvirus genomes. Viral BCL-2 proteins act as apoptosis inhibitors and their structure is similar to that of the cellular BCL-2 proteins. However, despite the preservation of the overall structure the unstructured loops that encode sites for phosphorylation and proteolysis, and which may be required for regulation, the function of the cellular proteins differs between vBCL-2 and cellular BCL-2 proteins. All vBCL-2s have a BH1 and at least one other BH2 or BH3 region. Mitochondrial apoptosis is a limiting factor in the life cycle of DNA viruses, because in cells deficient in BAX and BAK the onset of cytopathic effects is delayed and more viral replication can occur. In adenovirus the E1-B19K inhibits the formation of the highly oligomeric BAX/BAK complex at the mitochondrial membrane.

P027.48 The viral mitochondrial localized inhibitor of apoptosis encoded by HCMV (vMIA) also targets mitochondria but acts differently from the viral BCL-2 homologs (Goldmacher 2002). It has no BCL-2 homology domains and has no affinity to BAX. However, vMIA forms a complex with adenine nucleotide translocator (ANT), a component of the mitochondrial transition pore complex, which cooperates with BAX in the mitochondrial control of apoptosis (Marzo et al. 1998).

## H027.4 CELLULAR EFFECTOR CELLS OF INNATE IMMUNITY

### Natural killer cells, effector cells of innate immunity

P027.49 Natural killer cells are large granular lymphocytes that have potent antiviral effector mechanisms: they kill

infected cells using the perforin/granzyme pathway, and release copious amounts of IFN- $\gamma$ . In many virus infections, viral titers start to decline as a result of NK function well before the adaptive CD8 immune response has any effect. Unlike T cells, NK cells do not have clonally expressed antigen receptors with specificity for viral peptides. Instead, they use a series of germline-encoded receptors to detect the consequences of virus infection on cells, irrespective of the antigenic composition of the infecting virus. Some of these receptors are activating, and some are inhibitory. Signals from these receptors are integrated, leading either to killing and release of IFN, or no action.

For NK cells to control virus, they need (1) to be induced, (2) to traffic to the site of virus infection, (3) to be able to detect virus-infected cells, and (4) to perform their effector functions (killing cells and releasing IFN- $\gamma$ ). Discussion of interference with NK cells is usually limited to the third step – NK recognition of infected cells. NK trafficking and effector mechanisms are very similar to those of CD8 T cells, and are discussed below. NK cells are induced by cytokines – particularly type I interferons and IL-12, which are produced by infected tissues, and viral interference with production of those cytokines affects the NK response.

Natural killer cells integrate signals from activating and inhibitory receptors to achieve activation threshold (Colonna et al. 1999; Fong and Cambier 1999; Long 1999; Ravetch and Lanier 2000; Lanier 2001). It is likely that not all of these receptors and their ligands have been identified. Two important activating receptors are known: CD16 is an Fc receptor that enables NK cells to lyse cells with bound antibody, a process known as antibody-dependent cell-mediated cytotoxicity (ADCC) (see below under Inhibition of humoral immunity). ADCC is not generally considered to play a major role in antiviral immunity, and obviously plays no role in the NK control of the first few days of infection.

A second major activating receptor – NKG2D – has recently been described (Bauer et al. 1999; Wu et al. 1999). NKG2D is a C-type lectin that homodimerizes to generate a functional receptor. Most NK cells express NKG2D, making it an important activating receptor. Through a positively charged residue in its transmembrane domain, it interacts with the immunoreceptor tyrosine based activation motif (ITAM)-containing DAP10, to deliver a strong activating signal to the cell. In addition to NK cells, NKG2D is expressed on activated classic CD8 T cells and on  $\gamma\delta$  T cells: for these cells, it acts as a costimulatory molecule, i.e. signaling through NKG2D is not enough to activate the cell. For NK cells, however, NKG2D signaling is sufficient for activation. The ligands for NKG2D show loose homology to MHC class I. It is not yet clear whether all ligands have been identified, and the main ligands are different for humans and mice. In humans, MICA and MICB are stress-inducible molecules

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expressed mainly in epithelial tissues, and, interestingly, by most carcinomas (Bauer et al. 1999). MIC expression is induced by HCMV infection (Groh et al. 2001). A second set of receptors are the UL16-binding proteins (ULBP), discovered as the targets for HCMV *UL16* (see below) (McNees and Gooding 2002). The ULBP are homologous to one set of murine ligands for NKG2D – the retinoic acid early inducibles (RAE). The other known murine ligand is H60, a minor histocompatibility antigen expressed in BALB/c mice (Diefenbach et al. 2000)

P027.53 There are two families of inhibitory receptors for human NK cells: the killer immunoglobulin-like receptors (KIR) and the C-type lectin NKG2A/CD94 paired heterodimer. The KIR are members of the immunoglobulin superfamily, and are encoded in a locus on chromosome 19 (Vilches and Parham 2002). They interact with classical MHC class I molecules, different members having different specificities. In particular, important inhibitory KIR recognize a limited polymorphism on HLA-C and HLA-B molecules. KIR are expressed on subsets of NK cells; one theory of NK cell development suggests that during their education NK cells must express at least one inhibitory receptor capable of recognizing self-MHC. NKG2A/CD94 interacts with HLA-E, a nonpolymorphic class I molecule that binds peptides derived from the leader sequence of classic MHCI molecules (Braud et al. 1998a). Murine NK cells also have two families of inhibitory receptors. They share NKG2A/CD94 with humans and the murine homolog of HLA-E is Qa1. The second inhibitory receptor family is Ly49, a family of C-type lectins that also recognize polymorphic class I molecules. All of these inhibitory receptors recognize MHCI and they thus enable the NK cells to monitor the function of the class I pathway. Viruses that interfere with the class I antigen-presenting pathway run the risk of rendering infected cells susceptible to NK lysis.

P027.54 It is important to note that not all KIR and Ly49 molecules are inhibitory. Some lack cytoplasmic tails with immunoreceptor tyrosine-inhibition motifs (ITIM) and instead pair with adaptor molecules to activate cells. Furthermore, some NKG2 family members that recognize HLA-E molecules have an activating function. The function of the inhibitory members of these families in the control of NK lysis appears plausible; the function of the activating members is less clear (with the exception of Ly49H, discussed below).

### VIRAL INTERFERENCE WITH NK RECOGNITION OF INFECTED CELLS

P027.55 Several viruses have been described to interfere with the ability of NK cells to detect infection; this is summarized in Figure 31.4.

P027.56 Human cytomegalovirus UL18 is a class I homolog – it shares 25 percent amino acid homology with classic class I, and pairs with host  $\beta_2$ -microglobulin for expression on the surface of infected cells. When it became

appreciated that loss of cell surface class I, induced by HCMV infection, would make infected cells vulnerable to NK lysis, the idea that UL18 would engage NK inhibitory receptors and prevent NK lysis became an attractive hypothesis. Indeed Reyburn et al. (1997) used transfected cells expressing UL18 to demonstrate protection from NK lysis in vitro. However, it is controversial whether UL18 functions in this way in vivo. First, cell surface expression of UL18 in infected cells is very low. Second, none of the well-characterized NK inhibitory receptors have been shown to interact with UL18. Third, when a ligand for UL18 was identified (see section The leukocyte immunoglobulin-like receptors below), it was found to be mostly expressed on macrophages, B cells, and DCs, although it is also expressed on some NK cells (Cosman et al. 1997). The ligand, leukocyte immunoglobulin-like receptor 1 (LIR-1), is inhibitory, so it may indeed function to inhibit NK lysis.

Murine CMV also encodes a gene – *m144* – with homology to classic MHCI. The gene *m144* also pairs with  $\beta_2$ -microglobulin to produce a class I-like structure. Mice infected with a virus lacking *m144* control it better than they control wild-type virus (Farrell et al. 1997). The difference in titers is particularly evident 3 days after infection, when NK control is most important. Furthermore, mice depleted of NK cells show no difference in their ability to control wild-type virus or the *m144* deletion mutant. All this suggests that *m144* would inhibit NK recognition of infected cells. However, a direct demonstration that *m144* interacts with NK cells to inhibit their activation is lacking. Although it is clear that *m144* acts somewhere in the NK pathway, the exact site of action remains to be demonstrated. As with UL18, the discovery of other class I-interacting molecules (see The leukocyte immunoglobulin-like receptors below) raises possibilities other than direct interaction with NK inhibitory receptors.

Molluscum contagiosum virus is a poxvirus that establishes persistent infection, unlike other poxviruses. It also encodes an MHCI homolog of unknown function, which complexes with  $\beta_2$ -microglobulin (Senkevich and Moss 1998).

Another MCMV gene – *m152* – has been clearly shown to interfere with NK lysis. The gene *m152* is also loosely related to class I by secondary structure. Its best understood function is interference with antigen presentation to CD8<sup>+</sup> T cells (see below under Retention of class I molecules in pre-golgi compartments). However, Krmpotic et al. (2002) found that a virus lacking *m152* had much lower titers 3 days after infection – a time at which NK cells are the main antiviral mechanism – than wild-type virus. This was surprising because *m152* is known to decrease cell surface expression of classical MHCI, and it was thought that it should therefore make the virus more (not less) vulnerable to

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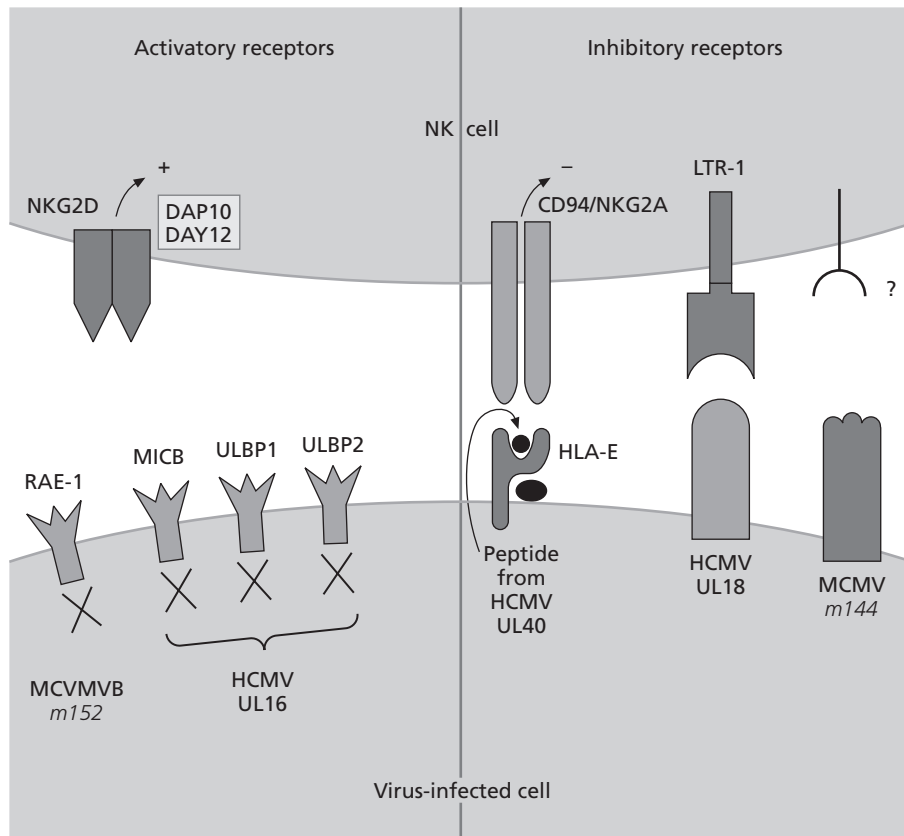


FIG027.4 **Figure 31.4** Viral interference with natural killer (NK)-activating receptors and provision of 'decoys' for inhibitory receptors. See text for abbreviations.

NK control. The riddle was solved when Krmptoc et al. found that *m152* also reduced the level of an NK ligand on the surface of infected cells. As described above, the RAEs and H60 are loosely class I-like molecules, and serve as a ligand for the important NK-activating receptor NKG2D. Cells infected with a virus lacking *m152* had high ligand levels and were more susceptible to NK lysis than those infected with wild-type virus.

P027.60 In HCMV a soluble form of the *UL16* gene product was found to interact with human ligands for NKG2D: MICB, ULBP1, and ULBP2 (Cosman et al. 2001). The ULBPs were actually identified by their interaction with UL16. UL16 retains MICB, ULBP1, and ULBP2 inside the cell. In fact, HCMV increases the transcription of all NKG2D ligands, and cell surface expression of MICA and ULBP3, the two ligands not targeted by *UL16*, is increased by HCMV infection. How these contradictory effects translate into susceptibility to or protection from NK lysis is still an open question.

P027.61 Another NK inhibitory effect has been described for HCMV. As described above, NKG2A/CD94 is an important inhibitory receptor on NK cells. NKG2A recognizes the nonpolymorphic HLA class I molecule HLA-E (Braud et al. 1998a). HLA-E binds peptides derived from the leader sequence of classical class I molecules, which must be transported back into the endoplasmic reticulum by transporter associated with

antigen processing (TAP) (Braud et al. 1998b). HLA-E cell surface expression is reduced if classical class I is absent or if TAP is impaired. It thus serves to monitor the integrity of the class I pathway. As the HCMV genes *US2* and *US11* cause rapid destruction of newly synthesized class I (see below under Degradation of newly synthesized MHC I molecules) and *US6* blocks the function of TAP, HCMV infection should render cells vulnerable to NK lysis through silencing of NKG2A/CD94 signaling. However, another HCMV protein, UL40, has in its leader sequence a peptide capable of binding to HLA-E (Tomasec et al. 2000). Furthermore, this peptide remains in the endoplasmic reticulum after signal sequence cleavage and is able to bind to HLA-E in the absence of functional TAP. Thus HCMV is able to circumvent the HLA-E pathway. It is still controversial whether the relative effects of either UL40 or class I downregulation dominate the susceptibility of HCMV-infected cells to NK recognition, i.e. whether the final effect is susceptibility or protection (Falk et al. 2002; Wang et al. 2002a). Again, the final outcome may be different in different cell types, times after infection, etc.

HIV does not directly interfere with NK recognition. However, investigators have noted that HIV-nef interference with class I cell surface expression affects HLA-A and -B molecules but not HLA-C (Collins et al.

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1998). As HLA-C isoforms are the most important ligands for KIRs, this may prevent HIV-infected cells becoming vulnerable to NK attack as a result of loss of KIR inhibition.

P027.63 KSHV-K5 is known to downregulate cell surface classic class I molecules. However, it also downregulates intercellular adhesion molecule 1 (ICAM-1) and B7-1, known to be important for NK cell lysis (Ishido et al. 2000). In consequence, K5 expression protects transfected cells from NK lysis.

P027.64 A viral gene has been reported which, instead of protecting from NK lysis, actually renders infected cells susceptible to a protective NK response. It was known that a gene in the NK locus has a major effect on the susceptibility of different mouse strains to MCMV. Mice from the resistant strains have a more effective NK cell response. It was recently discovered that the gene controlling resistance is *Ly49H* – an activating receptor found on a subset of NK cells. The surprising finding was that the ligand for *Ly49H* is not, as had been presumed, a host-encoded molecule upregulated by infection, but is actually a viral gene product encoded by the gene *m157* (Arase et al. 2002; Smith et al. 2002). The gene *m157* has no known other function and is dispensable for virus replication in vitro and in vivo. It also interacts with an inhibitory receptor, *Ly49C*, and investigators have postulated that this is its true evolutionary function.

## THE LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTORS

P027.65 Human CMV encodes a molecule – UL18 – with 25 percent homology to MHCII heavy chain. As described above, it was assumed that UL18 would interact with KIRs and prevent NK lysis of HCMV-infected cells. This idea was supported when it was shown that cells transfected with UL18 were resistant to lysis by some NK cells (Reyburn et al. 1997). However, identification of the ligand for UL18 led to the discovery of an entirely new gene family – the leukocyte immunoglobulin-like receptors (LIR) (Cosman et al. 2001). The LIRs are structurally related to the KIRs and human IgA Fc-receptor, and are encoded in close proximity to those genes on chromosome 19. There are eight LIRs – all but one are membrane glycoproteins; some contain ITIMs, whereas others have an arginine in their trans-membrane region which suggests that they pair with another chain and have an activating function (Fanger et al. 1999). The ligand identified for UL18 is LIR-1; its cytoplasmic tail contains ITIMs. It is expressed on most B cells and monocytes and a variable number of T cells and NK cells. In addition to UL18, LIR-1 binds to most HLA-A, -B, and -C molecules. The physiological function of this molecule, and hence what HCMV achieves by encoding a ligand for it, is currently unknown.

Most of the other LIRs are also expressed on monocyte and DCs but not on lymphocytes. LIR-2 also binds class I molecules, but the ligands for the other LIRs are not yet identified. The discovery of this family indicates that the role of MHCII in immunobiology is not yet fully understood, and this should be taken into account when considering the function of viral genes that inhibit class I expression.

P027.66

## EFFECTS ON THE ADAPTIVE IMMUNE RESPONSE

H027.5

### Viral interference with DC functions

The cellular arm of the adaptive immune response consists of CD4 and CD8 T cells. CD8 T cells are the most important effector arm of adaptive antiviral immunity. The effective antiviral T-cell response requires orchestration. Antigen-presenting cells (APC), usually DCs, capture antigen and travel from the site of infection to the draining lymph nodes, where they activate naïve T cells. Naïve T cells that recognize antigen on DCs remain trapped in the lymph node. They start to proliferate for several days, and acquire the effector phenotype. Activated effector T cells can enter tissues. This is essential for CD8 T cells to interact directly with and to lyse virus-infected cells. Infected tissues provide chemokine and cytokine signals that enable T cells to extravasate and direct them to the site of infection. In the tissues, CD8 T cells have two main effector mechanisms that are antiviral: cytolysis of infected cells and release of antiviral cytokines, especially IFN- $\gamma$ . CD4 T cells can make antiviral cytokines, but also provide critical help for CD8 T cells and for antibody production. Viruses have found several points of intervention within this process. These will now be dealt with individually: first, processes that would affect both CD4 and CD8 T cells, followed by mechanisms specific for each cell type.

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Among APCs, mainly DCs are able to activate naïve T cells. DCs are generated in the bone marrow, circulate in the blood, and enter tissues in an immature state. Immature DCs are active in phagocytosis, but are poor APCs as a result of intracellular retention of class I and class II molecules and low expression of costimulatory molecules such as CD80 and CD86. Immature DCs can phagocytose dying cells, cellular debris, immune complexes, and proteins. They can also be infected by many viruses. In the context of adequate mediators produced during infection, they differentiate to mature DCs that are extremely potent APCs and stimulators of T cells. Maturation also triggers their migration into the lymphatics and to the T-cell areas of draining lymph nodes. Maturation signals produced during virus infection include type I IFN from infected cells and TNF- $\alpha$  from tissue macrophages. DCs have two main ways of

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activating CD8 T cells to initiate the T-cell response to viruses. First, infected DCs can present antigen through the classic endogenous pathway of MHC I-restricted antigen presentation. Second, they can ‘crosspresent’ antigens that they acquire by phagocytosis of proteins, which are degraded to peptides for loading on to their class I molecules. Sources of material that can be ‘cross-presented’ include cells dying by either apoptosis or necrosis; cellular debris, immune complexes, and perhaps heat shock proteins from dying cells that have bound viral peptides. Crosspresentation is described in Figure 31.5. The relative importance of the two DC presentation pathways depends on (1) the ability of viruses to directly infect DCs and (2) the extent to which viruses interfere with the direct presentation pathway (Figure 31.5).

P027.69 For CD4 T cells, crosspresentation is the classical pathway of antigen presentation and is called exogenous presentation. Still controversial is the role of loading of class II molecules with endogenously synthesized viral proteins in infected class II-positive cells. This is discussed further below. Like CD8 T cells, naïve CD4 T cells are found only in blood and secondary lymphoid tissue, and they depend on trafficking of DCs to lymph nodes to be activated. Mature DCs that present viral antigen to naïve T cells are critical for the initiation of a T-cell response. There are three aspects of this with which viruses can interfere:

**1** Interference with DC trafficking: DCs enter uninfected tissue, but they enter sites of infection in much greater numbers. Interference with chemokines and cytokines could affect migration.

**2** Interference with the ability of DCs to present viral antigen: many viruses interfere with the class I antigen processing and presentation pathway. This would interfere with and perhaps prevent directly infected DCs from activating T cells, but would not interfere with crosspresentation. Although viruses might also be able to interfere with crosspresentation (perhaps via a secreted product), so far no such mechanism has been described.

**3** Interference with the ability of immature DCs to mature, which is essential for APC function. The normal response of a DC to virus infection is to mature into an APC. Type I IFNs, induced by most virus infections, are powerful DC maturation stimuli. However, several viruses have been described that do not initiate maturation of DCs, and in fact interfere with the ability of the DCs to mature in response to other stimuli (Figure 31.6).

A well-studied viral interference with DC function occurs during measles virus infection; this infection elicits good protective immunity, including CD8 T cells, and yet is associated with a profound immunosuppression which supports additional infection by other infectious agents. This superinfection contributes to the morbidity and mortality associated with measles. The interaction of measles virus and DCs is complex. Measles virus infects DCs and induces their maturation and IL-12 production (Schnorr et al. 1997). This stimulates virus replication in the infected DCs. However, measles virus-infected DCs inhibit rather than promote proliferation of T cells. A few infected DCs are enough to inhibit the ability of a large number of uninfected

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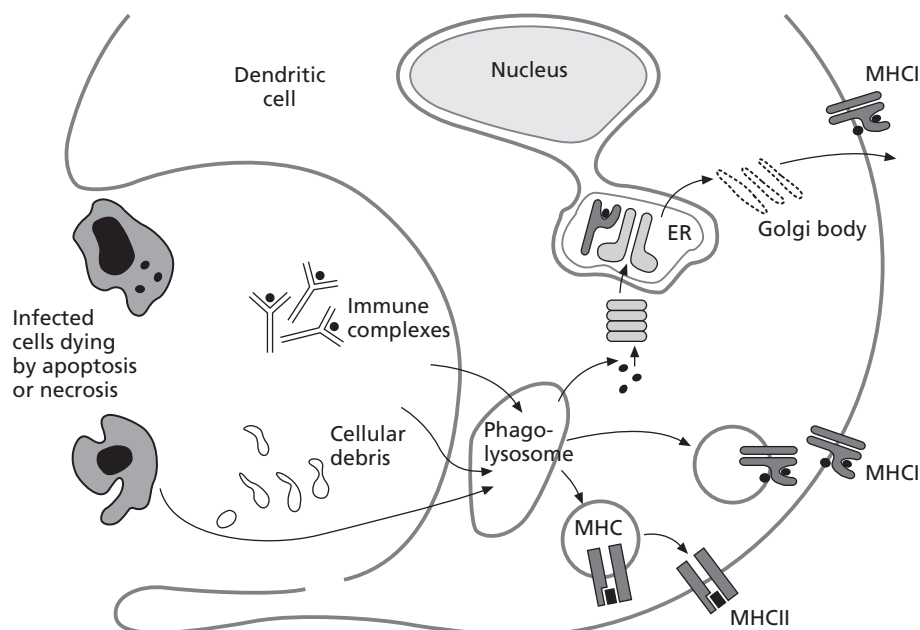


FIG027.5 **Figure 31.5** Crosspresentation of viral antigens to CD8 T cells: activation of antiviral T cells despite immune evasion genes. Phagocytosomal material is re-presented on the dendritic cell's MHC molecules. The pathways for crosspresentation via MHC I are still poorly defined.

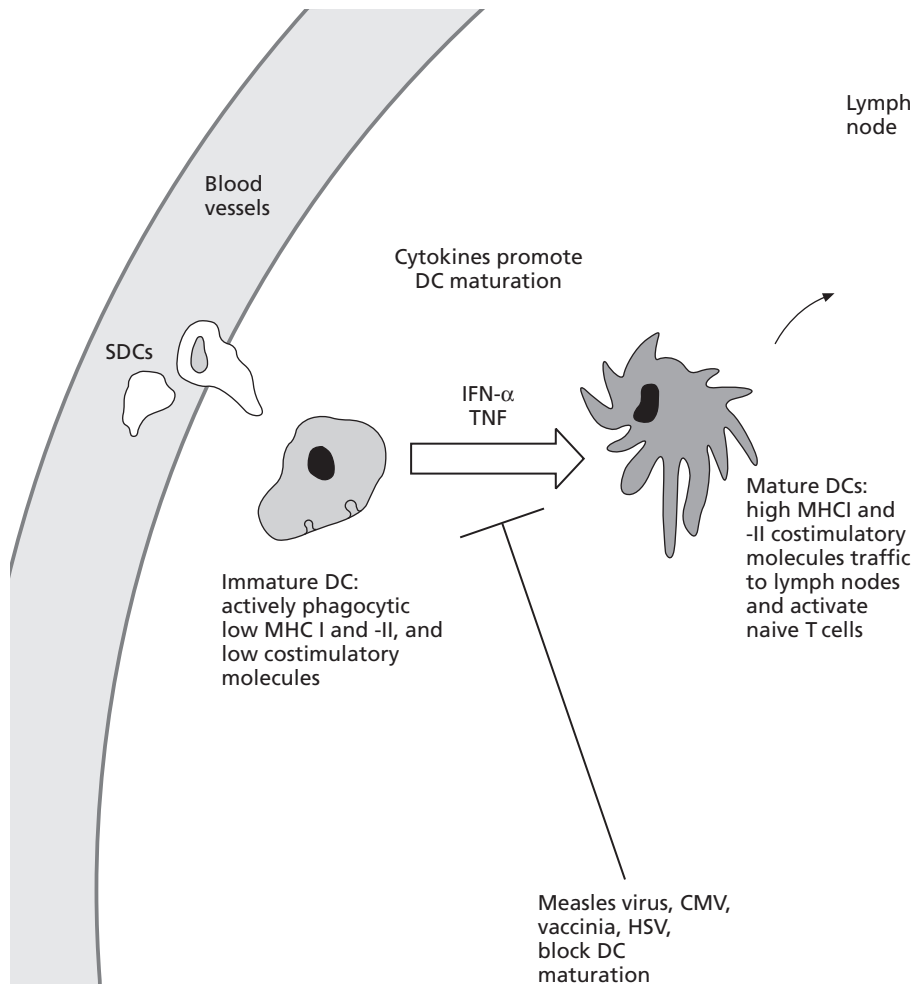


FIG027.6 **Figure 31.6** Viral interference with dendritic cell (DC) maturation or function. CMV, cytomegalovirus; IFN- $\alpha$ , interferon  $\alpha$ ; TNF, tumor necrosis factor.

DCs to promote T-cell proliferation (Grosjean et al. 1997; Schnorr et al. 1997). Measles virus induces the expression of the apoptosis-inducing ligand TRAIL on DCs (Vidalain et al. 2000); T cells and DCs cocultured with measles virus both undergo apoptosis (Fugier-Vivier et al. 1997; Servet-Delprat et al. 2000). Measles virus interference with DC function is thought to be the major mechanism of the generalized immunosuppression associated with MV infection.

P027.71 Engelmayer et al. (1999) reported that VV impaired maturation of human DCs, and concluded that this would impair immunity. Drillien et al. (2000) also noted that VV inhibited maturation of human monocyte-derived DCs. However, VV-infected DCs efficiently stimulate memory T cells from infected individuals. In fact, infected DCs have been observed interacting with T cells in vivo (Norbury et al. 2002). Another study in mice concluded that both direct presentation by virus-infected DCs and crosspresentation contribute to CD8 T-cell priming in VV-infected mice (Basta et al. 2002). VV, the vaccine for smallpox, is the poster child of good viral immunogens. If VV does impair DC function, this

effect is presumably mild and does not prevent an immune response.

Inhibition of DC maturation has also been reported for HSV (Salio et al. 1999) and MCMV (Andrews et al. 2001). The CD8 T-cell response to MCMV is thought to be largely achieved by crosspresentation (Gold et al. 2002), which suggests that functional DC impairment could play a role. HCMV is also thought to affect DC function. Moutaftsi et al. (2002) reported that HCMV impaired DC maturation. Mature DCs, on the other hand, were reported to increase costimulatory molecules after HCMV infection (Raftery et al. 2001). They also expressed the apoptosis-inducing ligands, FasL and TRAIL, suggesting that they might induce a generalized immunosuppression similar to measles.

P027.72

### Interference with antigen presentation in the MHC class I pathway

The most widely studied and best-appreciated viral immune evasion mechanisms have been found to affect

P027.73

CD8 T-cell recognition of virus-infected cells. There are two basic strategies: viruses can interfere with the MHC I pathway of antigen presentation, or they can mutate the epitopes that CD8 T cells recognize, forcing the immune system to generate new effectors from naïve precursors.

P027.74 Several viruses, mostly herpesviruses, have evolved genes that interfere with the MHC I pathway. Immune evasion genes of this type have been called viral genes that interfere with antigen presentation (VIPR) (Yewdell and Hill 2002). The pathway of MHC I-mediated antigen presentation is quite complex, and the past few years have seen a flurry of discoveries of viral gene products that interfere, to varying degrees of specificity, with almost every aspect of this pathway. Some viruses – notably CMV – actually encode several genes that all target this pathway. We begin this section by following the class I antigen presentation pathway and describing the various mechanisms that viruses use to attack it.

### ANTIGEN DEGRADATION

P027.75 The first step in antigen processing for the MHC I pathway is degradation of viral proteins in the cytosol, mostly by the proteasome. Proteasome function is vital for the health of the cells, and few viruses target this aspect. However, the EBNA-1 protein of EBV contains a long segment of repeated glycine and alanine residues, which renders the protein resistant to proteasomal degradation, and peptides for cytotoxic T lymphocyte (CTL) recognition are not generated (Levitskaya et al. 1995). EBNA-1 is the only protein that EBV needs to be expressed during latency, and this mechanism may help to prevent eradication of the latent virus pool.

P027.76 When HCMV infects a cell the phosphoprotein pp65, a structural protein that has kinase activity, is also introduced. This protein is apparently able to prevent presentation of the immediate early (IE) peptide of the immunodominant IE1 protein, perhaps by phosphorylating IE1 and altering its proteolysis (Gilbert et al. 1996) (Figure 31.7).

### TRANSPORT OF PEPTIDES BY TAP

P027.77 Peptides are transported by the TAP from the cytosol into the endoplasmic reticulum where they can bind to class I molecules. TAP is a member of the seven-transmembrane ATP-binding cassette family of transporters, and appears to have no other function. It can thus be incapacitated by viruses without impairing other cellular functions that the virus may need. TAP is specifically targeted by at least two VIPRs. The HSV IE gene *US12* encodes a polypeptide of 87 amino acids, ICP47, which is abundantly expressed in the cytosol (York et al. 1994). It binds to TAP and inhibits peptide transport, with the result that newly synthesized class I molecules are unable to leave the endoplasmic reticulum, and antigen presentation is prevented (Fruh et al. 1995; Hill et al. 1995). Both HSV-1 and HSV-2 encode ICP47; as a

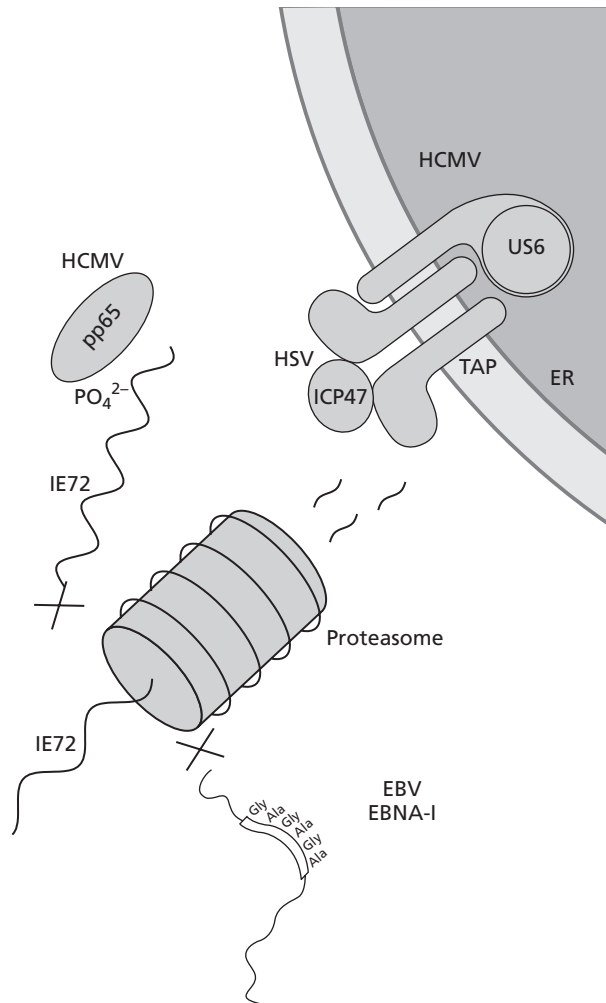


Figure 31.7 Interference with production of peptides and transport into the endoplasmic reticulum (ER). See text for abbreviations. FIG027.7

result of a frame shift mutation, they are homologous only in their first 35 amino acids, and this region of the molecule is all that is required for inhibition of TAP (Galocha et al. 1997).

The HCMV protein US6 also binds to TAP and inhibits peptide transport, although not as potently as ICP47 (Ahn et al. 1997; Hengel et al. 1997; Lehner et al. 1997). US6 is a transmembrane glycoprotein that binds TAP on the luminal side of the endoplasmic reticulum. P027.78

### THE PEPTIDE-LOADING COMPLEX

In the endoplasmic reticulum, MHC I molecules associate with TAP as part of a peptide loading complex that also includes tapasin, calreticulin, and a thiol oxidoreductase. This complex not only ensures that class I is in the optimal place to acquire peptides, but also orchestrates peptide loading and provides poorly understood quality control functions. Several VIPRs associate with this complex, but it is not understood whether this is a coincidental consequence of binding their P027.79

main targets or whether it plays a role in their interference with the pathway. The adenovirus protein Ad5E3 19K binds TAP and interferes with the ability of tapasin to bring class I and TAP together in the peptide-loading complex (Bennett et al. 1999). This function may supplement its ability to retain class I (see below under Retention of class I molecules in pre-golgi compartments).

### SYNTHESIS OF MHCI MOLECULES

P027.80 Many viruses interfere with host gene expression, either globally or selectively. As newly synthesized class I molecules are required for peptide loading, interference with synthesis of MHCI affects antigen presentation. However, the extent to which this mechanism would affect the antiviral efficacy of CTL depends on a number of timing issues: the half-life of class I RNA (if transcription but not RNA stability is affected); the average residence time of MHCI in the endoplasmic reticulum (which varies considerably between class I isoforms); the timing in the viral replication cycle of antigen expression (the earliest expressed proteins and structural viral proteins that can be processed immediately upon viral entry are less affected); and the length of the viral replication cycle (viruses with long replication cycles can gain more from mechanisms that would only impact CTL recognition after a lag time of many hours or days). One virus for which interference with MHCI synthesis has a clear impact on CTL efficacy is HSV. The viral host protein shut off (vhs) factor encoded by HSV UL40 is a virion structural protein. Upon virus entry it strips RNAs from ribosomes, allowing viral transcripts to be translated at the expense of host transcripts. The function is especially potent in HSV-2: within half an hour of virus infection synthesis of host proteins, including MHCI, essentially ceases. HSV UL40 has been shown to affect CTL recognition of infected cells (Tigges et al. 1996).

### DEGRADATION OF NEWLY SYNTHESIZED MHCI MOLECULES

P027.81 One of the most effective VIPR mechanisms is that employed by the HCMV proteins US2 and US11. These proteins bind MHCI in the endoplasmic reticulum and cause it to be transported back through the translocon. In the cytosol, MHCI is ubiquitinated and destroyed by the proteasome (Wiertz et al. 1996a,b). The process is very potent and in HCMV-infected fibroblasts metabolic labeling reveals very little class I (Beersma et al. 1993). These particular genes have been intensely studied, not just because of their immune evasion function, but also because they have helped to elucidate an important cell biological pathway – the degradation of endoplasmic reticulum proteins. US2 and US11 are both type I membrane glycoproteins, and both bind MHCI via their luminal domains. However, they are not structurally homologous, and show some differences in the way that

they achieve MHCI destruction, e.g. the carboxyl end of US2, but not US11, is required for MHCI degradation (Furman et al. 2002). The cytoplasmic tail of MHCI is, however, required (Story et al. 1999). These molecules show a specificity for class I isoforms and degrade HLA-A and -B, but not HLA-C, molecules (Schust et al. 1998). HLA-C can present peptide antigens to CTL, but it also has an important role as ligand for inhibitory KIR receptors on NK cells (see above).

The mouse  $\gamma$ 2 herpesvirus 68 also causes destruction of class I molecules. The K3 protein of MHV-68 is a ubiquitin ligase: it ubiquitinates the cytoplasmic tail of endoplasmic reticulum-resident MHCI, targeting it for destruction in the cytosol (Boname and Stevenson 2001). Interestingly, the highly homologous K3 and K5 proteins of KSHV also ubiquitinate MHCI, but this serves to target cell surface MHCI for degradation (Figure 31.8).

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### RETENTION OF CLASS I MOLECULES IN PRE-GOLGI COMPARTMENTS

Several VIPRs prevent MHCI from trafficking to the cell surface without causing their destruction. The adenovirus 5 protein E3 19K contains a di-lysine motif in its cytoplasmic tail; this motif binds to coat protein 1 (COP-1), which retrieves vesicles from the Golgi and returns them to the endoplasmic reticulum (Jefferies and Burgert 1990). In fact, this pathway was identified because of the E3 19K protein. The result is that MHCI is retained in the endoplasmic reticulum. Ad5E3 19K does not interact with all MHCI molecules equally, and the result is that some MHCI isoforms are more efficiently retained than others.

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The HCMV protein US3 also causes MHCI to be retained in the endoplasmic reticulum. US3 binds to peptide-loaded MHCI molecules. The mechanism of retention is not clear, because US3 does not contain an endoplasmic reticulum retention motif, and is in fact exported (Ahn et al. 1996; Jones et al. 1996). It is thought that the mechanism of retention involves repeated release and rebinding by newly synthesized US3 (Gruhler et al. 2000). As with most of the HCMV VIPRs, US3 has been studied primarily in transfected cells. In infected cells, the destruction of newly synthesized MHCI as a result of US2 and US11 is so rapid that the role of US3 is hard to appreciate. US3 is synthesized earlier in the viral replication cycle than US2 and US11, and it has been hypothesized that it prepares MHCI for destruction by US2 and US11 (Ahn et al. 1996).

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Murine CMV also encodes a VIPR that retains class I molecules. The *m152/gp40* causes MHCI to be retained in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and, as with US3, the mechanism is unclear (Ziegler et al. 1997). It has quite different efficacy against different MHCI isoforms (Kavanagh et al. 2001). Yet even for isoforms in which retention is not especially impressive (e.g. Kb, which is only 50 percent retained by *m152*), *m152* has a profound

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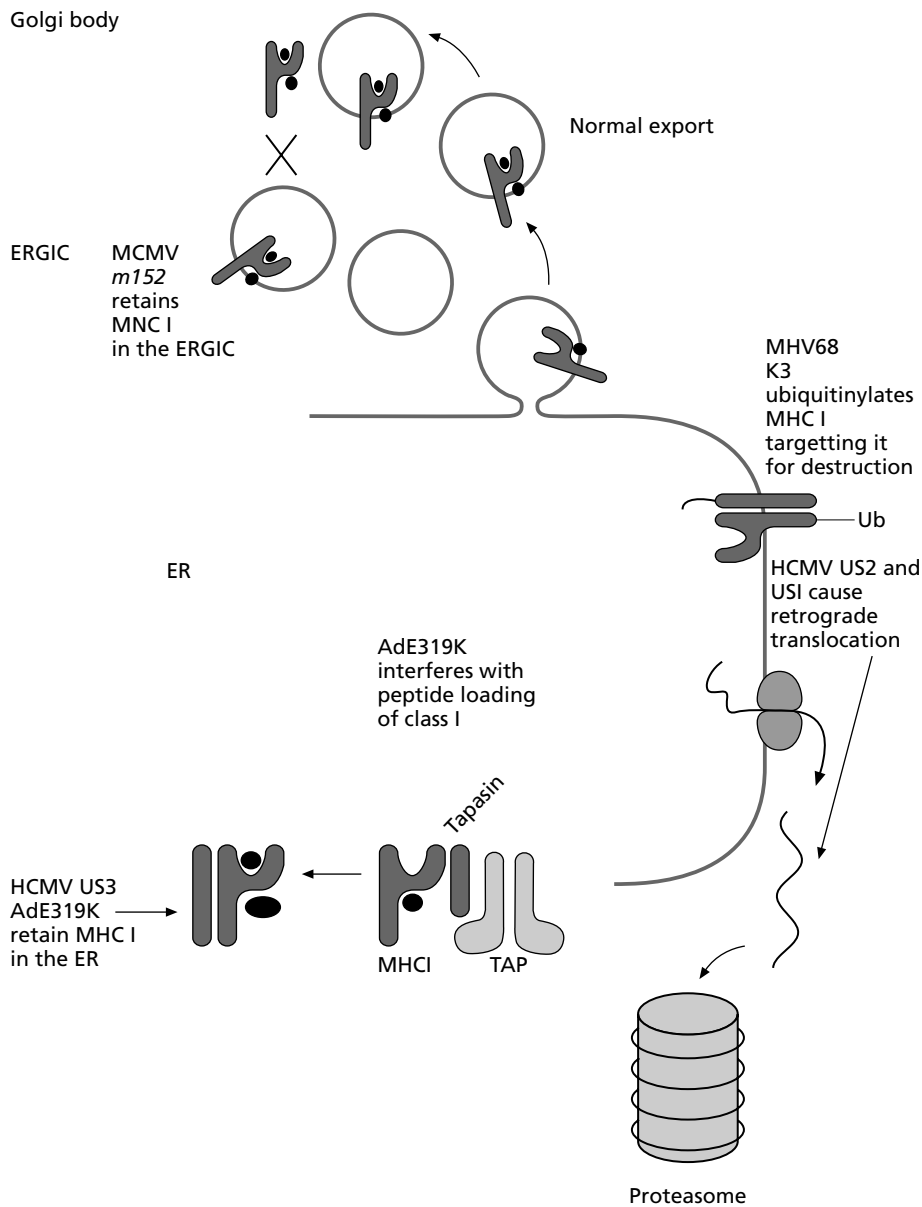


FIG027.8 **Figure 31.8** Interference with MHC class I in the endoplasmic reticulum (ER) and endoplasmic reticulum Golgi intermediate compartment (ERGIC).

effect on antigen presentation. Cells infected with wild-type virus are not detected by CTL, but cells infected with a virus lacking *m152* are readily detected. To prevent recognition of wild-type-infected cells by Kb-restricted CTL, the virus needs the two other MCMV VIPRs – *m4* and *m6* – the mechanisms of which are discussed below. Even so, the importance of *m152* remains enigmatic, and it may indicate that there is more profound synergism in the functions of these genes than is appreciated. Something similar may explain why HCMV needs its four VIPRs. As discussed above, in addition to retaining classical MHC I molecules, *m152* also prevents surface expression of molecules, which activates NK cells (Krpmotic et al. 2002).

### REMOVAL OF MHC I MOLECULES FROM THE GOLGI APPARATUS OR THE CELL SURFACE

Several VIPRs remove class I from the cell surface. The KSHV genes *K3* and *K5* both reduce cell-surface MHC I expression. *K3* ubiquitinylates MHC I after endoplasmic reticulum export. This signals MHC I internalization from the plasma membrane for endosomal degradation (Hewitt et al. 2002). The MCMV protein *m6* contains a di-leucine motif in its cytoplasmic tail, which sorts it to lysosomes. It binds MHC I in the endoplasmic reticulum, and targets MHC I to the lysosome for destruction (Reusch et al. 1999). Of the three MCMV VIPRs, *m6* has by far the most profound effect on cell-surface class I; from about 6 h after infection MHC I is rapidly lost

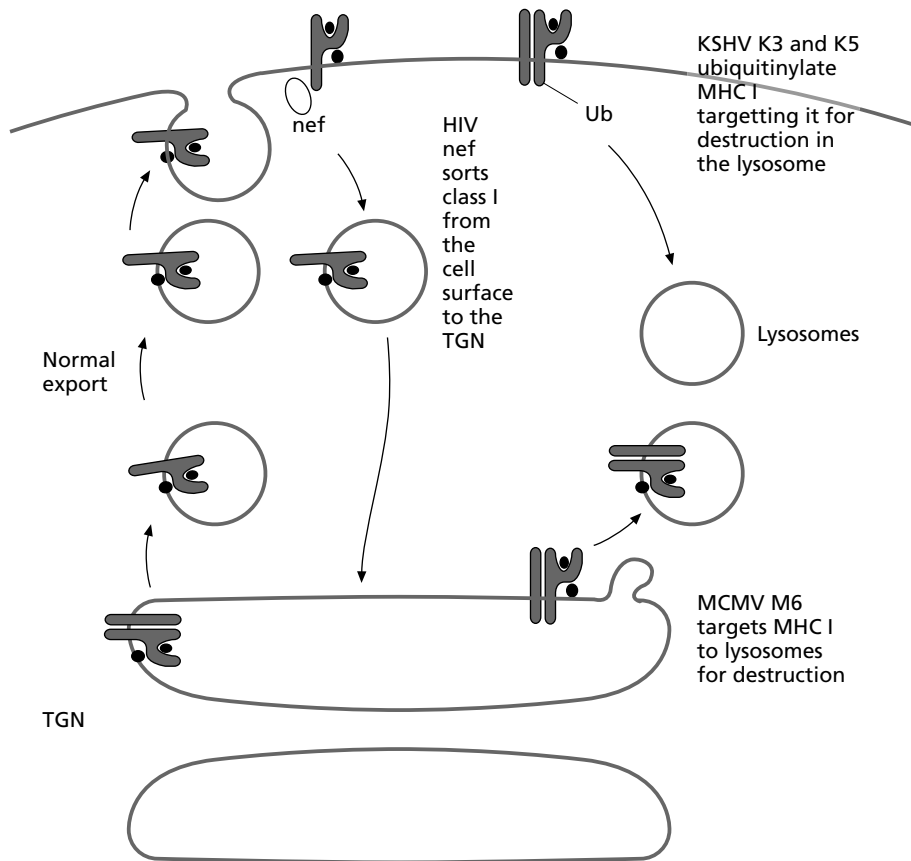


FIG027.9 **Figure 31.9** Interference with MHC class I at the Golgi body and cell surface.

from the cell surface if m6 is present (Wagner et al. 2002).

P027.87 The HIV (and simian immunodeficiency virus (SIV)) nef protein serves a number of functions. One of them is to remove MHC I from the cell surface and take it to the trans Golgi network (TGN) – an effect that has been shown to impair CTL destruction of HIV-infected cells. The mechanism of action of HIV nef is quite different from that of KSHV-K3. Nef binds to the PACS-1 protein that sorts molecules to the TGN: presumably it acts as a bridge between class I and PACS-1 (Piguet et al. 2000) (Figure 31.9).

P027.88 The MCMV protein m4/gp34 binds to MHC I both in the endoplasmic reticulum and at the cell surface. m4 is necessary to prevent lysis of infected cells by K<sup>b</sup>-restricted CTLs (Kavanagh et al. 2001). The mechanism of action of m4 is not understood, but it is likely that its association with class I at the cell surface prevents the productive engagement of the TCR.

## CD8 T-cell epitope variation

P027.89 With the exception of nef, all the VIPRs described above are encoded by large DNA viruses, the coding capacity of which gives them the luxury of such highly specialized immune evasion devices. Small RNA viruses have also other means of avoiding CTLs. As a result of their rapid

mutation rate, they exist in nature as a diverse genetic mix, often referred to as a ‘viral swarm’. Mutations in CTL epitopes should allow the selection of escape variants, unless the exact epitope sequence is crucial for viral replicative function. This means that, as CTL clones develop and become immunodominant, virus variants arise that escape detection. Whether CTL escape mutants help the virus to persist in a host has been a matter of debate for some time. However, recent evidence in both monkeys and humans demonstrates that mutants of a particular epitope have a selective advantage in hosts that possess the MHC I molecule capable of binding the epitope, but not in hosts that do not possess that allele (Allen et al. 2000; Goulder et al. 2001).

## Significance of viral interference with CD8 function in vivo

Most of the mechanisms reported above have been identified in vitro, and many of them have been described only using cells transfected with an isolated viral gene driven by a strong promoter. Their significance for infected cell recognition by CD8 T cells in vivo remains to be demonstrated. Obviously, true in vivo studies can be carried out only in animal models. The mouse is by far the most useful model for this purpose, although the importance of the HIV epidemic has led to studies of

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SIV in infected macaques. As a result of the high degree of variation between the MHCs of different species, and the specificity of VIPRs for MHCI isoforms, studies of immune evasion need to be conducted in the natural virus host. For mice, MCMV is the most useful model, although the vole virus MHV-68 infects laboratory mice well and has provided important insights.

P027.91 The first VIPR to be studied *in vivo* was the MCMV gene *m152*. Although wild-type MCMV-infected cells often escape detection by CTLs *in vitro*, cells infected with viruses lacking *m152* are always readily detected by CTLs. Krmpotic et al. showed that a virus lacking *m152* grew to about a log lower titer at day 7 (the time of peak CTL activity) than wild-type virus, but grew to the same titers in CD8 T-cell-deficient animals (Krmpotic et al. 1999). This effect was modest given the impact that *m152* has on CTL recognition *in vitro*, and challenges our concept of CD8 T-cell effector function *in vivo*. It has further been noted that a viral epitope whose presentation is completely abolished by *m152* *in vitro* is nevertheless equally immunodominant in mice infected with wild-type virus and with virus lacking *m152*, at least during the acute response. This suggests that cross-presentation is the main mode of antigen presentation driving the acute CTL response (Gold et al. 2002). However, viruses lacking *m152*, or indeed all three VIPRs, continue to provoke a very strong CTL response throughout the life of the animal. Therefore, it is unclear how the VIPRs of MCMV modulate the immunobiology of the virus.

P027.92 MHV-68 K3 downregulates MHCI and prevents CTL recognition of infected cells. MHV-68 has a complex pattern of infection, analogous to EBV (Doherty et al. 2001). After initial replication in the lungs, which is controlled by IFNs and to some extent by CD4 T cells and CD8 T cells, MHV-68 seeds the splenic B cells. These undergo a massive CD4-dependent proliferation, with concomitant amplification of the latent virus load. This situation is brought under control by the immune response, and a truly latent infection is established. Although expression of viral genes is limited during the splenic amplification phase, K3 is expressed. A virus lacking K3 grew to normal titers in the initial lung infection, but did not undergo splenic amplification unless CD8 T cells were removed (Stevenson et al. 2002). Thus this VIPR function is thought to be to prevent CD8 T-cell control of the splenic amplification stage.

P027.93 The selective advantage of CTL escape mutants in SIV and HIV infection *in vivo* has been clearly established, as described above under CD8 T-cell epitope variation. There is also evidence that the MHCI down-regulating ability of *nef* is important. As *nef* has multiple functions, a specific mutant that affected its ability to downregulate MHCI, but not CD4, molecules was made. The mutant and wild-type viruses grew equally well *in vitro*. However, because the mutation affected a single codon, a mutation back to the wild-type

sequence occurred in all four animals infected with the mutant virus within a few weeks (Munch et al. 2001). This suggested a strong selective pressure for the ability to downregulate MHCI.

## Modulation of MHCII expression

CD4 T cells play a role in antiviral immunity, but the exact role is controversial. They provide essential help for B cells, and effective CD8 memory also depends on CD4 T cells. Two mechanisms may be involved in CD4 T-cell help for CD8 T cells: secretion of growth-supporting cytokines such as IL-2 and activation of DCs for activation of CD8 T cells. This so-called 'licensing' role requires direct recognition of the DCs by the CD4 T cell. CD4 T cells may also be direct antiviral effectors in their own right. They synthesize antiviral cytokines, especially IFN- $\gamma$ , and if stimulated by professional APCs in the vicinity of the infection their role in secreting IFN- $\gamma$  may be significant. Also, some viruses replicate in professional APCs that express MHCII, and many somatic cells are induced to express MHCII by IFN- $\gamma$ . If these cells express class II loaded with viral antigen, CD4 T cells may have a direct antiviral effect by either killing the infected cell or secretion of cytokines.

Several viruses decrease the cell surface expression of class II by interfering with its synthesis. Most non-hemopoietic cells do not express MHCII, but (at least in humans) can be induced to do so by IFN- $\gamma$ . Viruses such as herpesviruses and adenoviruses that interfere with IFN- $\gamma$  signaling pathways will therefore interfere with induction of class II on these cells. MCMV induces the secretion of IL-10 by macrophages, and this leads to a decrease in macrophage MHCII expression (Redpath et al. 1999). Cells infected with HCMV also have decreased cell surface MHCII as a result of transcriptional inhibition of MHCII (Sedmak et al. 1994).

Some of the same HCMV proteins that interfere with MHCI expression have also been shown – in transfected cells – to interfere with MHCII in a similar manner. US2 causes HLA-DR $\alpha$  chains to be re-transported into the cytosol and degraded by the proteasome. Interestingly, it has the same effect on the  $\alpha$  chain of HLA-DM, the essential chaperone for peptide loading on to class II (Tomazin et al. 1999). US3 has also been shown to cause intracellular MHCII retention. (Hegde et al. 2002). From the above, it is clear that there may be some advantages to viruses in interfering with MHCII molecules, but in general the functional evidence for interference with presentation of endogenous viral antigens is lacking, and there are no *in vivo* data. The real significance of these phenomena remains to be demonstrated.

## Superantigens

Some viruses manipulate the immune system by stimulating T cells of irrelevant specificity. The B-type

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retrovirus, mouse mammary tumor virus (MMTV), encodes a superantigen that binds to certain TCR  $\beta$  chains and to MHCII, causing a massive proliferation of all CD4 T cells with the appropriate TCR. These activated T cells provide the necessary stimulation to drive proliferation of virus-infected B cells, which is essential for the infective process (Huber et al. 1994). EBV also has superantigen-like activity. Proliferation of CD4 T cells expressing the V- $\beta$ 13 TCR chain is a feature of EBV infection (Sutkowski et al. 1996). It has recently been shown that EBV activates an endogenous human retrovirus, HERV-K18, the envelope glycoprotein of which has superantigen activity (Sutkowski et al. 2001). Whether this activity plays a role in EBV pathogenesis is unclear but, as EBV undergoes latency in B cells, concomitant B-cell proliferation could aid the establishment of a latent virus pool.

## Inhibition of humoral immunity

P027.98 The production of antibodies specific to viral antigens may neutralize virus particles and thus prevent the infection of susceptible cells. Antibodies bound to viral antigens of cells can be detected by Fc-receptor-carrying effector cells. Viral antigens can activate the complement cascade, directly or in association with antibodies, leading to the destruction of infected cells or enveloped virus particles. Viruses with an RNA genome accumulate mutations caused by the low fidelity of the RNA polymerase. Virus particles with different antigenic properties may evade recognition by specific antibodies. Genetic variability may also generate variant peptide sequences representing new antigens or losing the ability

to bind to MHC molecules, thus evading recognition by the cellular immune system as well.

Herpes simplex virus and other viruses encode receptors for the Fc domain of IgG to evade the action of antibodies (Johnson and Hill 1998) (Figure 31.10).

In the case of HSV, glycoproteins gE and gI form a heterodimeric Fc receptor that participates in a process termed 'bipolar bridging', in which antibodies bind to their specific HSV antigen and simultaneously interact with gE–gI (Lehner et al. 1975; Frank and Friedman 1989). In this way, the viral Fc receptors inhibit complement-mediated antibody neutralization and antibody-dependent cellular cytotoxicity by cells carrying Fc receptors. HSV gE–gI may also bind other ligands and it has been suggested that it acts as a receptor-binding glycoprotein in the process of cell-to-cell spread.

Several members of the herpesvirus family encode homologs of complement regulatory proteins that block the complement cascade in several ways (Johnson and Hill 1998; Favoreel et al. 2003).

The HSV glycoprotein gC binds the complement factor C3; it protects cell-free virus from complement-mediated neutralization and inhibits the lysis of HSV-infected cells by antibody and complement (Lubinski et al. 1999). HSV gC also functions in virus attachment to cell-surface glycosaminoglycans, especially on the basolateral surfaces of epithelial cells (Sears et al. 1991); thus it has been difficult to interpret the attenuated phenotype of gC virus mutants in vivo (Lubinski et al. 1999). However, in C3 knock-out mice, the disease caused by the gC virus mutant was similar to that of wild-type HSV, suggesting a major role of gC in resistance to complement (Lubinski et al. 1999). HVS, a virus that infects monkeys, encodes a homolog of the

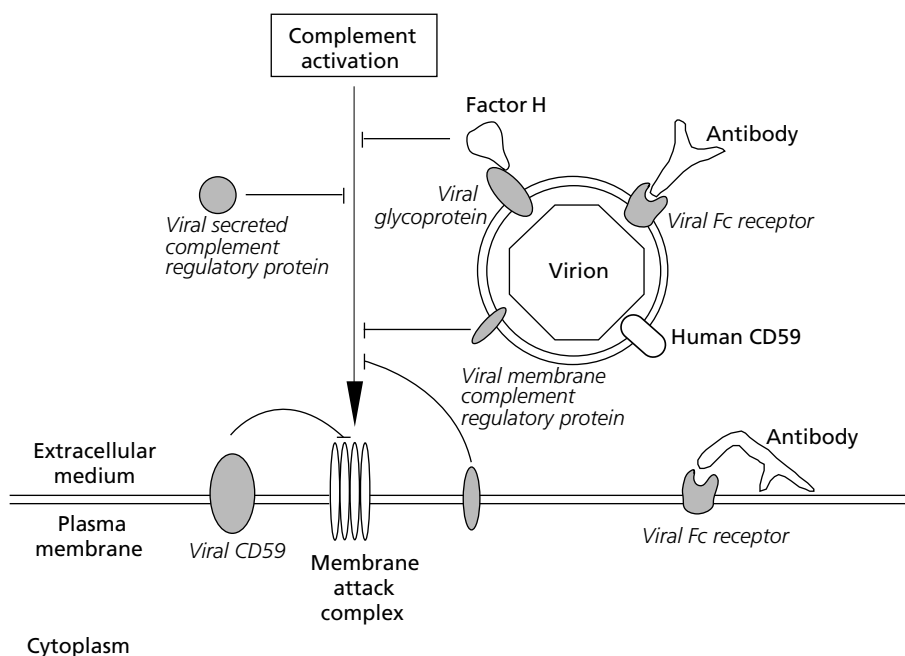


FIG027.10 **Figure 31.10** Viral modulation of complement and antibody responses.

inhibitor of the membrane attack complex CD59, which protects the infected cells from complement-mediated lysis (Rother et al. 1994).

P027.103 A strategy used by HIV is to recruit the soluble factor H that inhibits complement activation by interacting with the gp120–gp41 complex (Stoiber et al. 1996). Removal of factor H from human serum augments the effect of complement on HIV-infected cells. The poxviruses variola virus (VaV), VV, and CPV encode a secreted protein, known as viral complement control protein (VCP), which blocks the classic and alternate pathways of complement activation (Kotwal et al. 1990; Rosengard et al. 2002). Inactivation of the VCP gene in VV causes virus attenuation in animal models, demonstrating an important role of this protein in preventing host defense mechanisms and a role for complement in antiviral immunity (Isaacs et al. 1992). Interestingly, inactivation of VCP from CPV caused a substantial increase in the inflammatory response in infected mice compared with wild-type CPV infections (Miller et al. 1997). This illustrated that inhibition of the inflammatory response by viral proteins may reduce tissue damage and allow virus replication in host cells that would otherwise be eliminated by the immune response.

P027.104 Instead of encoding viral homologs of host proteins, HIV, VV, and HCMV, incorporate into the virion envelope complement regulatory proteins, such as CD56 and CD59, which confer complement resistance to the virus particle (Spear et al. 1995; Vanderplasschen et al. 1998)

## H027.6 CONCLUSIONS

P027.105 A simplistic view of a virus–host interaction is that of a fierce competition between the virus infecting and replicating in a cell until its death, and then spreading to uninfected cells and tissues, whereas the immune system of the host seeks to eliminate all infected cells. This may be true for viruses that are restricted in their growth to mucosal surfaces, those that replicate fast, and those that are directly transmitted. Many viruses replicate slowly and have a tropism only for specific tissues in certain organs to which they have to be transported or disseminated, and often the sites of entry and the sites of release differ. Cell tropism is defined not only by the virus' inability to infect other tissues, but also as a result of the stringent immune control of the host which limits viral spread. Under conditions of immune suppression herpesviruses can infect many tissues, showing that the restriction of the infection to certain sites reflects the virus–host balance of the immunocompetent host. Therefore, the diversity in the biology of closely related viruses has been shaped by evolution, and the biological diversity reflects the function of viral proteins that have been selected to modulate cellular functions.

P027.106 To describe viral functions as immunoevasive may have been correct for those that directly interfered with

antigen processing in the MHCI and MHCII pathways. The reader is cautioned to treat the growing list of viral immune evasion strategies with some skepticism. These strategies are best regarded as modifiers of the immune response, not as immune evasion, e.g. despite the mechanisms described for herpesviruses, they are highly susceptible to NK control *in vivo*. In fact, recurrent herpesvirus infection is the clinical hallmark of NK deficiency. Later, a host of viral effects on cell functions were also listed as immunoevasive. However, many of these functions affect not only cells of the immune system, e.g. viral functions that affect apoptosis may serve to inhibit proapoptotic molecules released from NK and T cells. However, in the absence of viral anti-apoptotic functions some herpesviruses completely fail to replicate in a number of cell types. Other herpesviruses use functions to keep their host cells in a state of continuous division and to prevent cell death at the same time. Thus, these functions select the viral host cell range and define the biology of a virus.

In a similar vein, the research on viral modulation of chemokine networks has led to hypotheses on Janus-like effects of these viral proteins. A nonfunctional receptor can neutralize the cell-attractive function of a chemokine for cells of the immune system, and thus truly serve an immunoevasive function. On the other hand, there are virus-encoded chemokines that attract host cells. It is thought that these cells serve the virus for reaching target tissues. A remarkable aspect is that some of the cells that provide the 'taxi' service are those that normally would fight the viral infection. Therefore, there is certainly a score that orchestrates the roles of viral chemokines and chemokine receptors in viral dissemination and persistence, but it will take years to understand the music.

Examples of viral proteins that modulate host immune functions presented in this section were mainly taken from viruses with large DNA genomes. In part this is the result of the limited expertise of the authors. Second, many of these functions were first detected in DNA viruses. There is no doubt that several RNA viruses that were not been listed also encode such functions. In general, it appears that viruses need functions to dampen the function of innate immunity and the powerful antiviral functions of the soluble mediators such as the interferons. The analysis of viral functions that affect soluble mediators and/or their subsequent signaling cascades is an area of active research. Another reason for reporting on DNA virus functions is the working hypothesis that in a large virus genome a protein would perhaps serve only one function. There is the tacit belief that the function seen for the isolated gene *in vitro* also represents the functions that it has in the viral context *in vivo*. This may probably turn out to be not entirely correct and examples have been mentioned where one protein affects different antiviral principles. If this is true, the pressure for multi-

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functionality of proteins is even higher in RNA viruses with their smaller genomes.

P027.109 In apparent contrast to the multifunctionality of proteins is the fact that some viruses encode multiple genes for similar functions, all of which attack the class I pathway. It is not always clear what the purpose of this multiplicity is. One purpose appears to be to deal with MHC polymorphism. MHCI molecules belong to the most polymorphic proteins, as a result of the three class loci – HLA-A, -B, and -C in humans – and to extensive polymorphism at each locus. The length of coevolution of class I and viruses – particularly herpesviruses – makes it likely that some of this polymorphism has evolved to counteract VIPRs. In fact, many VIPRs deal with different class I isoforms with different degrees of efficiency. Thus, the function of some viral proteins are considered complementary. Another function for multiple genes is cooperativity: neither gene is capable of inhibiting antigen presentation alone, but together they function effectively to prevent CTLs recognizing infected cells. A similar multiplicity of genes with related function is also seen with respect to genes controlling apoptosis, and genes controlling chemokine and cytokine networks.

P027.110 The analysis of the principles evolved by the viruses to target the same mechanism, e.g. the peptide transport or MHCI maturation, indicates that the biological problem – defense against CD8 T-cell control – has been independently addressed by the  $\alpha$  and  $\beta$  herpesviruses. Analogous but mechanistically completely different solutions were found. This indicates that the virus subfamilies were already separated before the acquisition of these functions. The primordial immune system fighting the archetype herpesvirus progenitor lacking this function was different as well. There is a strong belief that the constant attack by viruses has shaped both the virus and the mammalian immune system that we know today. Some viral immunomodulatory functions, e.g. some antiapoptotic genes, operate across species barriers, whereas others, e.g. MHCI modulators, often do not. This is explained by the coevolution of viruses with their hosts and could mean that the host-specific functions were acquired at a later stage.

P027.111 The true function of the immunomodulatory genes can be delineated only by in vivo studies using appropriate combinations of virus mutants and host mutants. Given the plethora of genes, the redundancy of related functions, the multifunctionality and cooperativity of proteins, and the intricate interplay of functions that act differently on different cells at different states of activation and differentiation, and with a different efficacy with respect to the genetic background of the host, it is clear that it will take a long time to test present concepts and to develop a conceptual network of hierarchical functions. This may be of use for modern medicine with its new concepts of treatment that affect immune functions and thus disturb the virus–host balance so carefully tuned by evolution.

## ACKNOWLEDGMENTS

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