Viruses use stealth technology to escape from the host immune system

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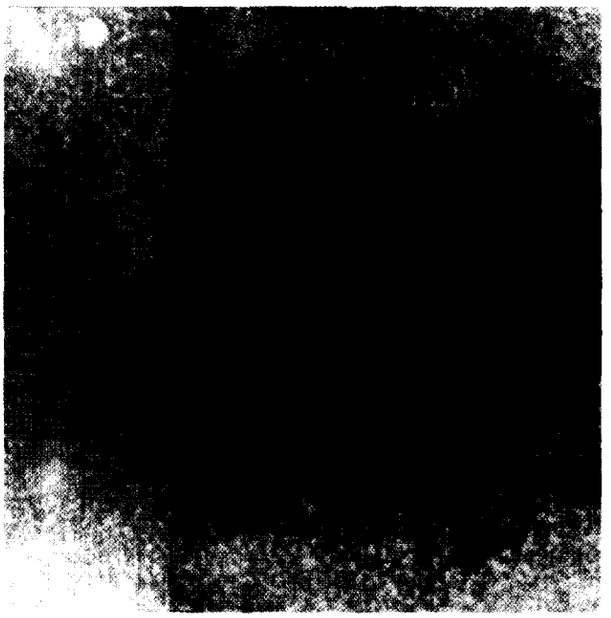

Electron micrograph of non-enveloped herpes virions. Kindly provided by Dr Paul Roholl, National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

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In this review, we focus on recent investigations that reveal novel mechanisms by which viruses evade detection and elimination by the host immune system. In particular, we consider the evasion mechanisms of five persistent viruses: herpes simplex virus, human cytomegalovirus, mouse cytomegalovirus, Epstein–Barr virus and adenovirus. Unravelling the strategies used by viruses to survive within the host could identify new targets for antiviral drugs and for improved vaccines. Identification of the mechanisms that underlie these strategies might also reveal new, fundamental features of biology that occur in uninfected cells and are exploited by viruses.

VIRUSES use host cells to replicate, and can have adverse effects on host physiology, but their fate is ultimately linked to the host's survival. Lethal viruses that eradicated entire host populations would ultimately eliminate themselves. Typically, viruses and their natural hosts coexist: all that is needed is a time-window within the host's life-cycle during which the virus can replicate and be transmitted.

This time-window determines the extent to which viruses interact with the host's defence mechanisms. Rapid, cytopathic viruses, which kill the cells that they infect, need to replicate and infect other organisms before the virus and virus-infected cells are eliminated by the host. Persistent viruses, which remain within cells without killing them, play out a different scenario. Some persistent viruses go into latency upon infection, expressing no viral genes (except those required purely for the maintenance of the latent state). Having remained quiescent within cells, viruses in their latent state can become acutely activated and begin to produce new virus particles. This cycle of latency and activation is, on occasion, repeated several times. For both cytopathic and persistent viruses, however, the host's defences limit the time available for the virus to multiply. Viruses that lengthen this time-window, by slowing down

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host defences or by making themselves undetectable by the host, increase their likelihood of transmission and survival.

Cytotoxic T lymphocytes (CTLs) play an important role in the elimination of virus-infected cells, which they recognize by the presence of virus-derived peptides in the antigen binding-site of major histocompatibility complex (MHC) class I molecules occurring on the surface of the infected cells[1,2]. Viral co-evolution with the host immune system is likely to favour the emergence of viruses that can elude recognition by CTLs. Viruses can manipulate not only peptide loading of the MHC class I molecules, but also the synthesis, assembly and surface expression of the MHC-encoded glycoproteins – the very molecules that signal the presence of a virus-infected cell to the immune system[3,4].

CTLs detect antigenic peptides bound to class I MHC molecules displayed on the surface of infected cells

Viral mRNAs are translated using the normal translation machinery of the cell. Like other proteins, cytosolic viral gene products are degraded, as part of normal protein turnover, by cellular proteases, among them, a multi-subunit catalytic protease complex known as the proteasome[5] (Box 1; Fig. 1a). Degradation of viral proteins in the cytosol leads to the formation of polypeptides that might then be transported to the lumen of the endoplasmic reticulum (ER) by a 'dedicated' transporter complex comprising two gene products: TAP1 and TAP2 (Fig. 1b), where TAP stands for transporter associated with antigen presentation[6]. In the ER, these peptides assemble with the membrane-bound heavy chain of the class I MHC molecules and the light chain, β_2 microglobulin (β_2m). The binding of peptide stabilizes the interactions of the MHC class I heavy and light chain and, as a result, a trimolecular complex of MHC class I, β_2m and peptide is formed (Fig. 1c)[7]. These MHC complexes, loaded with peptides, are then transported from the ER, through the Golgi apparatus to the plasma membrane (Fig. 1d). At the cell surface, they are available for surveillance by CTLs[8]. Upon recognition of the MHC–peptide complex on the plasma membrane of the virus-infected cell, the CTLs deliver a lethal 'hit', resulting in the lysis of the infected cell.

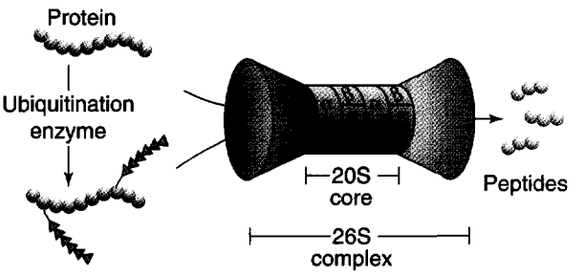
A herpes simplex virus-encoded protein, ICP47, inhibits TAP

The herpes simplex viruses HSV-1 and HSV-2 are closely related viruses that infect the oral and genital mucosa, respectively. HSV-1 can maintain latency in the trigeminal and cervical ganglia, HSV-2 in the sacral ganglia. The molecular basis of the establishment of HSV latency is still unclear. In the latently infected neurons, the virus is periodically reactivated and virus is transferred by axonal transport to cells innervated by the infected neurons. The severity of the lesions strongly depends on the host immune response. In normal individuals, small vesicles usually occur, but in immunosuppressed patients, severe lesions are frequently observed. HSV is relatively insensitive to antiviral antibodies. It is therefore likely that T cells play a role in controlling viral infection and reactivation.

To escape from immediate elimination by the host immune system, the virus has acquired a mechanism to reduce the expression of peptide–MHC complexes. As a result, within a few hours of infection with HSV, the infected cells are resistant to lysis by HSV-specific CTLs. The MHC class I molecules are synthesized normally but are retained in the ER[9]. This resistance to lysis by CTLs depends on a small (9 kDa) cytosolic protein, ICP47, encoded by HSV, and does not involve any direct interaction between ICP47 and class I molecules *per se*.

Box 1. The proteasome

The 26S proteasomal complex is a large (~1500 kDa) proteolytic complex[5] that contains numerous subunits, some of which have not yet been characterized. The core is formed by the 20S proteasome (~700 kDa), which consists of 14 α and 14 β subunits[51]. Proteins are targeted for degradation by attachment to chains of ubiquitin, an 8.4 kDa protein. Initially, the C-terminal Gly residue of ubiquitin is covalently linked to the ε-amino group of Lys side chains in the protein through an iso-peptide bond. Further ubiquitin moieties are added to the primary ubiquitin and the resulting branched chains of ubiquitin target the protein for ATP-dependent destruction by the 26S proteasomal complex. Non-ubiquitinated proteins can also be degraded by the proteasome. The proteasome is responsible for the normal turnover of proteins in the cytosol. Proteasomal degradation leads to the formation of peptides that can be transported into the endoplasmic reticulum (ER) where they bind major histocompatibility (MHC) class I molecules (reviewed in Refs 5, 52). In virally infected cells, peptides derived from viral proteins are generated by the proteasome and ultimately displayed by MHC class I molecules at the cell surface (Fig. 1). Recently, several very effective and specific inhibitors of proteasomal degradation have been described. These include peptide aldehydes and the antibiotic lactacystin. Leucyl-leucyl-norleucinal (LLNL), the calpain I inhibitor that was co-crystallized with the proteasome, is also highly effective, although it affects other cellular proteases as well. Blocking proteasomal function by these drugs interferes with MHC class I-restricted antigen presentation and illustrates the important role that the proteasomal complex plays in antigen processing[52].



As class I molecules require bound peptides and β_2m to form a stable trimolecular complex, the observation that the ER-retained MHC class I molecules from ICP47-expressing cells were unstable in detergent lysates suggested that the class I molecules lacked antigenic peptide. Because ICP47 is a cytosolic protein, it was possible that this HSV protein interfered with the generation of peptides or with the function of TAP, which moves peptides from the cytosol to the ER (Fig. 1b). The use of a semi-permeabilized cell system demonstrated a strong inhibition of translocation of peptides into the ER in the presence of ICP47 (Refs 10,11). Furthermore, a physical association of ICP47 with the human, but not rodent, TAP complex could be demonstrated[12]. The transporter consists of a heterodimer of TAP1 and TAP2, each containing 6–8 membrane-spanning regions and an ATP-binding domain. Competition experiments have suggested that the TAP complex contains a single peptide-binding site[13]. ICP47 and peptides compete for binding to

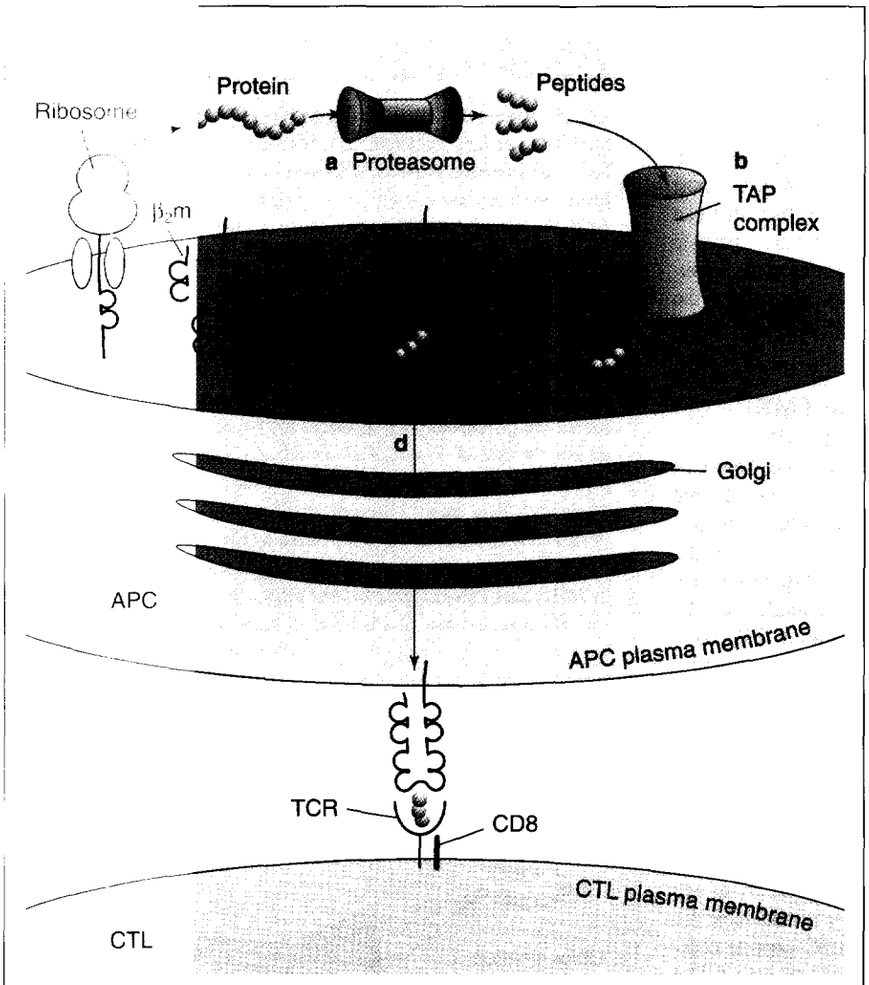


Figure 1. Major histocompatibility complex (MHC) class I-restricted antigen processing and presentation. Cytotoxic T lymphocytes (CTLs) recognize antigenic peptides presented by antigen-presenting cells (APCs) in the context of MHC class I molecules. The peptides are derived from cytosolic proteins, including virally encoded proteins. **(a)** The proteins are degraded by the proteasome and **(b)** the resulting peptides are translocated to the ER by the transporter associated with antigen presentation (TAP). **(c)** In the endoplasmic reticulum (ER), the peptides are loaded onto newly synthesized MHC class I molecules. **(d)** Properly assembled MHC class I heavy chain–β_2-microglobulin (β_2m)–peptide complexes are transported to the cell surface where they are available for interaction with T-cell receptors (TCR) and accessory molecules such as (in the case of CTLs) CD8.

TAP. This prevents the transport of peptides, making cytosol-derived peptides unavailable for binding to and display by MHC class I molecules (see Fig. 2a). Interestingly, the inhibitory effect of ICP47 can be mimicked with a 35-residue synthetic peptide corresponding to amino acid residues 1–35 of ICP47. The active site within this sequence is currently being mapped using a series of truncated synthetic peptides derived from ICP47 (B. Galocha, A. Hill and H. Ploegh, unpublished).

Because the only known role of the TAP complex is to transport peptides, inhibition of this function is unlikely to disrupt aspects of cellular physiology other than antigen presentation. Thus, inhibitors of TAP, based on active sequences in ICP47, might be immunosuppressive. Conversely, small molecules that prevent the TAP–ICP47 interaction might improve the immunogenicity of HSV-infected cells.

Human cytomegalovirus gene products target MHC class I molecules for destruction via a novel pathway

Primary infection with human cytomegalovirus (HCMV) is usually followed by a lifelong, asymptomatic persistence of the virus. However, serious morbidity and mortality occur in immunosuppressed adults and in infants infected *in utero*. CTLs play an important role in the maintenance of the host–virus equilibrium throughout the infectious cycle[14,15].

Infection with HCMV leads to a rapid decrease in the surface expression of MHC class I molecules. The drastic reduction of class I molecules abrogates the presentation of viral antigens by HCMV-infected cells. The class I heavy chains are not simply retained in the ER (as is the case in cells infected with murine CMV, see below), but instead are degraded soon after synthesis (Fig. 2b)[16–18]. The use of deletion mutants of HCMV has revealed two genes to be involved, *US2* and *US11*, each of which is sufficient to induce degradation of MHC class I molecules[18]. Despite the similarity in the modes of action of these two genes, there is no obvious homology in the primary structures of their products.

Cell lines stably transfected with *US11* were used to investigate the mechanism by which the class I heavy chains are targeted for degradation[19,20]. In the transfectants, as in HCMV-infected cells, the class I heavy chains were degraded with a half-time of less than 1 min. Surprisingly, inhibitors of the cytosolic proteasome blocked the degradation and, in the presence of these inhibitors, a breakdown intermediate was identified. Further characterization revealed that the observed intermediate was the class I heavy chain that had lost its single N-linked glycan. Unexpectedly, the removal of the sugar moiety involves an N-glycanase.

The precursor–product relationship, established by pulse–chase experiments, indicated that the deglycosylated intermediate was derived from fully glycosylated MHC class I heavy chains. Since the proteasome and an N-glycanase were involved in the degradation, it was likely that the process was occurring in the cytosol. Indeed, the cytosolic localization of the breakdown intermediate was confirmed by subcellular fractionation. Apparently, in the presence of the US11 glycoprotein, MHC class I molecules are translocated into the ER normally but are then 'dislocated' into the cytosol where they are degraded by the proteasome. We propose a model in which the class I heavy chains do not leave the translocation complex, but slide back to the cytosol as soon as the translation of the heavy chain is completed and the product has been released from the ribosome[19] (see Fig. 3).

At immediate-early times after infection with HCMV, a glycoprotein, US3, is expressed that shows significant homology to US2. Experiments with cell lines stably transfected with US3 indicate that this glycoprotein also causes downregulation of MHC class I molecules[21,22]. However, this gene product does not induce the rapid degradation of class I that is observed in the presence of US2 and US11. Instead, class I molecules are retained in the ER (Fig. 2b). Expression of US3 precedes and overlaps with the expression of US2 and US11. Apparently, HCMV downregulates class I expression using different glycoproteins that are likely to act synergistically when expressed simultaneously.

HCMV encodes another glycoprotein, UL18, which has been proposed to play a role in evasion of the immune response. UL18 demonstrates homology to MHC class I heavy chains[23]. When UL18 is coexpressed *in vitro* with β_2m, the molecules associate and the resulting complex is transported to the cell surface[24]. These findings led to the proposal that UL18 sequesters β_2m, thus preventing the formation of class I heavy chain–β_2m complexes. However, studies with UL18 deletion mutants indicated that this is unlikely to occur[25].

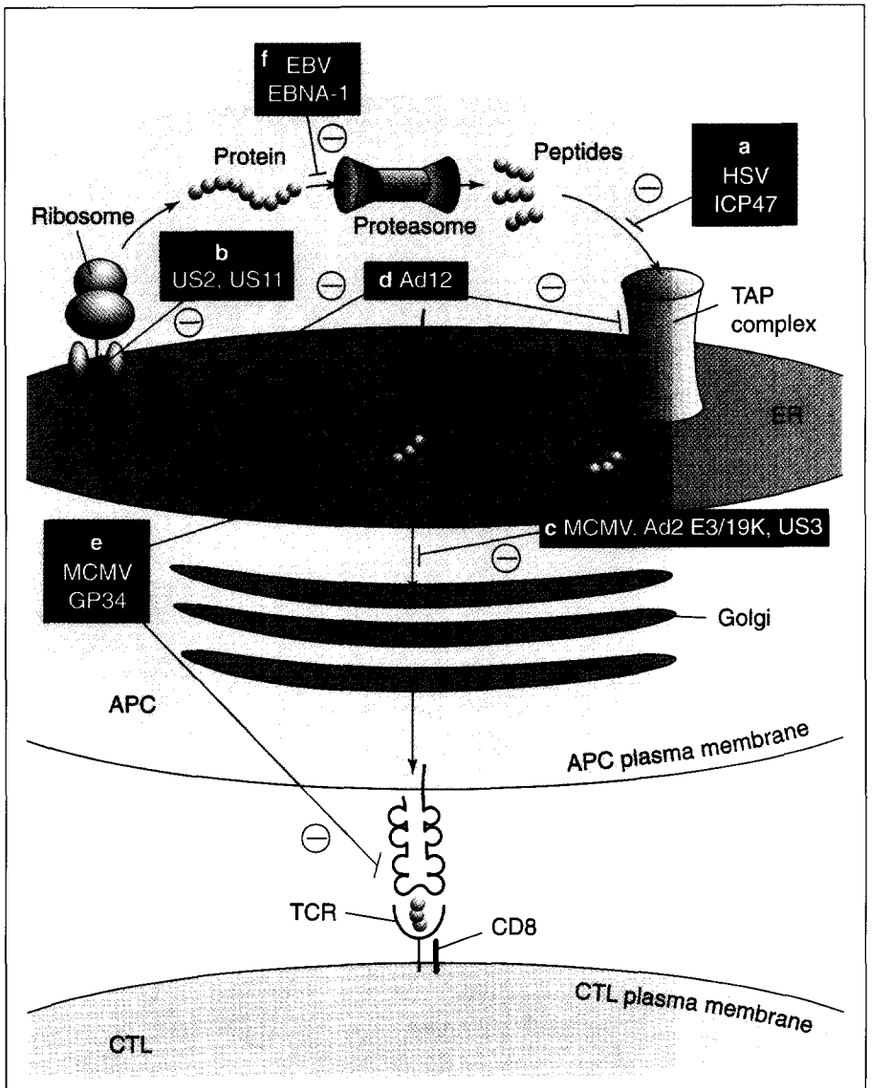


Figure 2. Interference by herpes simplex virus (HSV), human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), Epstein–Barr virus (EBV) and adenoviruses (Ad) in the MHC class I pathway. (**a**) HSV type 1 and 2 encode a small cytosolic protein, ICP47, that blocks the peptide transporter TAP. (**b**) HCMV expresses two different proteins, US2 and US11, which induce rapid degradation of major histocompatibility (MHC) class I molecules by a novel protein breakdown pathway (see also Fig. 3). (**c**) The HCMV-encoded US3 causes retention of MHC class I molecules in the endoplasmic reticulum (ER), and ER retention is also observed during infection with MCMV and Ad2. In the case of Ad2, retention is caused by an *E3*-encoded 19 kDa protein, E3/19k. (**d**) Ad12 *E1* gene products interfere with transcription of MHC class I and TAP genes. (**e**) MCMV GP34 binds to the the MHC class I heavy chain–β_2-microglobulin (β_2m) complex, thereby preventing it from presenting antigen to the cytotoxic T lymphocyte (CTL). (**f**) The EBV-encoded EBNA-1 protein evades CTL recognition, perhaps because it fails to be degraded by the proteasome.

Murine CMV prevents the surface expression of MHC class I molecules selectively retaining them in the ER

After infection, murine CMV (MCMV) persists in mice for life. As with other cytomegaloviruses, infection can be asymptomatic or accompanied by disease. CTLs generated by mice against MCMV are essential for controlling the infection and for resistance to lethal doses of virus[26]. Natural killer (NK) cells also play a role in controlling the infection[27].

In cells permissive for infection and production of virus, MCMV genes are expressed in a cascade characteristic of other herpesviruses. Within 1 h, immediate-early (IE) genes are expressed, which then control the expression of early (E) genes over the next 8 h after infection, culminating in the expression of late (L) genes at about 16 h after infection. Mice infected with MCMV generate CTLs that recognize a viral nucleophosphoprotein, pp89, an IE gene product. The presentation of epitopes from this protein to pp89-specific CTLs can be followed over the time-course of the infection by exposing MCMV-infected cells to CTLs at different time-points after infection[28]. While pp89-specific CTLs efficiently recognize an epitope from the protein within 1 h of infection, expression of some E gene(s) abrogates presentation of antigens to the lymphocytes over the next few hours[29,30].

To investigate this block to the presentation of antigens during the early phase of infection, the synthesis and maturation of MHC class I molecules was followed in the context of viral infection. This led to the discovery that MCMV expresses an E gene that selectively retains MHC class I molecules in the ER. Transit of the MHC molecule through the Golgi apparatus, and its eventual appearance on the cell surface, is blocked during this phase of the infectious cycle, thus thwarting the presentation of virally derived peptides (see Fig. 2c). Deletion studies have localized this retention gene in the MCMV

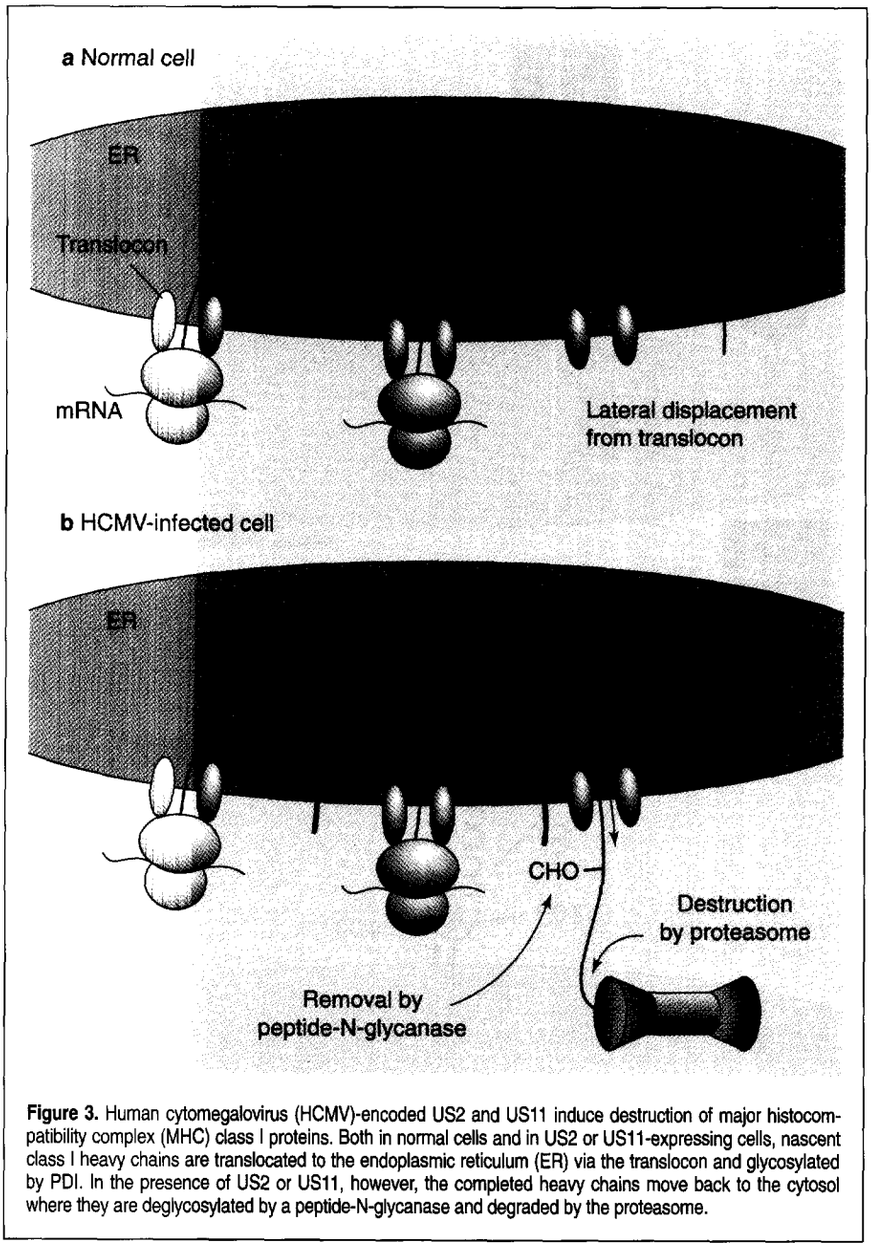


Figure 3. Human cytomegalovirus (HCMV)-encoded US2 and US11 induce destruction of major histocompatibility complex (MHC) class I proteins. Both in normal cells and in US2 or US11-expressing cells, nascent class I heavy chains are translocated to the endoplasmic reticulum (ER) via the translocon and glycosylated by PDI. In the presence of US2 or US11, however, the completed heavy chains move back to the cytosol where they are deglycosylated by a peptide-N-glycanase and degraded by the proteasome.

genome and have made its identification and characterization in the near future a possibility[31].

In addition to the retention mechanisms for MHC class I molecules, MCMV expresses other genes that might affect the ability of the host immune response to detect infected cells. In our efforts to characterize MCMV-encoded proteins that bind to MHC class I molecules, we have discovered and cloned the gene for a 34 kDa, membrane-bound glycoprotein, gp34, which co-precipitates with MHC molecules. This MCMV protein binds to MHC class I molecules in the ER and then moves to the cell surface in association with these molecules (Fig. 2d). Only folded MHC class I molecules, containing both heavy chain and β_2m, are bound by gp34 (Ref. 32). Whether gp34 protects the virus against host immunity is unknown.

The Epstein–Barr virus Gly-Ala repeat might conceal a viral antigen from recognition by the immune system

Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus that infects B cells and some epithelial cells. The infection is usually asymptomatic in children; in some previously unexposed adults, however, infection causes EBV mononucleosis. Most infections progress to the asymptomatic state, in which the virus exists with little or no consequence for the host. The virus is occasionally shed from the oropharynx of carriers and can spread through the exchange of saliva.

EBV-infected B cells grown *in vitro* carry the virus in a latent state; in these cells, a limited repertoire of nine viral gene products is expressed. Immunocompetent carriers of EBV will raise $CD8^+$ CTLs to eight of these nine proteins: EBNA2–6, LMP-1, LMP-2a and LMP-2b[33]. It is likely that B cells of the host that are latently infected by EBV express these genes and, as a result, are recognized and eliminated by CTLs specific for these eight antigens[34]. The existence of a vigorous CTL response to antigens from EBV has led to the hypothesis of a viral 'reservoir' in which the virus can persist without detection and elimination by host lymphocytes[35]. The virus might move continuously from this reservoir to other cellular compartments, into B cells and epithelia, the virally infected cells of which are constantly being recognized and eliminated by circulating CTLs.

The ninth antigen, EBNA-1, has several peculiar characteristics. First, the virus can express only EBNA-1 without expressing the other eight viral genes. Indeed, a small subpopulation of infected B cells in carriers has been found that expresses only *EBNA-1* (Ref. 36). It is possible that the 'EBNA-1-only-expressing' B cells represent the reservoir where the virus persists, concealed from the immune system.

The candidacy of the EBNA-1-only B cells for the persistent reservoir has been strengthened by another unique immunological feature of EBNA-1. EBV-infected individuals mount a CTL response to all the other eight EBV antigens, but not to EBNA-1. The absence of a cytotoxic response to EBNA-1 would seem advantageous to the virus: B cells expressing EBNA-1 only would constantly elude detection from CTLs and allow EBV to persist.

The inability to find human CTLs that recognize epitopes from EBNA-1 raised an interesting immunological question: how did EBNA-1 evade detection by such T cells? It is possible that EBNA-1 contains no natural epitopes that will bind to any human MHC molecules. However, peptides derived from EBNA-1 that bind with high affinity to several human MHC alleles have been described[37]. Alternatively, it is possible that while EBNA-1 contains peptides that bind to MHC class I molecules, these epitopes are not generated efficiently by the

antigen-processing and presentation machinery. This has been investigated by placing natural epitopes from EBNA-4 (another EBV antigen) into the reading frame of EBNA-1. When such an epitope was placed into various parts of the reading frame of EBNA-1, CTLs that recognized the EBNA-4 epitope could no longer detect the EBNA-1–EBNA-4 chimera. This suggested that *EBNA-1* encodes some mechanism to inhibit the generation of epitopes placed in its reading frame, including, potentially, epitopes contained in EBNA-1 itself[38].

To confirm this, mouse CTLs were raised against an epitope contained within an 80-residue fragment from the C-terminal region of EBNA-1. The CTLs were tested to see whether they could recognize EBV-infected cells expressing full-length EBNA-1 or mouse cells transfected with *EBNA-1*. In both cases, cells expressing the full-length molecule were not lysed by CTLs. Once again, this suggested the presence of inhibitory sequences in EBNA-1 that prevented the generation of epitopes from the protein.

To characterize the nature of this inhibitory domain, we and other investigators have made several deletions in EBNA-1. The EBNA-1 molecule contains an N-terminal domain followed by a polymorphic Gly-Ala co-repeat unit (the length of which varies among EBV strains) and a conserved C-terminal domain. Deletion of the Gly-Ala repeat restored recognition of EBNA-1 by CTLs. This provided direct evidence of the inhibitory effect of the repeat unit in the generation of epitopes from the full-length protein (S. Mukherjee *et al.*, unpublished). Conversely, it has been demonstrated that transplanting the Gly-Ala repeat onto other antigens abrogates the presentation of epitopes from these antigens[38].

The mechanism through which the Gly-Ala repeat conceals EBNA-1 from CTLs is unknown. It is possible that EBNA-1 is not efficiently degraded by the proteasome and thus peptides derived from it are not generated in sufficient quanitity to be detectable in cells or to prime an immune response. This is consistent with the unusually long half-life of the protein. The Gly-Ala repeat could act by placing steric constraints on the accessibility of EBNA-1 to the normal degradation apparatus (see Fig. 2e). By transplanting the repeat to other antigens and by placing degradation sequences in EBNA-1, we are investigating the possible link between the structure of this viral antigen and its resistance to recognition by CTLs.

Adenoviruses block the transcription of MHC class I molecules and prevent their appearance on the cell surface

Adenoviruses (Ad) have evolved two distinct strategies to avoid detection by host CTLs. Both mechanisms reduce the availability of MHC class I molecules on the surface of infected cells. Genes encoded in the *E1a* region in the genome of adenovirus strain 12 (Ad12) were shown to reduce the expression of MHC class I molecules by interfering with the transcription of class I genes[39] (Fig. 2f). Expression of TAP molecules is also reduced by this mechanism. It is not known, however, whether peptide transport is reduced to the extent that assembly of MHC class I–peptide complexes is affected[40]. Class I expression in cells is regulated by several proteins, among them NFκB, which bind to an element in the MHC class I promoter. The *E1a* gene product inhibits the processing of precursors of NFκB, and consequently reduces the level of these two transcriptional activators[41]. Conversely, different regions in the class I promoter responsible for the Ad12 *E1a*-mediated downregulation have been identified. While the identity of the transcription factor that is the predominant target for the *E1a* gene is disputed, the expression of MHC class I molecules in Ad12-infected cells renders them partially resistant to lysis by CTLs[42,43].

Glossary

Cytotoxic T lymphocytes (CTLs) – A sub-population of small lymphocytes, most of which are $CD8^+$. CTLs recognize antigenic peptides in the context of major histocompatibility (MHC) class I molecules and play an important role in elimination of virus-infected cells. In addition, CTLs are involved in rejection of foreign tissue grafts.

Major histocompatibility complex (MHC) class I molecules – Proteins that present antigenic peptides to $CD8^+$ CTLs. They consist of a transmembrane glycoprotein heavy chain of about 45 kDa, covalently associated with a 12 kDa light chain, β_2 microglobulin (β_2m). Properly folded class I molecules contain a polymorphic cleft that can bind antigenic peptides of 8–10 amino acids. In general, class I molecules are loaded with peptides in the endoplasmic reticulum (ER). Most peptides are derived from cytosolic proteins that have been degraded by the proteasome.

MHC class II molecules – Proteins consisting of two non-covalently associated polypeptide chains, referred to as the α and β chains. Class II molecules present antigenic peptides to $CD4^+$ T helper cells. While peptides presented by MHC class I molecules are usually derived from proteins endogenously synthesized by the antigen-presenting cell, MHC class II-presented peptides originate from endocytosed, soluble proteins, degraded in an intracellular lysosomal compartment.

Transporter associated with antigen presentation (TAP) – A multimeric complex, presumably a heterodimer, composed of the TAP1 and TAP2 subunits (both ~70 kDa). TAP transports peptides from the cytosol to the ER in an ATP-dependent fashion.

The E3 region of the virus encodes a 19 kDa glycoprotein, E3/19k, which binds to the protein products of several human and mouse MHC class I alleles. In cells expressing the gene for E3/19k, there is reduced expression of MHC class I molecules on the cell surface, although the synthesis of MHC class I heavy and light chains remains unaltered[44,45]. Examination of the maturation status of N-linked glycans on MHC class I molecules recovered from such cells revealed that their egress from the ER had been blocked[46] (Fig. 2c). This retention in the ER, caused by E3/19k binding, was sufficient to reduce the availability of MHC molecules on the cell surface and consequently abrogate the lysis of infected cells by CTLs (see Fig. 1)[47]. The sequence responsible for retaining E3/19k in the ER has been identified[48] and the disruption of the retention signal resulted in E3/19k molecules that matured to the cell surface and could not retain MHC class I molecules in the ER[49].

Viruses use diverse mechanisms to evade host immunity

Viruses have evolved unique strategies for immunosubversion. The mechanisms utilized by a particular virus depend on the cell-type that is infected, the kinetics of infection, the life-cycle of the virus and the predominant aspects of host immunity responsible for resistance (NK cells, CTLs or antiviral antibodies). Antigenic variation, as typified by influenza virus, is a classical example of such a viral strategy[50]: the host immune system that efficiently recognizes one strain of virus might be unable to detect a strain in which antigenic regions have been altered by mutations. Interference with host cytokines has been

described in the context of vaccinia virus and HIV infection. The viruses described in this review (HSV-1 and -2, MCMV, HCMV, EBV and adenovirus) use a very different strategy: they block some of the crucial steps in antigen processing and presentation within infected cells. Targets include TAP and the proteasome, and MHC class I molecules are attacked at various stages of their biosynthesis. Several other steps in the MHC class I-restricted antigen presentation pathway are potential candidates for viral disruption, although natural instances of such interference have not been described yet. We suggest the following list of strategies that viruses could employ to evade the immune response.

First, viruses could block the ubiquitin conjugation system, which targets proteins for degradation. Second, they could inhibit the isopeptidases that remove the branched ubiquitin moieties from poly-ubiquitinated proteins before degradation. Third, viral gene products could inactivate the intracellular chaperone molecules that deliver peptides to, and help fold, nascent MHC class I molecules. Fourth, viruses could prevent the maturation of MHC class I molecules and transport to the cell surface by interacting with components of the secretory pathway. Finally, viral proteins could bind directly to MHC class I molecules and travel to the with them cell surface, where they could interfere with the recognition of the MHC–peptide complexes by CTLs.

Viruses that infect cells expressing MHC class II molecules could also inhibit presentation of viral peptides to the immune system by interfering with the MHC class II-restricted pathway. Their potential targets in this 'strategy' could include (1) MHC class II molecules themselves, (2) enzymes in the lysosomal compartment that catalyse production of the peptides presented by class II molecules and (3) intracellular trafficking of class II molecules to the cell surface. Consequently, organisms infected by such viruses might not be able to recruit T-cell help to generate a coordinated immune response to the infection.

Clinical applications

Elucidation of the mechanisms by which viruses elude the immune system will facilitate the development of vaccines and drugs to treat infection with such viruses. For example, the genes of HCMV that encode the glycoproteins that cause retention (US3) and destruction (US2 and US11) of MHC class I molecules can be deleted from the viral genome. Likewise, in HSV-1 and -2, the gene encoding ICP47 can be eliminated. The resulting viruses will no longer interfere with MHC class I expression; consequently, presentation to CTLs of peptides derived from other viral proteins will be restored. These mutant viruses represent interesting vaccine candidates. It is hard to predict what the efficacy of such vaccines will be. Theoretically, virus-infected cells in which 100% of the MHC class I molecules have been destroyed cannot be eliminated by CTLs induced by the vaccine. It is most likely, however, that immunization will shift the balance of the virus–host equilibrium towards a more rapid and efficient control of the infection.

Alternatively, the mechanisms used by the viruses described in this review could also be used to suppress an unwanted immune response, for example in the case of autoimmune diseases or rejection of allogeneic tissue transplants. Interference with MHC class I-restricted antigen presentation in cells that are targets for a harmful attack by CTLs might rescue these cells from elimination. Possible applications of ICP47 and US2/US11 for the purpose of immune suppression are currently being investigated in several laboratories.

One of the major problems encountered in gene therapy with viral vectors is the elimination of successfully transduced cells by the host immune system. Antigens derived from the viral vector are presented at the cell surface in the context of MHC class I molecules and the infected cells are destroyed by CTLs. As a possible solution, the HCMV-encoded US2/US11 molecules or the HSV-derived ICP47 could be included in the viral vector to downregulate MHC class I expression in infected cells, thereby rescuing the cells from elimination by CTLs.

The outstanding questions

- How do the human cytomegalovirus *US11* and *US2* gene products selectively target major histocompatibility complex (MHC) class I molecules for degradation?
- How does the Gly-Ala repeat of the Epstein–Barr virus antigen EBNA-1 prevent the presentation of epitopes from this viral protein?
- What is the role of the murine cytomegalovirus-encoded MHC class I-binding glycoprotein p34?
- Could the viral escape mechanisms be inhibited so that the viruses become more immunogenic? Viruses that have lost their immunosuppressive properties represent interesting vaccine candidates.
- What other cellular pathways are targets for viruses to allow them to escape detection by host immunity?

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