Immune evasion strategies of the herpesviruses

Ian A York

All viruses can deal with the immune response to some extent, and herpesviruses are exceptionally sophisticated in this ability. Recent work has uncovered some of the mechanisms by which herpesviruses subvert the antigen-presentation systems of their host cells.

Address: Department of Lymphocyte Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02215, USA.

Chemistry & Biology May 1996, **3**:331–335

 ISSN 1074-5521

Every animal virus must be able to overcome its host's immune response to some extent. Some viruses 'hit and run', completing replication before the immune response is fully aroused, and others escape the consequences of the responses they generate by altering their surface antigens. But there are much more sophisticated ways for viruses to avoid, evade, or modify the immune system. Identification of these strategies can help us to decipher the origins of viral pathogenesis; it can also help us to understand antiviral immunity, by identifying the aspects of the immune system the virus deems most dangerous, and the elements of the immune system that evolved to overcome or circumvent the evasion systems. Furthermore, since many viral molecules seem to efficiently block components of the immune response while having little effect on other systems, it may be possible to take therapeutic and experimental advantage of millions of years of viral evolution.

To be successful, viruses must deal with both the humoral (B lymphocyte and antibody) and cellular (T lymphocyte) branches of the immune system. The humoral system is primarily useful in attacking extracellular pathogens. Intracellular pathogens such as viruses are vulnerable to the humoral system during the initial entry into the body and while spreading from one cell to another, but are more effectively controlled by the cell-mediated immune system, in particular cytotoxic T lymphocytes (CTL). CTL recognize virus-infected cells through interactions of their T cell receptor and CD8 surface molecule with class I major histocompatibility complexes (MHC class I) on the infected cell's surface.

The MHC class I complex consists of a polymorphic transmembrane heavy chain (HC) associated with a non-polymorphic light chain (β_2-microglobulin) and a peptide derived from proteolysis of cytoplasmic proteins. By transferring these peptides from the cytoplasm to the cell surface, the MHC class I antigen presentation system (reviewed in [1,2]; see Fig. 1) provides CTL with a means of inspecting the intracellular environment.

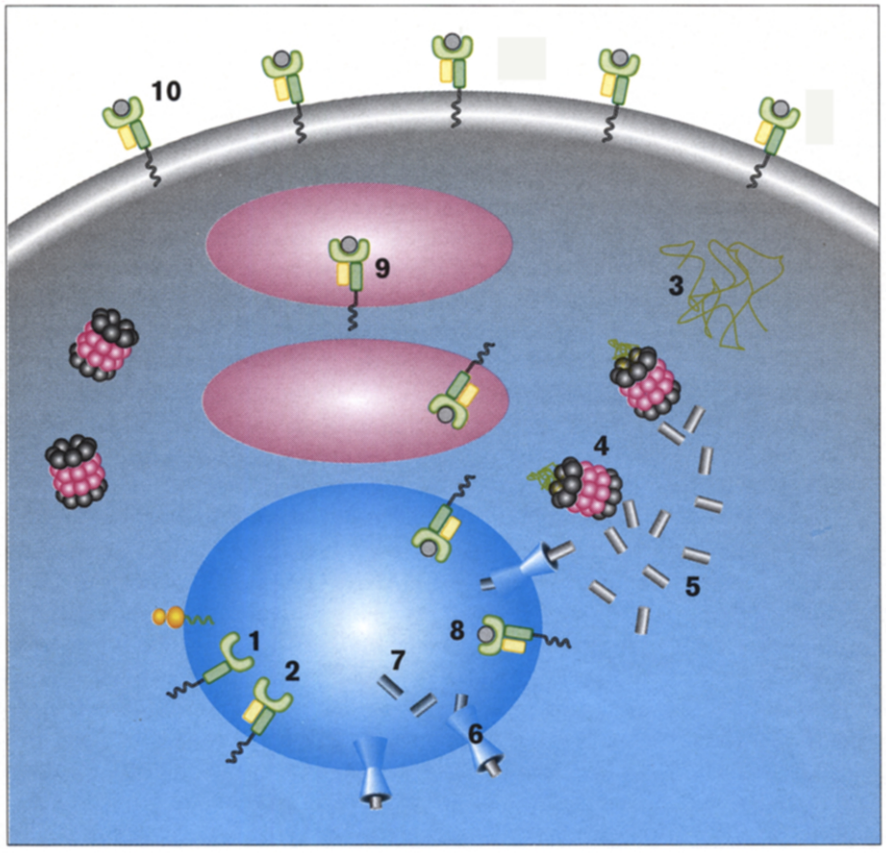
The production and delivery of potentially antigenic peptides has several stages. Proteins are degraded in the cytoplasm, mainly by the proteasome, and the peptides thus produced bind to the cytoplasmic face of TAP (transporter associated with antigen processing), a heterodimer of transporter proteins of the ATP-binding cassette family (reviewed in [3]). TAP prefers to bind peptides of between 8 and 16 amino acids in length, but is quite promiscuous regarding peptide sequence. After binding peptides, TAP transports them across the endoplasmic reticulum (ER) membrane. In the ER lumen, the interaction of peptides with newly synthesized MHC I molecules allows the complex of MHC I and peptide to exit the ER. If the peptide was originally derived from a normal cellular protein, the phenomenon of self-tolerance [4] ensures that CTLs ignore it. If the peptide was derived from a viral protein, however, CTLs can recognize the combination of foreign peptide and MHC I at the cell surface and are triggered to lyse the target cell, thus blocking further viral replication. Not surprisingly, many viruses have evolved mechanisms for evading the CTL recognition and clearance system.

Here I focus on the immune-evasion strategies of three members of the herpesviridae. These are large viruses, with genomes ranging from 100 kDa to 250 kDa or more, which characteristically infect their hosts for life. Many of them can spread from cell to cell without entering the extracellular environment, so that the humoral immune system has little opportunity to clear an infection. There are scores of human and animal herpesviruses (reviewed in [5]); here I will touch on some of the mechanisms used to evade CTL by three ubiquitous human herpesviruses: the closely related herpes simplex viruses (HSV) types 1 and 2, and human cytomegalovirus (HCMV).

Herpes simplex virus

HSV types 1 and 2 replicate in superficial tissues, typically the lip or genitals. After the initial infection, they establish a latent infection in neurons; as few if any viral proteins are expressed during latency and neurons are relatively sheltered from the immune system, latent HSV is unlikely to be eliminated. The viruses can reactivate from latency and reinfect superficial tissues in spite of the immune response, which is at most moderately suppressed. Although the HSVs express a number of gene products involved in evasion of the humoral immune response, these are most likely to be important in the initial infection before the

Figure 1



The class I major histocompatibility complex antigen presentation pathway. The class I MHC is composed of a transmembrane heavy chain (1) bound to a soluble protein, β_2-microglobulin. These proteins are co-translationally translocated across the ER membrane, and bind in the lumen of the ER (2). Cytoplasmic proteins (3) are degraded by the proteasome (4) to produce peptides (5). These peptides bind to the cytoplasmic face of TAP (6), which then transfers the peptides into the lumen of the ER (7). In the ER, peptides bind to newly synthesized class I MHC to form a trimeric complex of heavy chain, β_2-microglobulin and peptide (8). This complex exits the ER, passes through the Golgi complex (9), and reaches the cell surface (10), where it can be surveyed by cytotoxic T lymphocytes.

virus first enters cells, rather than in the ongoing infection (reviewed in [6]).

The cellular immune response to HSV in humans is unusual in that $CD8^+$ CTL specific for HSV are relatively rare and often recognize peptides derived from virion structural proteins brought into the cell in the process of viral invasion, instead of the much more abundant products of *de novo* viral protein synthesis [7]. These observations suggest that the HSVs may actively perturb the MHC class I/CTL system. Indeed, MHC class I in HSV-infected human fibroblasts is misfolded and retained within the ER [8,9], because peptide is not supplied to the nascent MHC class I complex within the ER. This blockade of peptide supply is mediated by a viral protein, ICP47 [9], a small cytoplasmic protein that prevents TAP from transporting peptide into the ER lumen [10,11].

ICP47 is a member of the immediate-early class of viral genes, and is expressed very shortly after viral infection; thus, antigen presentation is effectively blocked as soon as viral genes are expressed. This means that CTL are limited to recognition of the viral proteins present in the cell before ICP47 expression (mainly those present in the infecting virion particle), accounting for the scarcity of human CTL directed against non-structural HSV proteins (Fig. 2).

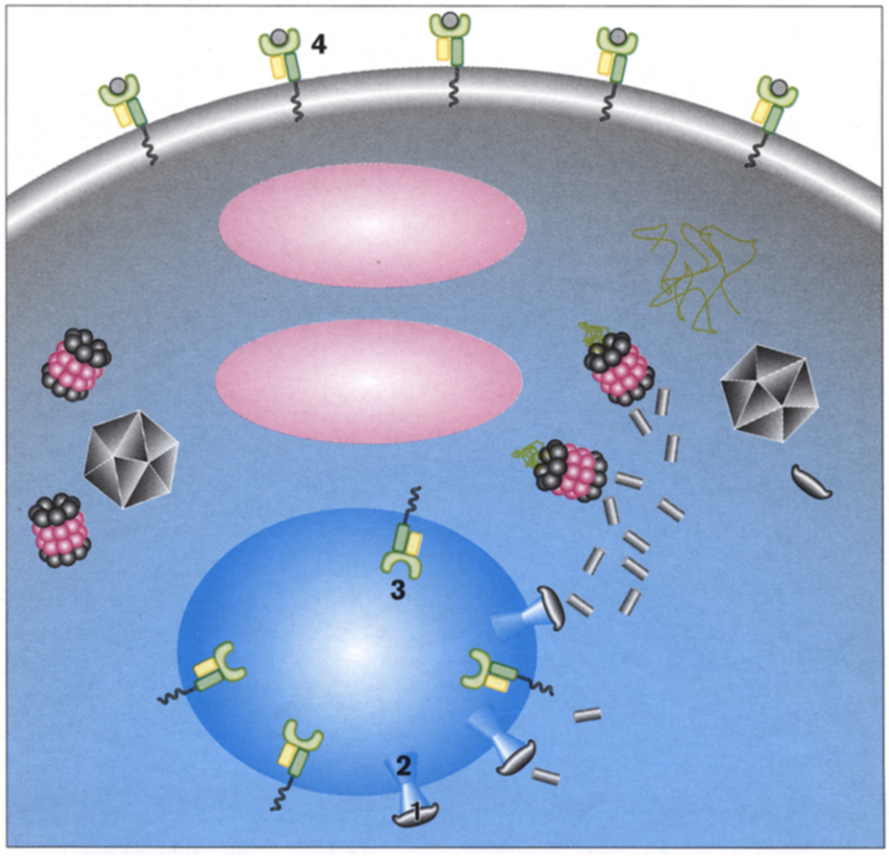
The mechanism of ICP47's action has recently been partially elucidated. ICP47 binds with high affinity to human TAP, inhibiting peptides from binding to the transporter [12,13] and therefore preventing peptide translocation. The inhibition of peptide binding is competitive, suggesting that ICP47 binds to the peptide-binding site of TAP, but ICP47 binding is clearly not identical to peptide binding. ICP47 is 88 amino acids in length (87 amino acids in HSV-2) and peptides longer than about 16 amino acids seem to bind TAP with low affinity [14], yet ICP47 binds TAP with at least 10-fold higher affinity than do peptides and, unlike peptides, is not transported across the ER membrane [12,13]. Presumably ICP47 contacts TAP residues adjacent to the peptide-binding site as well as those of the peptide-binding site itself.

Human cytomegalovirus

HCMV, like HSV, establishes a lifelong infection even in immune-competent people, but rarely causes disease except in those who are immunosuppressed. HCMV is faced with an even more challenging immunological environment than

Figure 2

Effect of HSV on antigen presentation. In cells infected with HSV, the cytoplasmic protein ICP47 (1) binds to the cytoplasmic face of TAP (2), blocking the peptide-binding site and preventing peptides from entering the ER lumen. Because the MHC class I complex (3) cannot exit the ER until it has associated with peptide, it is retained in the ER in HSV-infected cells and no newly formed MHC class I (which could carry viral peptides) reaches the cell surface. Only those MHC class I complexes formed prior to viral infection (4) are on the cell surface.



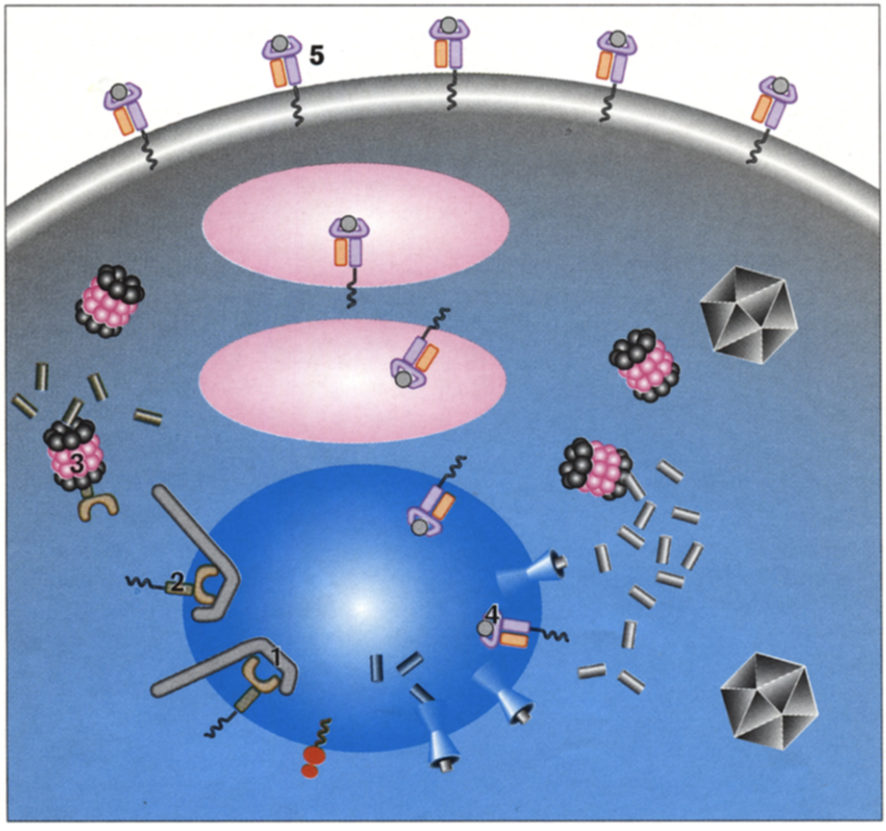
is HSV. It infects a wide range of cell types, including such efficient antigen-presenting cells as macrophages and lymphocytes as well as epithelial cells and fibroblasts, and takes considerably longer than HSV to replicate, so that infected cells are vulnerable to lysis by CTL for a longer time.

A partial explanation for HCMV's immunity to the immune system lies in its ability to inhibit CTL recognition. Fibroblasts infected with HCMV have low cell-surface MHC class I [15,16], due to rapid degradation of the MHC class I heavy chain [17]. HC is normally quite stable, with a half-life measured in hours, but in HCMV-infected cells its half-life can be as short as one minute. Cells infected with a mutant HCMV lacking nine genes failed to induce HC degradation, suggesting that at least one of those genes is involved in the process, and a cell line transfected with one of the genes, US11, showed rapid degradation of heavy chain [18].

The degradation of HC induced by US11 can be blocked by inhibitors of the proteasome, suggesting that degradation might occur in the cytoplasm. Subcellular fractionation in the presence of proteasome inhibitors confirmed that HC was present in the cytoplasm and not the ER in US11-expressing cells. But it appeared to have been N-glycosylated and then deglycosylated, suggesting that it had temporarily reached the ER lumen before entering the cytoplasm [19]. It seems that, in the presence of US11, the HC, despite its transmembrane anchor region, falls or is pushed out of the ER membrane to become a soluble cytoplasmic protein (Fig. 3). Not surprisingly, in this alien environment, where the HC is presumably misfolded, it is rapidly degraded.

The mechanism by which US11 causes HC to enter the cytoplasm is not yet known. Proteins destined for the ER are co-translationally translocated across the ER membrane in a proteinaceous channel; transmembrane proteins are believed to move laterally out of this channel to insert their transmembrane anchor into the ER membrane (reviewed in [20]). HC seems to be displaced to the cytoplasm as fast as it is synthesized [19], suggesting that it may never have the chance to insert itself into the membrane. US11 may be forcing HC into a non-physiological route, or, perhaps more likely, it may be taking advantage of a normal pathway; in either case, this represents a previously unknown route from the ER to the cytoplasm.

Figure 3



Effect of HCMV on antigen presentation. In cells infected with HCMV, the viral protein US11 (1) induces the MHC class I heavy chain to be released from the ER membrane and enter the cytoplasm (2), where it is rapidly degraded by the proteasome (3). MHC class I at the cell surface is lost due to normal turnover. As NK cells normally lyse cells with low cell-surface MHC class I, this would eventually cause these cells to be susceptible to NK lysis. A second viral protein, UL18 (4) has homology to the MHC class I heavy chain, binds β_2-microglobulin and peptide, and reaches the cell surface (5); this protein may act as camouflage, inhibiting NK cells from lysing the infected cell, yet failing to stimulate CTL.

Multiple lines of defense

Surprisingly, HCMV mutants lacking US11 still prevent the surface expression of MHC class I [18]. Another gene product, US2, is also capable of causing rapid degradation of the heavy chain, perhaps by a different mechanism than that used by US11 (E. Wiertz, T. Jones and H. Ploegh, unpublished data). Nor is this the limit of HCMV's anti-CTL arsenal; US3 binds to MHC class I and retains it in the ER, and at least one other gene independently prevents MHC class I surface expression (K. Ahn, A. Angulo, P. Ghazal, P.A. Peterson, Y. Yang and K. Früh, unpublished data). Why does HCMV need so many proteins to inhibit MHC class I antigen presentation, when US11 seems to be effective alone? Similarly, several observations suggest that ICP47 is the only HSV protein affecting MHC class I antigen presentation [9–11]; if HCMV requires four or more proteins to adequately defend itself against CTL, why does HSV only need one?

Part of the reason may be that the different proteins are active at different phases of the viral life cycle. US11 expression begins within a few hours of infection and decreases after two or three days [21], well before HCMV replication is complete. Other gene products may be effective later than US11, while US3, an immediate-early gene, is expressed earlier than US11. ICP47, in contrast, is probably present for almost all of the two-day HSV replication cycle. Another possible explanation is that HCMV has to contend with antigen presentation in more cell types than does HSV: although HSV does infect several cell types, the main site of HSV latency is neurons, which normally express little or no MHC class I. The HCMV strategy of infiltration into the opposing corps, infecting so-called 'professional' antigen-presenting cells like macrophages, may require a more profound block than HSV's approach of repeated raids from ambush.

The long infectious cycle of HCMV raises a problem for the virus that HSV may not have to deal with. Natural killer (NK) cells are lytic lymphocytes which, instead of recognizing peptide in the presence of MHC class I, preferentially lyse cells with low MHC class I expression; their lytic ability is inhibited by MHC class I molecules (reviewed in [22,23]). As cell-surface MHC class I molecules can have a half-life of 12 h or more, the HSV replication cycle can be completed before the level of MHC class I molecules on the cell surface is significantly reduced. HCMV-infected cells, on the other hand, will have low cell-surface MHC class I levels well before new infectious HCMV particles are formed. Intriguingly, HCMV expresses a protein, UL18, which not only shows sequence

similarity to the MHC class I heavy chain, but also binds β_2-microglobulin and peptide [24]. Fahnestock and co-workers suggest that this protein may camouflage infected cells, inhibiting NK cells without stimulating $CD8^+$ CTL.

Significance and implications

Although HSV and HCMV normally block CTL recognition of infected cells, severe herpesvirus disease is rare in people with normal immune systems. Does this mean that CTL are not important in controlling these viral infections? Not necessarily — although human $CD8^+$ CTL specific for HCMV are mainly directed against virion structural proteins, they are readily detectable and are associated with protection against infection [25]. HSV-specific $CD8^+$ CTL are relatively rare, though still detectable, and their importance is less clear. Other components of the immune system, such as NK cells or $CD4^+$ CTL, may be particularly important in controlling herpesvirus infections, but the species specificity of the viruses and their immune evasion proteins (neither ICP47 [8–11] nor US11 [E. Wiertz, T. Jones and H. Ploegh, unpublished data] are fully effective in mouse cells) make *in vivo* experiments difficult.

This review has only dealt with three of the eight human herpesviruses, and a few of the tactics they use to evade one arm of the immune system. It seems possible that virtually every component of the immune system will be affected by some viral gene product. These gene products have obvious potential for dissecting immune pathways, and may also be useful in inhibiting unwanted immune responses. For example, the survival of transplants or gene therapy vectors might be extended by blocking CTL recognition with US11 and/or ICP47, or by modifying immune responses with viral cytokine homologs [26].

Acknowledgements

I thank Drs Peter Schiepers and Chris Counter for critical comments, and Dr K. Früh and E. Wiertz for sharing work before publication. Support from the National Cancer Institute of Canada is gratefully acknowledged.

References

1. Heemels, M.T. & Ploegh, H. (1995). Generation, translocation, and presentation of MHC class I-restricted peptides. *Annu. Rev. Biochem.* **65**, 463–491.
2. York, I.A. & Rock, K.L. (1996). Antigen processing and presention by the class I major histocompatibility complex. *Annu. Rev. Immunol.* **14**, 369–396.
3. Howard, J.C. (1995). Supply and transport of peptides presented by class I MHC molecules. *Curr. Opin. Immunol.* **7**, 69–76.
4. Nossal, G.J. (1994). Negative selection of lymphocytes. *Cell* **76**, 229–239.
5. Roizman, B. (1990). Herpesviridae: a brief introduction. In *Virology* (Fields, B.N. & Knipe, D.M., eds), pp. 1787–1793, Raven Press Ltd., New York.
6. York, I.A. & Johnson, D.C. (1995). Inhibition of humoral and cellular immune recognition by herpes simplex viruses. In *Viroceptors, Virokines and Related Immune Modulators Encoded by DNA Viruses.* (McFadden, G., ed.), pp. 89–110, R.G. Landes Co., Austin, TX.
7. Tigges, M.A., Koelle, D., Hartog, K., Sekulovich, R.E., Corey, L. & Burke, R.L. (1992). Human $CD8^+$ herpes simplex virus-specific cytotoxic T-lymphocyte clones recognize diverse virion protein antigens. *J. Virol.* **66**, 1622–1634.
8. Hill, A.B., Barnett, B.C., McMichael, A.J. & McGeoch, D.J. (1994). HLA class I molecules are not transported to the cell surface in cells infected with herpes simplex virus types 1 and 2. *J. Immunol.* **152**, 2736–2741.
9. York, I.A., Roop, C., Andrews, D.W., Riddell, S.R., Graham, F.L. & Johnson, D.C. (1994). A cytosolic herpes simplex virus protein inhibits antigen presentation to $CD8^+$ T lymphocytes. *Cell* **77**, 525–535.
10. Hill, A., *et al.*, & Johnson, D. (1995). Herpes simplex virus turns off the TAP to evade host immunity. *Nature* **375**, 411–415.
11. Früh, K., *et al.*, & Yang, Y. (1995). A viral inhibitor of peptide transporters for antigen presentation. *Nature* **375**, 415–418.
12. Tomazin, R., *et al.*, & Johnson, D. (1996). Stable binding of the herpes virus ICP47 protein to the peptide binding domain of TAP. *EMBO J.*, in press.
13. Ahn, K., *et al.*, & Früh, K. (1996). Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus protein ICP47. *EMBO J.*, in press.
14. Heemels, M.-T. & Ploegh, H.L. (1994). Substrate specificity of allelic variants of the TAP peptide transporter. *Immunity* **1**, 775–784.
15. Barnes, P.G. & Grundy, J.E. (1992). Down-regulation of the class I HLA heterodimer and β_2-microglobulin on the surface of cells infected with cytomegalovirus. *J. Gen. Virol.* **73**, 2395–2403.
16. Yamashita, Y., Shimokata, K., Mizuno, S., Yamaguchi, H. & Nishiyama, Y. (1993). Down-regulation of the surface expression of class I MHC antigens by human cytomegalovirus. *Virology* **193**, 727–736.
17. Beersma, M.F., Bijlmakers, M.J.E. & Ploegh, H.L. (1993). Human cytomegalovirus down-regulates HLA class I expression by reducing the stability of class I H chains. *J. Immunol.* **151**, 4455–4464.
18. Jones, T.R., Hanson, L.K., Sun, L., Slater, J.S., Stenberg, R.M. & Campbell, A.E. (1995). Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *J. Virol.* **69**, 4830–4841.
19. Wiertz, E.J.H.J., Jones, T.R., Sun, L., Bogyo, M., Geuze, H.J. & Ploegh, H.L. (1996). The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* **84**, 769–779.
20. Ng, D.T. & Walter, P. (1994). Protein translocation across the endoplasmic reticulum. *Curr. Opin. Cell Biol.* **6**, 510–516.
21. Jones, T.R. & Muzithras, V.P. (1991). Fine mapping of transcripts expressed from the US6 gene family of human cytomegalovirus strain AD169. *J. Virol.* **65**, 2024–2036.
22. Lanier, L.L. & Phillips, J.H. (1995). NK cell recognition of major histocompatibility complex class I molecules. *Semin. Immunol.* **7**, 75–82.
23. Chambers, W.H. & Brissette-Storkus, C.S. (1995). Hanging in the balance: natural killer cell recognition of target cells. *Chemistry & Biology* **2**, 429–435.
24. Fahnestock, M.L., Johnson, J.L., Feldman, R.M., Neveu, J.M., Lane, W.S. & Bjorkman, P.J. (1995). The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides. *Immunity* **3**, 583–590.
25. Li, C.R., Greenberg, P.D., Gilbert, M.J., Goodrich, J.M. & Riddell, S.R. (1994). Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. *Blood* **83**, 1971–1979.
26. Qin, L., *et al.*, & Bromberg, J.S. (1996). Retrovirus-mediated transfer of viral IL-10 gene prolongs murine cardiac allograft survival. *J. Immunol.* **156**, 2316–2323.