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Mechanisms of interference with the MHC class I-restricted pathway of antigen presentation by herpesviruses

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Summary Herpesviruses are an ancient, ubiquitous family of DNA viruses, most of which share a lifestyle of latently or persistently infecting a young host, and spreading to infect a new host a generation later. Most herpesviruses interfere with antigen presentation via the MHC class I-restricted pathway of antigen presentation, suggesting that impairment of the cytotoxic T lymphocyte response is necessary for the maintenance of this lifestyle. The diverse molecular mechanisms that have so far been discovered employed by Epstein–Barr virus, herpes simplex viruses, and human and murine cytomegaloviruses are described in this review.

Key words: antigen presentation, cytomegalovirus, cytotoxic T lymphocyte, Epstein–Barr virus, herpes simplexvirus, herpesvirus, latency, MHC class I, natural killer cell, transporter associated with antigen processing.

The herpesviruses: what defines them as a family

Herpesviruses are a phylogenetically ancient family of large DNA viruses. The family recognized as herpesviridae includes the mammalian and avian viruses, which share a core set of recognizably homologous genes as well as a conserved genome organization.[1] Even more ancient phylogenetically is the conserved herpesvirus virion structure. Herpesviruses were divided initially on the basis of cell tropism, and later on sequence comparison into three subfamilies, alpha, beta and gamma.[2] Alpha herpesviruses are neurotropic; in humans these are herpes simplex viruses (HSV) types 1 and 2, and Varicella-zoster virus (VZV). The betaherpesviruses infect a multitude of cell types *in vivo*; these are the cytomegaloviruses (CMV). More recently two newly discovered herpesviruses, human herpesvirus 6 (HHV-6) and HHV-7, have been classified as betaherpesviruses. The gammaherpesvirus of humans is the Epstein–Barr virus (EBV).

Herpesviruses also have much in common in terms of their lifestyle, that is, the mechanism by which they survive and propagate within a species. Most herpesviruses infect their hosts in the first decade of life; the initial infection usually causes mild or no symptoms. The virus establishes latent or persistent infection of the host and, at least for the well characterized viruses (HSV-1 and 2, CMV, VZV and EBV), the cellular site of latency or persistence is different to the primary cell in which efficient productive replication occurs. Throughout the active healthy life of the host new virus production occurs, not to an extent that it is noticed by the host but enough to produce an inoculum capable of infecting a new individual. Most infection of new hosts probably occurs from the adults in a child's immediate environment. Thus most new herpesvirus infection is from a host who was him/herself infected some decades earlier. This lifestyle has been highly successful for the herpesviruses: most of the human herpesviruses affect most individuals of our species and, given the phylogenetic history of the herpesviruses, the spread from generation to generation would appear to have occurred successfully for tens to hundreds of millions of years.

This extraordinary coexistence involves a close accommodation with the host immune system. In order to achieve the generation to generation spread, herpesviruses must avoid destruction by the immune system, and indeed must be able to replicate sufficiently to produce an infective inoculum in the face of a fully primed immune system. Herpesviruses have evolved a multitude of mechanisms to interfere with various aspects of the host immune response. Interference with the MHC class I-restricted pathway of antigen presentation is particularly common, and is the subject of this review.

However, the successful herpesvirus lifestyle is also utterly dependent on the normal functioning of the immune system. Primary herpesvirus infection in an immunodeficient infant is often fatal. When immunodeficiency occurs later in life, the inability to maintain control of latent/persistent virus makes herpesvirus infections, particularly CMV, one of the most common causes of morbidity and mortality in the immuncompromized host. Thus the herpesvirus survival strategy, which depends on the infected host surviving to reproduce and pass the infection on to a new generation, requires a healthy immune system. The dual nature of this interaction with the immune system needs to be borne in mind when attempting to

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understand the complexity of mechanisms with which herpesviruses interfere with immune function.

The class I antigen presentation pathway

The MHC class I-restricted antigen presentation pathway is the immune system's way of monitoring the internal contents of intact cells. MHC class I molecules bind peptides derived mostly from cytosolic and nuclear proteins and transport them to the surface of cells where they can be detected by the T cell receptor of $CD8^+$ T cells.[3] $CD8^+$ T cells upon activation are capable of killing cells bearing the MHC class I-peptide complex they recognize, and hence are commonly known as cytotoxic T lymphocytes (CTL). However, they also secrete a number of cytokines and chemokines, and this function is also critical for their antiviral effectiveness.

The processing of antigen for presentation by MHC class I molecules begins in the cytosol, where protein is degraded by the proteasome into short peptides. Peptides are then transported into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). The details of targeting of proteins for degradation and of the linkage between the proteasome and TAP are not known. TAP transports preferentially peptides that are of the right size and upwards for binding to class I MHC molecules, and which bear an appropriate C terminus for binding to the class I molecules of that species. Peptides delivered by TAP can bind to waiting nascent class I molecules.[4]

Class I molecules consist of a polymorphic heavy chain, a light chain (beta-2 microglobulin) and the bound peptide. Class I heavy chain is a type I membrane glycoprotein, co-translationally translocated into the ER where it acquires one or two N-linked glycans. After initial trimming of glycan residues, class I associates with the chaperon calnexin, where it folds with beta-2 microglobulin (beta-2-m). On association with beta-2-m the complex achieves its final conformation (as detected by antibodies) but the association is unstable in the absence of peptide binding. In human (but not mouse) class I may pass to another chaperon at this point. The entire complex then associates with TAP. Again, several lines of evidence suggest that at least one other molecule is involved in the process at this point. Upon binding of a suitable peptide, heavy chain/beta-2-m and calnexin dissociate from TAP; the class I molecule then dissociates from calnexin and is able to leave the ER. Without peptide, most class I molecules remain in the ER. After leaving the ER, class I travels through the Golgi, where its glycans are modified, and arrives at the cell surface, where it can interact with the TCR and CD8 molecules of CTL. (The assembly of class I molecules has been recently reviewed in ref. 5.)

The class I-restricted antigen presentation pathway thus serves to provide the immune system with a continuous survey of the protein content of the cytosol and nucleus. The immune system apparently has a backup system if the class I reporting fails. Some receptors of NK cells deliver an inhibitory signal upon binding to class I molecules. The end result is that these NK cells will lyse cells with inadequate levels of class I.[6] This NK 'backup' presumably constrains the effectiveness of viral interference manoeuvres which would abolish class I transport.

Molecular mechanisms of interference with class I in herpesvirus infections

Epstein–Barr virus

EBV is a herpesvirus which enters a truly latent state, that is, one in which no virus replication occurs. Several patterns of gene expression exist for EBV latency, but in all of them the gene EBNA-1 is expressed, and in one of them EBNA-1 is the only gene expressed. EBNA-1 is essential for maintenance of the viral episome during latency. It is also the only one of the latency associated proteins against which no cytotoxic T cells have ever been detected. EBNA-1 contains a long stretch of repeated glycine and alanine residues. When this gly-ala repeat stretch is removed, EBNA-1 specific responses can be elicited in mice. Furthermore, if the gly-ala repeat stretch is inserted into another, normally antigenic protein, EBNA-4, the recombinant EBNA-4 is no longer able to be recognized by CTL. Thus EBNA-1 is able to selectively prevent its own processing for presentation by CTL while leaving the presentation of other proteins intact. Cells latently infected with EBV expressing EBNA-1 are thus invisible to the immune system.[7]

Herpes simplex virus

HSV types 1 and 2 encode a small cytosolic protein, ICP47, which binds to TAP and completely blocks its ability to transport peptides.[8,9] ICP47 is encoded by an immediate early gene, US12, and the blockade is effective within 2 h of infection of human fibroblasts with HSV.[8,10] Blockade of TAP by ICP47 abolishes antigen recognition by CTL. ICP47 is an 87 amino acid cytosolic protein. It blocks TAP by binding to it with high affinity and a slow dissociation rate. In order to co-precipitate TAP with ICP47, both subunits of the TAP heterodimer must be present. Both subunits of TAP are also required to transport peptides, and competition studies between ICP47 and peptide for binding to TAP indicate that TAP and peptides compete for a single binding site.[11] Comparison of sequences of ICP47 from HSV types 1 and 2 reveals a high degree of homology in the N-terminal two-thirds of the molecule, and almost no homology in the C terminal third (B Manning & D McGeoch, unpubl. data, 1993). A synthetic polypeptide corresponding to the N-terminal 53 amino acids blocks TAP function as effectively as the full length molecule in a peptide transport assay using permeabilized cells (B Galocha, A Hill & H Ploegh, unpubl. data, 1996).

In mouse models of HSV infection, HSV-specific CTL have been easy to detect, a situation that contrasts with the difficulty in detecting HSV-specific CTL in infected humans. ICP47 blocks antigen presentation to CTL in human but not mouse fibroblasts. Direct studies of TAP inhibition by ICP47 show that human TAP is blocked by ICP47 with a half maximal efficiency of around 0.5 micro-

molar. In contrast, mouse TAP is not blocked by concentrations of 10 micromolar.[11] Studies of other species show that old world primate TAP are blocked in a similar manner to humans, but with increasing phylogenetic distance the concentration of ICP47 causes 50% inhibition increases (P Jugovic & A Hill, unpubl. data, 1996).

HSV establishes latent infection in neurones, where no viral proteins are expressed. In its latent state the virus is hidden from the immune system both by virtue of the privileged status of the neuron with regard to the immune system, and because of the paucity of antigen it produces. In contrast to EBNA-1 of EBV described above, ICP47 acts to prevent CTL recognition not during latent infection, but during the productive replication cycle of the virus, which occurs *in vivo* in epithelial cells. We have suggested that this may be necessary for the virus to replicate in sufficient numbers to infect a new host in the face of a fully primed immune system.

Human cytomegalovirus

In human fibroblasts infected with human CMV (HCMV), class I heavy chain is synthesized at a normal rate but is degraded within minutes of synthesis.[12–14] Two HCMV gene products are independently capable of mediating this effect; they are encoded by the genes US2 and US11.[15] In US11 transfectants, class I is translocated into the ER; it achieves its full length, is glycosylated, and a small proportion becomes associated with beta-2-m. It is then dislocated into the cytosol by an unknown mechanism, where it is deglycosylated by an N-glycanase and degraded by the proteasome.[16] US2, an apparently unrelated gene, mediates the same effect.

US2 and US11 are early genes, that is, they depend on immediate early encoded transcription factors for their own transcription. Within the same genomic segment, Klaus Fruh *et al.* found two other genes encoding proteins which bind to class I MHC.[17] These are US3 and US6. US3 is an immediate early gene and, in US3 transfectants, class I MHC is retained in the ER. Thus in cells undergoing full lytic cycle infection with HCMV, class I is first retained by US3, then degraded by US2 and US11.[17] The US6 gene product also binds to class I; its function is not known.

Finally, in a different region of its genome, HCMV encodes a gene (UL18) which is homologous to class I heavy chain.[18] It has been difficult to demonstrate a function for this gene, or even to detect significant protein product in HCMV-infected fibroblasts. However, the UL18 product associates with beta-2-m[19] and binds peptides.[20] Recent experiments have demonstrated that in transfectants UL18 exerts a powerful inhibitory signal to human NK cell clones which recognise lack of self class I (HLA-C) (H Reyburn & J Strominger, unpubl. obs., 1996). It is thus likely that UL18 acts as a decoy to prevent NK-mediated destruction of HCMV-infected cells which have lost class I expression due to the actions of US2, 3 and 11. The NK decoy function of UL18 remains to be demonstrated in the context of real infection with HCMV.

The existence of this array of class I modulating genes in HCMV provokes the question: why does the virus need such a complex arsenal? Questions on the role of each of these genes in the context of a real virus infection, in primary infection, in latent/persistent infection and in the cell types involved therein, and in reactivated productive infection, are difficult to address in HCMV infection. To understand the role of class I manipulation in the host-virus relationship I have turned in my own work to the murine cytomegalovirus model.

Murine cytomegalovirus

CMV are highly species-specific and are thought to have co-evolved with their hosts. Thus in evolutionary time murine CMV (MCMV) is as distant from HCMV as is mouse from human. Because of the much higher replication rate of viruses compared to their hosts in this time the genetic distance between the viruses is vastly greater than between their hosts; conserved features can be assumed to result from powerful selective pressure for their conservation. It is therefore interesting to note that the natural history of MCMV in terms of its host relationship, types of cells infected and pathogenesis is similar to that of HCMV.

MCMV also interferes with antigen presentation through the class I pathway,[21–25] although at present there would seem to be little similarity in molecular mechanisms with HCMV. An early gene of MCMV causes fully peptide loaded class I molecules to be retained in the ER. This results in lack of presentation of epitopes within the immediate early protein pp89, despite the continued synthesis of pp89. The mechanism by which MCMV retains class I in the ER is not known.

We have identified a second gene product in MCMV, encoded by the gene *m04*, which binds to class I MHC (Kleijnen MF *et al.*, unpubl. data, 1996). *M04* encodes a 34 kDa glycoprotein, most of which remains localized to the ER. Gp34 binds to properly folded (beta-2-m associated) class I in the ER, and travels with it to the cell surface, where they remain tightly associated. The retention of class I in the ER precedes the action of gp34; later in infection gp34 is synthesized in large quantities and less class I is retained in the ER. It remains to be tested whether gp34 actually functions to rescue retained class I from the ER. To date we have been unable to determine a functional effect of gp34 in immune function. Nevertheless it appears that, like HCMV, MCMV's intervention in the class I pathway is multifaceted and finely regulated. The MCMV model offers the opportunity to determine the effect of these manoeuvres on the host virus relationship.

Finally, as has been reported, like HCMV, MCMV also encodes a class I homologue.[26,27] Again, the experimental manipulability of the MCMV model should enable the biological function of this molecule to be readily determined

Conclusion

Investigation of the interaction of herpesviruses with the class I antigen presentation pathway has revealed an un-

expected diversity of mechanisms, and there are undoubtedly many more to be discovered. It seems probable that these mechanisms will throw light on those parts of the class I antigen presentation pathway which are not yet fully described. HCMV US2 and US11 will almost certainly reveal a normal cellular mechanism by which unwanted proteins in the ER are degraded. In addition, it is to be hoped that elucidating these mechanisms will help us to understand how herpesviruses maintain their lifestyle of persistence within a host and spread to new hosts across the generations.

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