Review

Modulation of HLA Expression in Human Cytomegalovirus Immune Evasion

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Human cytomegalovirus (hCMV) has evolved multiple mechanisms to escape the host immune recognition and innate or adaptive immune responses. Among them, hCMV has developed strategies to modulate the expression and/or function of human leukocyte antigens (HLAs), including by encoding series of infection stage-dependent hCMV proteins to detain and destroy the expression of HLA molecules on the surface of infected cells. This disturbs the antigen presentation and processing, by encoding MHC class I homologues or selective up-regulation of particular HLA class I molecules binding to NK cell inhibitory receptors, and by encoding specific ligand antagonists to interfere with NK cell activating receptors. Here we discussed the molecular mechanisms utilized by the hCMV to alter the formation, transportation and expression of HLA antigens on the infected cell surface. The knowledge about hCMV modulating HLA expression could benefit us to further understand the pathogenesis of viral diseases and may eventually develop novel effective immunotherapies to counteract viral infections and viral associated diseases. *Cellular & Molecular Immunology*. 2007;4(2):91-98.

Key Words: HLA, human cytomegalovirus, NK cell, T lymphocyte

Introduction

hCMV is a world wide human pathogen with the ability to persist as a lifelong latent infection, resulting in subclinical or clinical infections. In the developing fetus and immuno-compromised patients, however, it may contribute to significant morbidity and mortality rates (1). Though the human immune system has the innate and adaptive antiviral responses which include IFN-α/β repressing the replication of the virus, cytolysis mediated by NK cells, or both specific cellular and humoral immunity, especially the $CD8^+$ T cell mediated cytotoxicity. However, hCMV has evolved multiple strategies (Figures 1 and 2) to efficiently escape from host immune surveillance and defense system resulting in lifelong latent infections (2, 3).

Cell surface expression of antigen presenting HLA molecules, which plays a crucial role in the detection and elimination of viral infected cells, is modulated by the concerted action of a set of hCMV encoded unique short (US) proteins (US2, US3, US6, US10, US11) which expressed differentially on different stages of the viral infection (4). With these proteins which were summarized in Table 1, hCMV could interfere with the HLA restricted pathway of antigen presentation retention by degradation and internalization of HLA class I or class II molecules, thus confers from hCMV to escape the host T lymphocyte-mediated antiviral immune responses (5).

When expression of HLA molecules on cell surface is devoided or down-regulated, cells may become susceptible to NK cell lysis. Several strategies by blocking expression of ligands that activate NK cells or preserve expression of ligands that inhibit NK cell triggering, have been developed by which hCMV can sidetrack NK cell attack. Encoded within the unique long (UL) region of the hCMV genome, hCMV proteins such as UL16, UL18 and UL40 could directly contribute inhibitory signals to NK cells or allow locus-restricted surface expression of HLA molecules such as HLA-E to block the activation of NK cells (6, 7).

Together, hCMV has developed multiple efficient strategies including down-regulation of particular surface HLA expression to prevent viral antigen presentation to CTLs, induction of HLA-E or HLA surrogate expression to escape NK cell attack.

Down-regulation of the expression of HLA class I molecules

Impairing the transportation and maturation of HLA class I

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Received March 14, 2007. Accepted April 11, 2007.

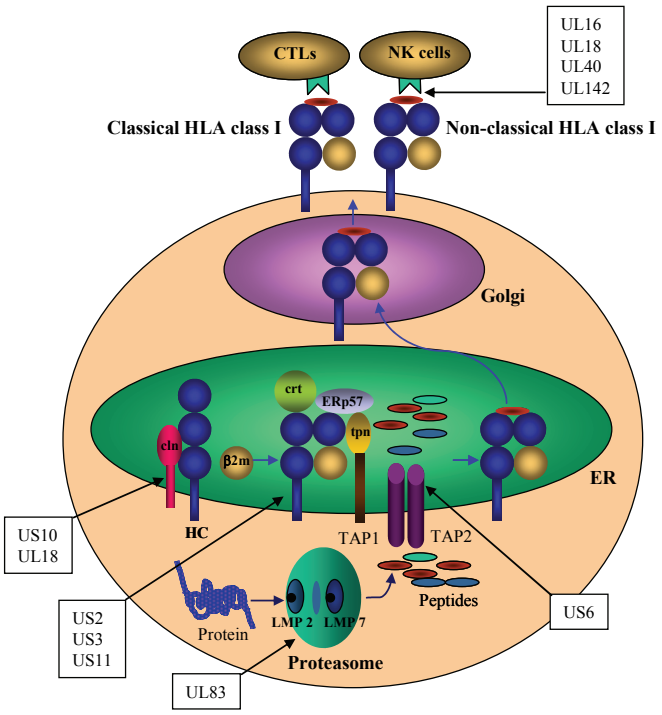

Figure 1. hCMV interferes with class I MHC antigen presentation and inhibits cytotoxicity by CD8$^+$ T cells and NK cells. HC, heavy chain; TAP, transporter associated with antigen presentation; ER, endoplasmic reticulum; CRT, calreticulin; Tpn, tapasin; β_2m, β_2 microglobulin.

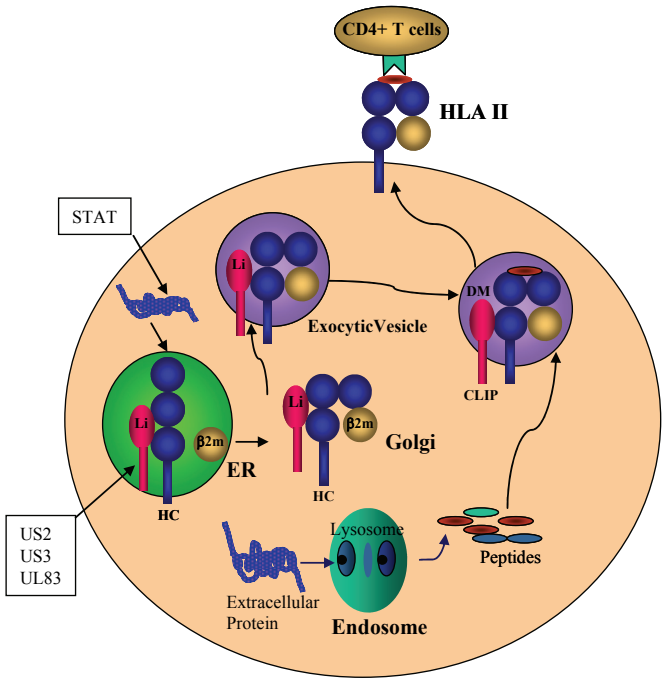

Figure 2. hCMV interferes with class II MHC antigen presentation and inhibits CD4$^+$ T cells. HC, heavy chain; STAT, signal transducers and activators of transcription.

heavy chains

HLA complex consists of the HLA encoded glycoprotein heavy chain, and a small soluble polypeptide, β_2-microglobulin (β_2m). HLA I molecule expression on cell surface depends on the coexistence of HLA heavy chain and β_2m. Nascent HLA class I heavy chain translocated to the endoplasmic reticulum (ER) and stabilized by chaperons such as tapasin, assembled and bound β_2m to form the integrated HLA I molecules. Newly synthesized HLA class I heavy chain associated with β_2m in the ER and then the heterodimer associated with TAP and other antigen processing machinery components (APM) to form a "peptide-loading" complex. Processed antigen peptides bound to TAP and translocated to the ER lumen. Peptide binding confered HLA heavy chain/β_2m heterodimer stability, and finally, the trimeric complex transported through the *cis*, medial and *trans* Golgi compartments to the cell surface (8).

hCMV encodes various proteins that interfere with cross talk between infected cells and immune effector cells through modulation of surface expression of HLA class I molecules. The success of immune escape through modulation of MHC class I surface expression is likely to be influenced by the efficiency, as well as by the specificity, of this down-modulation by hCMV encoded different US proteins (9).

hCMV can detain properly assembled HLA class I molecules at the site of synthesis by sequentially encoding US2, US3, US6, US10 and US11 glycoproteins through multi-step process which leads to the HLA molecules retain in the ER and can not reach cell surface (4, 9). The similar sequence among US2, US3, and US11 indicates that those proteins may evolve from a common precursor. However, each of them has distinct phase-dependent expression and function during the viral infection (9).

US3 is an hCMV encoded immediate-early (IE) glycoprotein, transcribed abundantly at an immediate-early stage but markedly decreased later after infection. US3 forms a complex with β_2m associated HLA class I heavy chain with high affinity, thus impair maturation and intracellular transportation of HLA heavy chain by binding to the HLA heavy chain/β_2m heterodimer prior to peptide loading in the ER. Consequently, the assembled HLA/peptide complex is retained in the ER, preventing its translocation to the cell surface (10, 11). However, this mechanism could only affect HLA allelic products that are dependent on tapasin for their surface expression, other HLA allelic products which are tapasin independent could express on the surface of US3-expressing cells (12). Although US3 share many features with US10, in this context, it is noteworthy that US10 binds HLA I heavy chain, thereby delaying, but not preventing, the egress of folded and peptide loaded HLA I molecules from the ER (4).

US2 and US11 are the products of hCMV early genes, each of which is sufficient to dislocate the unassembled heavy chain or the heavy chain assembly with β_2m but

Table 1. hCMV proteins interfering with host immune evasion

Proteins	Major functions	Ref
US2	Targets HLA class I to proteasome	14, 18
US3	Retention of folded class I complexes	10, 11
US6	Blocks TAP from ER-lumenal side	20
US10	Delay of the egress from the ER	4
US11	Targets MHC class I heavy chains for degradation	13, 16
UL16	Inhibits NK cell lysis (binding to ULBPs to disrupt activating receptor NKD2D)	39
UL18	Inhibits NK cell lysis (MHC class I homolog binds to inhibitory receptor ILT2)	35
UL40	UL40 leader sequence mimics that of HLA-C provides signal peptide ligand for HLA-E	66
UL83	Block of protein entry into the proteasome to inhibit generation of antigenic peptides	56
UL142	Down-regulates the NKG2D receptor ligand MICA	46
IE/E product	Interferes with MHC class II up-regulation (IFN-γ signal transduction cascade)	73

interact with different sites from the ER to the cytosol, where they are rapidly subjected to proteasomal degradation (13, 14). US2 results in destabilization of the HLA I heavy chain, but HLA-B7, HLA-Cw3 and HLA-E are insensitive to the US2 degradation (15-17). Previous studies indicated that the sensitivity to US2 is largely determined by the junction of the α2/α3 domain around the region comprising residues 176~183 which account for sensitivity differences of HLA I locus to US2 medicated degradation (14). With the crystal structure data of HLA-A2-β_2m-US2 complex and sequence alignment of the HLA I in the US2 binding site, variation in the α2/α3 domain ER-lumenal region of the heavy chain could be an explanation for the allelic specific effects of the US2 (14, 19). US11 encodes a type I membrane glycoprotein that resides in the ER and causes rapid dislocation of newly synthesized HLA I heavy chains from ER to the cytosol, where they are degraded by an N-glycanase and proteasome. Studies demonstrated that α1/α2 domain of the HLA molecules is important for the affinity of the US11 association and cytosolic tail residues are critical to the degradation of the HLA molecules. However, not all HLA class I locus products are equally sensitive to this down-regulation (13, 18). Among HLA I molecules, interestingly, only HLA-G and HLA-E are completely insensitive to US11 mediated down-regulation, the reason could be that the essential residues necessary for US11 to target should bear a minimum of 29 HLA I tail residues going with a favorable ER-lumenal region or with lysine and valine tail residues. This was supported by a recent report that subtle sequence variation among HLA-E locus product markedly influenced sensitivity to US11 mediated degradation, where HLA-E became sensitive to US11 when its cytoplasmic tail C-terminus extended with two additional amino acids lysine and valine (18).

As mentioned above, hCMV proteins US2, US3 and US11 expressed on different stage of infection, form a delicate regulation network to decrease HLA class I cell surface expression. The gene products US2 and US11 redirect nascent HLA class I heavy chains into the cytosol for degradation, whereas US3 retains HLA class I complex in the ER. All of those mechanisms result in a decreased HLA class I cell surface expression, rending the hCMV to escape $CD8^+$ cytotoxic T lymphocyte recognition.

Inhibiting TAP-dependent endogenous peptide translocation

Transporter associated with antigen processing (TAP) consists of two subunits, TAP1 and TAP2, which form a heterodimer, playing a critical role in transportation of peptides. Peptides processed in the cytosol by proteasome are delivered to the lumen of the ER by the TAP complex, which is physically linked to the HLA I molecule through the ER resident protein tapasin, which facilitates peptide loading. When properly assembled and loaded with peptide, class I molecules are then released from ER and finally displayed at the cell surface in a form of "peptide-loading" trimeric complex (20).

hCMV protein US6, contains a double-arginine motif, an ER retention signal in the C-terminal cytoplasmic domain. Rather than blocking peptide binding to TAP complex, US6 inhibits the ATP binding to the subunit TAP1, but not TAP2. As a consequence, US6-mediated impairing TAP function prevents the delivery of cytosolic peptides into the ER. HLA complex class I molecules are thus unable to load TAP-dependent peptides, resulting in the retention of HLA class I molecules in the ER, with a consequent reduction in class I molecules at the cell surface (21-23). In spite of this, surface expression of HLA-E molecules remains unaltered which is in a TAP independent peptide-binding manner (24, 25). The hCMV infected cells, therefore, are unable to present endogenous antigen to cytotoxic T lymphocytes while preserving cell surface HLA-E expression, which entitles the infected cells with ability to resist cytotoxic T lmyphocyte and NK cell lysis.

Inhibition of NK cell-mediated lysis

The function of NK cell is controlled by the expression of NK cell receptors interacting with ligands including HLA I molecules. Three superfamily receptors specific for HLA I molecules have been identified, killer cell immunoglobulin-like receptors (KIRs), immunoglobulin-like transcripts (ILTs) and C-type lectin receptors. KIRs are specific for different classical HLA I molecules (HLA-A, B, C) and non-classical HLA I molecule HLA-G. The C-type lectin complex CD94/NKG2A is specific for non-classical HLA I molecule HLA-E which requires binding of a nonamer peptide derived from positions 3~11 of the signal sequence of classical HLA I molecules and HLA-G (26). ILT receptor family is with a

broad HLA specificity (27-29).

Viruses have evolved multiple strategies to escape CTL responses by interfering at different steps with the HLA class Ia antigen presentation pathway. Decreased expression of HLA class I molecules at the cell surface could make the target cell more susceptible to the NK cell mediated lysis. However, hCMV also developed additional strategies to counter NK cell mediated lysis. UL18 was identified as a HLA I homologue during the analysis of the strain AD169 sequence and Cosman et al. for the first time, addressed that UL18 was specially recognized by ILT2 (30, 31). UL18, a 348-residue type I transmembrane glycoprotein, whose extracellular region shares 25% amino acid sequence identity with the extracellular regions of human class I molecules. As class I HLA molecule, UL18 associates with β_2m. UL18 binds a mixture of endogenous peptides with characteristics similar to those of peptides eluted from class I molecules, that is, "anchor" residues, and a predominance of short peptides derived from cytoplasmic proteins (32-34).

The HLA class I homologue UL18 is able to substitute for HLA I molecule function as it binds to the inhibitory receptor ILT2 with much higher affinity than that for HLA I molecules, thus even low level of UL18 could compete efficiently for binding, consequently, prevent NK cell lysis (35). ILT2 is expressed on cells other than NK cells, including monocytes, indicating that UL18-mediated immune regulation might occur at several points in the innate responses (36). However, studies of UL18 yielded contradictory results. Reyburn et al. have shown that lymphoblast cell line 721.211 transfected with the gene encodeing UL18 can inhibit lysis of NK cell expressing CD94/NKG2A receptor, indicating that the UL18 may prevent NK cell lysis of hCMV infected cells (37). Leong et al. using fibroblasts infected with either wild-type or UL18 knockout CMV virus, and cell lines transfected with UL18 gene, however, showed that expression of UL18 resulted in the enhanced killing of target cells and drew a conclusion that KIRs and CD94/NKG2A inhibitory receptor for HLA class I do not play a role in affecting susceptibility of CMV-infected target cells to NK-mediated cytotoxicity (38). Obviously, the conclusions between Reyburn and Leong remain discrepant. Because the cell line 721.221 expressing low levels of HLA-E and -F may be responsible for the protection against NK cell lysis. On the other hand, ILT2 is expressed predominantly on a subset of NK cells and the leader peptide of UL18 does not conform to the preferred peptide bound by HLA-E. For these reasons, Leong suggested that Reyburn might simply select a variant of 721.221 expressing higher levels of the endogenous HLA-E protein rather than UL18 which prevent NK cell-mediated lysis (38). A reasonable explanation for this controversy rose recently that spontaneous mutations in the hCMV protein UL18 affect its binding to the inhibitory receptor ILT2 which may result in different UL18-mediated effects on ILT2 positive cells during the course of hCMV infection.

Unlike UL18, UL16 escapes NK cell activation by employing disruption of NK cell activating receptor and ligand interaction. NKG2D is an activating receptor expressed on NK cells and subsets of T cells that could bind to the UL16-binding proteins (ULBP1~4) that are built up by an HLA class I-like α1 and α2 domain, but lack an α3 domain. NKG2D also engages the stress inducible human MHC class I-related molecules (MICA and MICB), transmembranous proteins with HLA class I-like α1, α2, and α3 domains. Through the adaptor protein DAP10, ligation of NKG2D results in NK cell cytotoxicity (39-41). hCMV encodes the soluble form of UL16 which selectively binds to ULBP1, ULBP2 and MICB, but not ULBP3, ULBP4 and MICA (42-44). Interestingly, MICA and MICB are very similar by amino acid sequence and tertiary structure. Distinctive binding of UL16 to MICA *vs* MICB indicates that UL16 could discriminate delicate structure basis between the MICA and the MICB. In fact, a recent study reported evidence that UL16 binding to MICB is critically determined by residues in the MICB α2 domain and that MICA escaping from UL16 binding is due to its altered α2 domain (45). Furthermore, co-expression of UL16 strongly reduced MICB, ULBP1 and ULBP2 surface expression by altering the trafficking of the NKG2D ligand (43), consequently, interfered with the recognition of activating receptor NKG2D on NK cells. It is noteworthy that MICA could be down-regulated in a UL16-independent pathway. Thus, it's reasonable to postulate that hCMV might utilize other gene products to down-regulate surface expression of the NKG2D ligands (such as MICA) not affected by UL16. This hypothesis was realized with the evidence that UL142, a novel hCMV-encoded HLA class I-related molecule, inhibits NK cell killing in a clonally dependent manner (46). UL142 is able to down-regulate the cell surface expression of full-length MICA, but not the truncated and population prevalent allele of MICA*008 (47).

Modulation of the non-classical HLA I molecule expression in pregnancy

hCMV infection is a notorious factor for congenital disorders such as spontaneous abortion or pre-term delivery by disturbing expression of HLA class I molecules at the surface of placental extravillous trophoblast (EVT) cells. EVTs do not express HLA-A, HLA-B and HLA II molecules but do express an unusual combination of HLA-E, HLA-F and HLA-G, in addition to low levels of HLA-C. Convincing evidence indicated that these HLA molecules expressed on EVT play a key role in the fetal-maternal maintenance (48).

HLA-G contributes to uterine and placental immune privilege by targeting various immune component cells and driving the targeted cells into immune suppressive modes, indicating that HLA-G plays a crucial role in the maintenance of the genetically semi-allograft fetal-maternal tolerance (49). Contrast to its roles in immunotolerance *via* inhibitory receptors such as ILT2 and ILT4, mounting evidence postulated that HLA-G could serve an activating functionality through the receptor KIR2DL4 which bears both activating and inhibitory motif in its structure. Interaction with KIR2DL4 expressed by decidual NK cells has been shown to

induce IFN-γ and TNF-α secretion, an antiviral cytokine in resting NK cells, and cytotoxicity further arised in IL-2 activated NK cells (50-52). Similar to the classical HLA I molecules, HLA-G are peptide receptors presenting peptides derived from cellular proteins at the cell surface with a specific sequence motif of XI/LPXXXXXL (53). Peptide presentation by HLA-G expressed on the trophoblast might allow T cell-mediated surveillance of viral infection. HLA-G binds intracellular processed nonamer peptides and can serve as a restriction element for hCMV-derived peptides in transgenic mice, and the HLA-G restricted T lymphocytes had been reported with the hCMV derived peptide pp65 (UL83), indicating that HLA-G can select anti-hCMV-restricted CTLs *in vivo* on the development of an antiviral CTL response, although the potency of this cytolytic response is limited (54-56).

Information on regulation of HLA-G by hCMV encoded US proteins has been documented using different cell lines and experimental set-ups to express them independently, unfortunately, remains to be further elucidated. Schust et al. addressed that unlike HLA-A and -B, both HLA-C and HLA-G expressed in a human trophoblast cell line were fully resistant to the rapid degradation associated with US2 and US11 (57). Others pointed out that US2, US3 and US6 proteins, but not US11, affect cell surface expression of HLA-G (19, 58), that US3 impairs maturation of newly synthesized HLA-G class I heavy chains, and that US6 prevents peptide loading by blocking the TAP and thus inhibits the intracellular trafficking of HLA-G (58). Additionally, US2 was found to be able to degrade and down-modulate the membrane-bound HLA-G molecule while the US11 protein has no effect on surface expression of membrane-bound HLA-G (19).

To eliminate limitations caused by particular US proteins for fully understanding the effects of hCMV on HLA-G surface expression modulation, studies in the context of viral infection, which ensures expression of all these US proteins, had been performed. Down-regulation of HLA-G in trophoblast following hCMV infection independent of the US proteins was observed (59). These data differed from results obtained in the choriocarcinoma cell line JEG-3 where US3 and US6 proteins were found to down-modulate HLA-G cell surface expression (58). Current studies indicated that hCMV infection down-modulated HLA-G cell surface expression, but later and to a lesser extent than HLA-A2, which could be the result of the unique short cytoplasmic tail of HLA-G involving in the resistance of HLA-G to viral down-modulation, particularly, for efficient US11-induced degradation (60, 61). Opposite results have been obtained by Terauchi et al. who did not find any down-regulation of HLA I molecules at the surface of EVT (62). However, the authors of this study used the W6/32 mAb to detect expression of HLA-G which is a pan-HLA class I and can detect HLA-E, -F, and -C in addition to HLA-G. Therefore, it could be that the staining of these different HLA I molecules may have masked the specific labeling of HLA-G. Expression of HLA-G on cytotrophoblast cells down-regulation by hCMV could prevent interaction with uNK cell receptor KIR2DL4 which could induce high cytotoxicity and cytokine production when engaging membrane-bound HLA-G, thus impaired uNK cell lysis and prohibited cytokine production, which is necessary during early pregnancy (51).

HLA-E is the specific ligand for receptor CD94/NKG2A/B, which delivers inhibitory signals to NK cells. HLA-E cell surface expression requires binding of a nonamer peptide derived from residues 3~11 of the signal sequence of HLA-I molecules, especially the HLA-G. Therefore, HLA-E expression allows NK cells to monitor expression of HLA-I molecule expression on cells with a consequence to control the function of NK cells (63, 64). After hCMV infection, protein US6 directly binds to the TAP, which inhibits the delivery of leader peptides from the cytosol to ER. Without the leader peptides, at the first sight, HLA-E cell surface expression seems impaired. However, hCMV found another way to escape NK lysis by producing a TAP-independent HLA-E ligand, UL40, which allows HLA-E expression when the TAP-dependent pathway is shut down (65, 66). UL40 has an NH2-terminal 37-amino acid signal sequence containing the peptide homologue to the HLA-E binding peptide. UL40 encoded by hCMV strain AD169 includes an HLA-E ligand, a 9-amino acid residue, identical to the leader sequence of most HLA-C alleles. Strain Tolendo also has a sequence matched an HLA-A2 leader peptide that was known to bind to HLA-E (67). Therefore, hCMV protein UL40 could up-regulate the expression of HLA-E in a TAP-independent way, thus inhibit NK cell lysis with the interaction between HLA-E and its receptor CD94/NKG2A/B.

Modulation of the HLA class II molecule expression

The priming of HLA II-restricted $CD4^+$ T cells by professional APCs is crucial for control of hCMV infection with respect to these cells are direct antiviral effectors and also provide help for maintenance of $CD8^+$ T cell antiviral response. Exogenous antigen was endocytosed by antigen presenting cell (APC) and destroyed by the endosomal and lysosomal proteases into peptide fragments containing 13~18 amino acid residues. The nascent assembled HLA II molecule associated with an accessory polypeptide, the invariant chain or Li occupied the binding groove in the ER where it prevents the HLA-II molecule from loading exogenous peptide in lumen, and directs its export through the Golgi apparatus to endosome, where the Li was destroyed by proteases to yield a fragment called CLIP bond to the HLA II molecules, then the CLIP is removed by the HLA-DM, freeing the HLA-II molecules to load peptides and the HLA-II/peptides complex are transported to the cell surface where it can be recognized by $CD4^+$ T cells (68).

hCMV encoded protein US2, US3, UL83 destroy the HLA-DR with specific pathways. US2, UL83 down-regulate cell surface expression of HLA-DR by accumulation of HLA-DR in lysosomes and degraded (69, 70). Meanwhile, US2 causes degradation of HLA-DM heavy chain leading to the dysfunction of exogenous antigen procession and

presentation. US3 applies a novel mechanism for evading the MHC class II antigen presentation by inhibition of HLA-DR assembly, transportation, and antigen loading (71).

hCMV also disturbs expression of HLA II molecules by alternating the regulatory factors at transcription level. IFN-γ can efficiently induce HLA II expression (72). IFN-γ binding to its extracellular receptor IFN-γR causes the phosphorylation of the intracellular JAK1/2, which activate the phosphorylation of signal transducers and activators of transcription (STAT). The phosphorylated STAT migrates to the nucleus where it binds to IFN-γ activation sequence elements present in the promoters of class II transactivator (CIITA), thereby drives expression of class II molecules. In infected cells, proteins expressed at the immediate early and early phase of an hCMV infection tamper with the IFN-γ signaling cascade and prevent the expression of CIITA, as a consequence, disruption of inducible HLA class II expression at the transcription level (73, 74). These mechanisms, combined with the interruption of HLA I molecule-restricted antigen presentation pathway, further demonstrate the remarkable diversity of hCMV immunoevasive strategies.

Concluding remarks

hCMV has developed multiple strategies to establish long-term infection in hosts by encoding a series of stage-dependent proteins interfering with HLA molecule surface expression, by which hCMV could escape CTL and NK cell antiviral responses. Other viruses such as adenovirus, EB virus, HSV and even HIV virus may adopt a similar way to counteract the host immune attack, which is crucial for viral survival. The study on the viral protein and the mechanisms of immune modification by the infected viruses will benefits us in further understanding the pathogenesis of viral caused diseases and developing new approaches to vaccine research and disease therapy.

Acknowledgements

This work was supported by the grants from Natural Science Foundation of Zhejiang Province, China (Y205531, Y205575) and the grant from Ministry of Personnel, China.

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