Acta Biochim Biophys Sin 2012, 44: 551–554 | © The Author 2012. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gms032.
Advance Access Publication 9 May 2012

Review

Human leukocyte antigen E in human cytomegalovirus infection: friend or foe?

Fang Gong[1], Shengli Song[2], Guozhong Lv[3], Yuhong Pan[1], Dongqing Zhang[2], and Hong Jiang[2*]

[1]Department of Laboratory medicine, The Third Hospital Affiliated to Nantong University, Wuxi 214041, China
[2]Department of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[3]Department of Microbial Pathogenesis, The Third Hospital Affiliated to Nantong University, Wuxi 214041, China
*Correspondence address. Tel/Fax: +86-21-63846590-776633; E-mail: Hongjiang1949@yahoo.com.cn

Human cytomegalovirus (HCMV) is a well-studied β-herpesvirus virus, which adopts a variety of strategies to evade immune surveillance. It has been reported that in HCMV-infected cells, classical major histocompatibility (MHC) class I molecules are down-regulated, but the MHC class Ib molecule human leukocyte antigen (HLA)-E is normally expressed or even overexpressed on the cell surface. HLA-E has been first described to interact with CD94/NKG2 receptors expressed mainly on the surface of natural killer (NK) cells, thus confining its role to the regulation of NK-cell function. The engagement of CD94/NKG2A with HLA-E, with a signal peptide of the HCMV glycoprotein UL40, usually induces inhibitory signals. However, HLA-E also serves as a ligand for the TCR expressed by $\alpha\beta CD8^+$ T cells. Recognition of peptides presented by HLA-E may result in $CD8^+$ effector T-cell activation. These findings will help to understand more on both pathogenic and protective roles of HLA-E in HCMV infection. In this review, we discussed recent studies about the roles of HLA-E in HCMV infection.

Keywords major histocompatibility Ib; human leukocyte antigen-E; cytomegalovirus

Received: January 2, 2012 Accepted: March 13, 2012

Introduction

Human cytomegalovirus (HCMV) is the most extensively studied member of the β-herpesvirus subfamily [1]. HCMV infection is usually subclinical in healthy individuals. However, in immunocompromised hosts, HCMV can cause pathology in multiple organ systems with high mortality rates. It is a risk factor for congenital birth defects when the mother experiences primary infection during pregnancy.

The cellular immune response to HCMV plays a crucial role in controlling primary infection and reactivation from latency [2]. The virus has developed a variety of mechanisms to subvert host defenses. Until recently, more intensive researches focus on the inhibition of the host major histocompatibility (MHC) class I antigen processing and presentation pathway, thereby reducing the presentation of virus-derived epitopes on the surface of the infected cells [1]. It was reported that cell surface expression of antigen presenting MHC molecules is modulated by the concerted action of a set of HCMV-encoded unique short (US) proteins (US2, US3, US6, US10, and US11), which is expressed differentially at different stages of the viral infection [3–10]. With these proteins, HCMV could interfere with the MHC-restricted pathway of antigen presentation retention by degradation and internalization of MHC class I molecules, thus confer from HCMV to escape the host T-lymphocyte-mediated antiviral immune responses [1]. There comes a question. Why can such infected cells in the body survive in the condition of natural killer (NK)-controlled immunosurveillance because an effective shutdown of MHC class Ia presentation should sensitize these cells for NK attack? Part of the answer is provided by studying the HLA-E displayed on HCMV-infected cells. This short review will focus on the biological features of HLA-E and summarize recent advances in the pathogenic and protective role of HLA-E in HCMV infection.

Biological Features of HLA-E

HLA-E is perhaps the least polymorphic of all the MHC class I molecules and only nine alleles have been identified to date, including three non-synonymous alleles, i.e. HLA-E*0101, HLA-E*0103, and HLA-E*0104 (the HLA-E*0102 allele has been deleted from the IMG/HLA database, release 3.4.0). The HLA-E*0101 and HLA-E*0103 alleles are found at nearly equal frequencies in various populations whereas HLA-E*0104 is considered to be a rare allele [11]. Different from classical MHC class I, MHC class Ib binds a narrow set of nonameric peptides, mostly derived from the leader sequences of classical

MHC class Ia molecules. Under physiological conditions, HLA-E is associated with peptides derived from residues 3–11 of the leader sequence of most HLA-A, -B, -C, and -G molecules [12]. In some situations, such as cellular stress and induced expression of heat-shock proteins, HLA-E can bind other self-peptides such as heat shock protein-derived peptides [13].

HLA-E serves as a ligand for C-type lectin CD94/NKG2 heterodimer in NK and $CD8^+$ T cells [14] (**Fig. 1**). NKG2A contains an intracellular immunoreceptor tyrosine-based inhibitory motif-mediating inhibitory signals [15], whereas NKG2C, E, or H contains the immunoreceptor tyrosine-based activating motif bearing adaptor molecule DAP-12, and mediates positive signaling [15]. In most cases, NKG2A exerts a dominant inhibitory effect, showing as both increased expression levels and a relatively high binding affinity of CD94/NKG2A compared with CD94/NKG2C, E, or H [16]. Thus, under physiological conditions, the engagement of CD94/NKG2A with HLA-E, loaded with peptides derived from MHC class I leader sequences, usually induces inhibitory signals [17].

It is a new role for HLA-E as a ligand for the TCR expressed by $\alpha\beta CD8^+$ T cells. Recognition of peptides presented by HLA-E may result in $CD8^+$ effector T-cell activation [18–24] (**Fig. 1**). Several studies have characterized HLA-E-restricted T cells both *in vitro* and *in vivo*. HLA-E-dependent presentation of bacteria-derived antigens to human $CD8^+$ T cells has been documented, including *Mycobacterium tuberculosis* [18,19], cytomegalovirus [20–22] and *Salmonella typhi* [23].

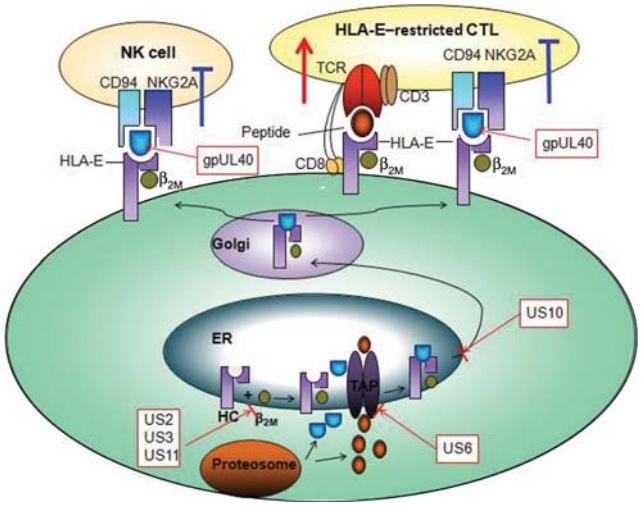

Figure 1 HCMV interferes with class I MHC antigen presentation and inhibits cytotoxicity by $CD8^+$ T cells and NK cells The MHC class I-mediated peptide presentation process involves several steps, many of which can be inhibited by HCMV gene products. MHC class I molecules are targeted by US2 and US11 for degradation. US3 prevents maturation of class I MHC molecules by retaining them in the ER. Transport of peptides by TAP is inhibited by US6. US10 can delay the egress from the ER. HLA-E serves as a ligand for C-type lectin CD94/NKG2 receptors on NK and $CD8^+$ T cells. The engagement of CD94/NKG2A with HLA-E, loaded with a signal peptide of the HCMV glycoprotein UL40 (gpUL40) which is TAP-independent, usually induces inhibitory signals. HLA-E also serves as a ligand for the TCR expressed by $\alpha\beta CD8^+$ T cells. Recognition of peptides presented by HLA-E may result in $CD8^+$ effector T-cell activation. ER, endoplasmic reticulum.

Immune Response to HCMV Infection

The vertebrate host responds to HCMV infection using both innate and adaptive immune systems. It is recognized that activation of the innate immune system is important in the defense against HCMV and in priming the adaptive immune response. During viral infection, NK cells offer an important first-line defense that limits viral expansion when specific immunity has not yet fully developed [25]. Despite a large number of HCMV-encoded gene products target the MHC class I and MHC class II antigen presentation pathway with an attempt to avoid $CD8^+$ and $CD4^+$ T-cell recognition, it becomes increasingly apparent that HCMV-specific $CD8^+$ T cells, $CD4^+$ T cells, γδ T cells, and B cells are all important for controlling and restricting viral replication in hosts with persistent infection [26,27]. $CD8^+$ and $CD4^+$ T cells can directly kill the virus-infected cells or produce cytokines that exerts direct and indirect effects on the immune responses [26].

HLA-E and Immune Escape of NK Cells

It is reported that in HCMV-infected cells, while classical MHC class I molecules are down-regulated, HLA-E is normally expressed or even overexpressed on the cell surface [2,28,29]. As for the resistance of HLA-E molecules to US2-, US6-, and US11-mediated down-regulation, previous reports have indicated that there are allelic differences among MHC class I molecules [10,30,31]. However, the picture is still far from complete. Previous reports showed that a signal peptide of the HCMV glycoprotein UL40 (gpUL40) is identical to the peptide from the leader sequence of HLA-Cw alleles [2]. More importantly, with high amounts available during HCMV infection, gpUL40 binds to HLA-E molecules and enables their expression on the cell surface, even when HCMV suppresses transporter associated with antigen presentation (TAP)-mediated transport from cytosol to endoplasmic reticulum [2,28,29]. As a consequence, HCMV itself, could supply proteins such as gpUL40 to block the activation of NK cells via CD94/NKG2A receptors **(Fig. 1)** [2,28–30]. Thus, induction of HLA-E surface expression in HCMV-infected cells may represent an escape route for HCMV.

Although the ability of viruses to preserve HLA-E seems to be an attractive mechanism to avoid NK cells via the

inhibitory CD94/NKG2A receptor, its functional impact is questionable because the activating receptor CD94/NKG2C also binds to HLA-E [14]. Epidemiological studies have shown that a subset of CD94/NKG2C$^+$ is increased in HCMV-seropositive healthy individuals [32,33], in patients with chronic hepatitis virus infection [34] and in solid-organ transplant recipients with active HCMV infection [35]. These results are consistent with the finding that the NKG2C$^+$ cells expanding after co-culture with HCMV-infected fibroblasts that rarely co-express NKG2A [36]. The expanded NKG2C$^+$ NK cells shows a terminally differentiated phenotype with strong functional responses against HLA-E expressing targets [34,37]. Interestingly, NKG2C$^+$ NK cells have a clonal expression of self-specific KIRs that blocked NKG2C-mediated activation. The mechanism behind the expansion of NKG2C$^+$ NK cells bearing self-specific KIR remains elusive [34]. It may be a tactic that HCMV employs to both invite and evade immune detection through NKG2, as part of a strategy to persist without killing the host.

HLA-E Restricted CD8$^+$ T Cells in HCMV Infection

CD8$^+$ T cells can recognize HLA-E loaded with the UL40-derived peptide [20,21] **(Fig. 1)**. Benefiting from a transfectant from the TAP-deficient RMA-S murine cell line, which is co-transfected with HLA-E*01033 and human β2-microglobulin (RMA-S/HLA-E), it is demonstrated that this subset of T cells is capable of lysing the RMA-S/HLA-E transfectant loaded with saturating amounts of different HLA-E-binding peptides, including peptides derived from the UL40 protein isolated from different HCMV strains [21]. Because these cells display a broad cytolytic activity against various allogeneic NK-susceptible tumor cell lines (a function referred to as NK-like activity), they are named NK cytotolytic T lymphocytes (NK-CTLs) [21]. In addition, this subset of T cells is already present in a sizeable fraction in the peripheral blood of HCMV-seropositive healthy individuals [25]. These cells display an effector-memory surface phenotype (CD27$^-$CD28$^-$CD45RA$^+$CCR7$^-$) and express intracellular cytotoxic granules containing perforin and granzymes. Thus, HLA-E-restricted CD8$^+$ T cells could represent an additional type of effector cells playing a role in defense against HCMV that can escape recognition mediated by both conventional CTLs and NK cells [22].

Concluding Remarks

During the last few years, a series of relevant discoveries unraveled several important functions of the poorly known MHC class Ib molecule HLA-E in humans. HLA-E may play a relevant role in both innate and adaptive immunity. First, HLA-E molecules interact in an 'innate' immune manner, through one molecular, with members of the heterodimeric CD94/NKG2 receptor family. Second, there is a convincing evidence that HLA-E can present peptide antigens for TCR recognition, thus exert function on adaptive immunity. A number of studies revealed that HLA-E-restricted CTLs recognize a number of HLA-E-binding, pathogen-derived peptides, thus revealing the likely roles played by these cells in host defenses. Remarkably, in HCMV infection, down-regulation of MHC class Ia molecules and up-regulation of HLA-E mean that HLA-E-restricted CTLs might play a major role in antivirus defenses. Along this line, a better definition of the functional potential of the HLA-E-restricted CTL subset may help to clarify their role in defense mechanisms and to exploit their properties in immunotherapy, in view of their anti-viral activity.

Funding

This work was supported by the grants from the National Key Basic Research Program of China (2009CB522409) and Major Program of the National Natural Science Foundation of China (30830093).

References

1 Griffin BD, Verweij MC and Wiertz EJ. Herpesviruses and immunity: the art of evasion. Vet Microbiol 2010, 143: 89–100.
2 Tomasec P, Braud VM, Rickards C, Powell MB, McSharry BP, Gadola S and Cerundolo V, *et al*. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. Science 2000, 287: 1031.
3 Ulbrecht M, Hofmeister V, Yuksekdag G, Ellwart JW, Hengel H, Momburg F and Martinozzi S, *et al*. HCMV glycoprotein US6 mediated inhibition of TAP does not affect HLA-E dependent protection of K-562 cells from NK cell lysis. Hum Immunol 2003, 64: 231–237.
4 Misaghi S, Sun ZY, Stern P, Gaudet R, Wagner G and Ploegh H. Structural and functional analysis of human cytomegalovirus US3 protein. J Virol 2004, 78: 413–423.
5 Liu Z, Winkler M and Biegalke B. Human cytomegalovirus: host immune modulation by the viral US3 gene. Int J Biochem Cell Biol 2009, 41: 503–506.
6 Dugan GE and Hewitt EW. Dependence of the localization and function of the human cytomegalovirus protein US6 on the transporter associated with antigen processing. J Gen Virol 2009, 90: 2234–2238.
7 Dugan GE and Hewitt EW. Structural and functional dissection of the human cytomegalovirus immune evasion protein US6. J Virol 2008, 2: 3271–3282.
8 Furman MH, Dey N, Tortorella D and Ploegh HL. The human cytomegalovirus US10 gene product delays trafficking of major histocompatibility complex class I molecules. J Virol 2002, 76: 11753–11756.

9 Park B, Spooner E, Houser BL, Strominger JL and Ploegh HL. The HCMV membrane glycoprotein US10 selectively targets HLA-G for degradation. J Exp Med 2010, 207: 2033–2041.

10 Barel MT, Pizzato N, van Leeuwen D, Bouteiller PL, Wiertz EJ and Lenfant F. Amino acid composition of alpha1/alpha2 domains and cytoplasmic tail of MHC class I molecules determine their susceptibility to human cytomegalovirus US11-mediated downregulation. Eur J Immunol 2003, 33: 1707–1716.

11 Strong RK, Holmes MA, Li P, Braun L, Lee N and Geraghty DE. HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. J Biol Chem 2003, 278: 5082–5090.

12 Lee N, Goodlett DR, Ishitani A, Marquardt H and Geraghty DE. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. J Immunol 1998, 160: 4951–4960.

13 Michaelsson J, Teixeira de Matos C, Achour A, Lanier LL, Karre K and Soderstrom K. A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition. J Exp Med 2002, 196: 1403–1414.

14 Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS and Lazetic S, *et al*. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 1998, 391: 795–799.

15 Yokoyama WM and Plougastel BF. Immune functions encoded by the natural killer gene complex. Nat Rev Immunol 2003, 3: 304–316.

16 Vales-Gomez M, Reyburn HT, Erskine RA, Lopez-Botet M and Strominger JL. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2-A and the activating receptor CD94/NKG2-C to HLA-E. EMBO J 1999, 18: 4250–4260.

17 Lu L, Werneck MB and Cantor H. The immunoregulatory effects of Qa-1. Immunol Rev 2006, 212: 51–59.

18 Joosten SA, van Meijgaarden KE, van Weeren PC, Kazi F, Geluk A, Savage ND and Drijfhout JW, *et al*. *Mycobacterium tuberculosis* peptides presented by HLA-E molecules are targets for human CD8 T-cells with cytotoxic as well as regulatory activity. PLoS Pathog 2010, 6: e1000782.

19 Heinzel AS, Grotzke JE, Lines RA, Lewinsohn DA, McNabb AL, Streblow DN and Braud VM, *et al*. HLA-E-dependent presentation of Mtb-derived antigen to human $CD8^+$ T cells. J Exp Med 2002, 196: 1473–1481.

20 Romagnani C, Pietra G, Falco M, Mazzarino P, Moretta L and Mingari MC. HLA-E-restricted recognition of human cytomegalovirus by a subset of cytolytic T lymphocytes. Hum Immunol 2004, 65: 437–445.

21 Pietra G, Romagnani C, Mazzarino P, Falco M, Millo E, Moretta A and Moretta L, *et al*. HLA-E-restricted recognition of cytomegalovirus-derived peptides by human $CD8^+$ cytolytic T lymphocytes. Proc Natl Acad Sci U S A 2003, 100: 10896–10901.

22 Mazzarino P, Pietra G, Vacca P, Falco M, Colau D, Coulie P and Moretta L, *et al*. Identification of effector-memory CMV-specific T lymphocytes that kill CMV-infected target cells in an HLA-E-restricted fashion. Eur J Immunol 2005, 35: 3240–3247.

23 Salerno-Goncalves R, Fernandez-Vina M, Lewinsohn DM and Sztein MB. Identification of a human HLA-E-restricted $CD8^+$ T cell subset in volunteers immunized with *Salmonella enterica* serovar Typhi strain Ty21a typhoid vaccine. J Immunol 2004, 173: 5852–5862.

24 Jiang H and Chess L. Qa-1 restricted CD8 Treg and self-nonself discrimination-An essay on peripheral T-cell regulation. Hum Immunol 2008, 69: 721–727.

25 Orange JS, Fassett MS, Koopman LA, Boyson JE and Strominger JL. Viral evasion of natural killer cells. Nat Immunol 2002, 3: 1006–1012.

26 Revilleza MJ, Wang R, Mans J, Hong M, Natarajan K and Margulies DH. How the virus outsmarts the host: function and structure of cytomegalovirus MHC-I-like molecules in the evasion of natural killer cell surveillance. J Biomed Biotechnol 2011, 2011: 724607.

27 Vermijlen D, Brouwer M, Donner C, Liesnard C, Tackoen M, Van Rysselberge M and Twité N, *et al*. Human cytomegalovirus elicits fetal gammadelta T cell responses in utero. J Exp Med 2010, 207: 807–821.

28 Ulbrecht M, Martinozzi S, Grzeschik M, Hengel H, Ellwart JW, Pla M and Weiss EH. Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. J Immunol 2000, 164: 5019–5022.

29 Wang EC, McSharry B, Retiere C, Tomasec P, Williams S, Borysiewicz LK and Braud VM, *et al*. UL40-mediated NK evasion during productive infection with human cytomegalovirus. Proc Natl Acad Sci U S A 2002, 99: 7570–7575.

30 Barel MT, Pizzato N, Le Bouteiller P, Wiertz EJ and Lenfant F. Subtle sequence variation among MHC class I locus products greatly influences sensitivity to HCMV US2- and US11-mediated degradation. Int Immunol 2006, 18: 173–182.

31 Prod'homme V, Tomasec P, Cunningham C, Lemberg MK, Stanton RJ, McSharry BP and Wang EC, *et al*. Human cytomegalovirus UL40 signal peptide regulates cell surface expression of the NK cell ligands HLA-E and gpUL18. J Immunol 2012, 188: 2794–2804.

32 Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N and Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood 2004, 104: 3664–3671.

33 Monsiváis-Urenda A, Noyola-Cherpitel D, Hernández-Salinas A, García-Sepúlveda C, Romo N, Baranda L and López-Botet M, *et al*. Influence of human cytomegalovirus infection on the NK cell receptor repertoire in children. Eur J Immunol 2010, 40: 1418–1427.

34 Beziat V, Dalgard O, Asselah T, Halfon P, Bedossa P, Boudifa A and Hervier B, *et al*. CMV drives clonal expansion of highly differentiated NKG2C(+) NK cells expressing self-specific KIRs in patients with chronic hepatitis virus infection. Eur J Immunol 2011, 42: 447–457.

35 Lopez-Vergès S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA and Houchins JP, *et al*. Expansion of a unique $CD57^+$NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. Proc Natl Acad Sci U S A 2011, 108: 14725–14732.

36 Guma M, Budt M, Saez A, Brckalo T, Hengel H, Angulo A and Lopez-Botet M. Expansion of CD94/$NKG2C^+$ NK cells in response to human cytomegalovirus-infected fibroblasts. Blood 2006, 107: 3624–3631.

37 Foley B, Cooley S, Verneris MR, Pitt M, Curtsinger J, Luo X and Lopez-Vergès S, *et al*. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated $NKG2C^+$ natural killer cells with potent function. Blood 2012, 119: 2665–2674.