

Review Article

The Emerging Role of HLA-E-Restricted CD8⁺ T Lymphocytes in the Adaptive Immune Response to Pathogens and Tumors

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Human leukocyte antigen (HLA)-E is a nonclassical major histocompatibility complex (MHC) class I molecule of limited sequence variability that is expressed by most tissues albeit at low levels. HLA-E has been first described as the ligand of CD94/NKG2 receptors expressed mainly by natural killer (NK) cells, thus confining its role to the regulation of NK-cell function. However, recent evidences obtained by our and other groups indicate that HLA-E complexed with peptides can interact with $\alpha\beta$ T-cell receptor (TCR) expressed on CD8⁺ T cells. Although, HLA-E displays a selective preference for nonameric peptides, derived from the leader sequence of various HLA class I alleles, several reports indicate that it can present also “noncanonical” peptides derived from both stress-related and pathogen-associated proteins. Because HLA-E displays binding specificity for innate CD94/NKG2 receptors, as well as all the features of an antigen-presenting molecule, its role in both natural and acquired immune responses has recently been re-evaluated.

1. Introduction

Major histocompatibility complex (MHC) class I molecules may be subdivided into two families, MHC class Ia (classical) and MHC class Ib (nonclassical). Both classical and nonclassical MHC class I molecules are expressed at the cell surface in association with β 2-microglobulin. MHC class Ia molecules (human leukocyte antigen (HLA)-A, -B, and -C, in humans) [1] play a central role in adaptive immunity. They interact directly with T-cell receptors (TCRs) and with the coreceptor molecule CD8 on cytotoxic T cells (CTLs). In humans, the MHC class Ib family members include HLA-E, -F, -G, and HFE (HLA-H) [2]. They are best known for their capability of regulating innate immune responses. Nevertheless, there is now accumulating evidence that, like the MHC class Ia molecules, certain class Ib molecules can play a role also in regulating acquired immune responses

to bacteria and viruses [2, 3]. While, classical MHC class I molecules are extremely polymorphic, nonclassical MHC class I molecules are poorly polymorphic and, in most instances, exhibit a narrow tissue distribution. In addition, cell surface expression of MHC class Ib molecules is generally lower than that of MHC class Ia molecules. In humans, HLA-E is the least polymorphic of all the MHC class I molecules [4]. Several studies reported that only two alleles predominate in the Caucasian population. These two alleles HLA-E*0101 (HLA-E^{107R}) and HLA-E*0103 (HLA-E^{107G}) differ at only one amino acid position. Thus, an arginine at position 107 in HLA-E*0101 (HLA-E^{107R}) is replaced by a glycine in HLA-E*0103 (HLA-E^{107G}). In most cases the HLA-E^{107G} allele is expressed at significantly higher levels than the HLA-E^{107R} allele on normal cells [5]. The differences in surface expression of the two alleles do not reflect only the higher affinity of HLA-E^{107G} for available

peptides, but also the higher stability of surface HLA-E^{107G}/peptide complexes. HLA-E displays a relatively high degree of peptide binding ability [6]. Under physiological conditions, cell surface expression of HLA-E depends on binding of nonamer peptides derived from the signal sequence of MHC class I molecules [7]. The assembly of HLA-E with the signal peptide-derived ligands is strictly dependent on a functioning processing machinery (i.e., proteasome and the transporter associated with antigen processing, TAP) [7, 8]. Unlike the other MHC class Ib molecules, HLA-E is transcribed virtually in all human tissues and cell lines, although at lower levels than MHC class Ia antigens [9, 10]. Recently, unusually high levels of HLA-E have been detected in neoplastic cells [11, 12]. Although, HLA-E was first described as the ligand for CD94/NKG2A (inhibitory) and CD94/NKG2C (activating) NK receptors [13–15], thus mainly confining its regulatory function to NK cell population, emerging evidence (obtained by our and other groups) clearly support the notion that HLA-E can serve as a restricting element for the TCR of some $\alpha\beta$ T cells [16–20]. Along this line, this MHC class Ib molecule plays an important role in both natural and acquired immune responses [21].

2. HLA-E Does Bind Different Sets of Peptides

As indicated above, HLA-E is a conserved class Ib molecule characterized by a limited polymorphism. It binds non-amer leader peptides primarily derived from amino-acid residues 3–11 of the signal sequences of most HLA-A, -B, -C and -G molecules (e.g., VMAPRTLIL, VMAPRTLVL, VMAPRTLLL, VMAPRALLL, and VMAPRTLFL) [7]. Peptide binding stabilizes the HLA-E protein, allowing it to migrate to the cell surface. A functioning TAP molecule is required to transport these peptides into the endoplasmic reticulum (ER), where they can interact with HLA-E [7]. Although, HLA-E appears to bind a narrow peptide repertoire, recent evidences revealed that several proteins other than MHC class I molecules encode peptides that can bind to HLA-E. Among these peptides the best characterized are the VMAPRTLVL and the VMAPRTLIL peptides derived from the gpUL40 leader sequences (gpUL40₁₅₋₂₃) of two different human cytomegalovirus (CMV) strains (i.e., Toledo and AD169 strains). These peptides match exactly the leader sequence peptides of various HLA-A and -Cw alleles, respectively. Importantly, unlike the MHC-derived peptides, the gpUL40-derived ones are assembled with HLA-E via a TAP-independent mechanism [22]. In addition, peptides derived from the human heat shock protein 60 (hsp60₁₀₋₁₈, QMRPVSRVL) [23], the ATP-binding cassette transporter, multidrug resistance-associated protein 7 (MRP7₄₉₆₋₅₀₄, ALALVRMLI) [24], the human immunodeficiency virus (HIV) gag protein (HIV p24₁₄₋₂₂, AISPRTLNA) [25], the *Salmonella enterica* serovar Typhi GroEL protein (GroEL₁₅₋₂₃, KMLRGVNVL) [20], and gliadin (gliadin α 2 chain₅₂₋₆₀, SQQPYLQLQ) [26] have also been shown to bind HLA-E.

Interestingly, binding to HLA-E has been demonstrated also for viral peptides previously shown to bind classical

HLA class I molecules. For example, peptides from the Epstein-Barr virus (EBV) BZLF-1 protein (BZLF-1₃₉₋₄₇, SQAPLPCVL), the influenza matrix protein (InflM₅₉₋₆₇, ILGFVFTLT) [27], and the Hepatitis C virus (HCV) core protein (HCV Core₃₅₋₄₄, YLLPRRGPR) [28] are viral peptides capable of binding to both HLA-A2 and HLA-E. Along this line, we have recently demonstrated that also peptides encoded by cellular-associated proteins can display a dual binding specificity (namely, they bind to both HLA-A2 and HLA-E). Thus, two alternative splicing isoforms of mutant peroxiredoxin 5 (Prdx5) encode two nonapeptides (Prdx5 splice variant (Prdx5 Δ 2)₅₂₋₆₀, AMAPIKTHL and Prdx5 splice variant (Prdx5 Δ 2, 3)₅₂₋₆₀, AMAPIKVRL) able to bind to both HLA-A2 and HLA-E and to allow their cell surface expression [29]. It is of note that the amino acid sequences of some of these HLA-E-binding peptides (derived from pathogen-associated, stress-related or normal proteins) differ markedly in sequence from the canonical MHC class I-derived leader sequence peptides.

3. HLA-E Is Recognized by T Cells via their $\alpha\beta$ TCR

CD8⁺ CTLs expressing $\alpha\beta$ TCR generally recognize antigen peptides in association with MHC class Ia molecules [30]. MHC class Ia molecules are central to allow CTL-mediated discrimination between self and nonself. Thus, they present antigens, in the form of 8–10 amino acid-long peptides, to the $\alpha\beta$ TCR expressed by CD8⁺ T cells. HLA class Ia- $\alpha\beta$ TCR interactions induce T lymphocytes to kill cells that are infected by viruses or some intracellular bacteria. MHC class Ia peptide epitopes, in most instances, are generated from the proteasomal degradation of cytosolic proteins and their loading into MHC class Ia is dependent on TAP function. The ability of MHC class Ib molecules to stimulate T-cell responses emerged only in recent years. Various experimental evidences revealed that MHC class Ib molecules may be recognized by T cells. Thus, while it is well established that HLA-E plays an important role in innate immunity, functioning as ligand for the CD94/NKG2 killer lectin-like receptors [13, 31] expressed by most NK cells [32] and a subset of CTLs [33–35], it is now clear that it can present peptide antigens for $\alpha\beta$ TCR-mediated recognition [16–20, 36]. Several studies have characterized HLA-E-restricted T cells. HLA-E dependent presentation of bacteria-derived antigens to human CD8⁺T-cells has been documented. In particular, bacteria, including *Mycobacterium tuberculosis* (Mtb) and *Salmonella enterica* serovar Typhi GroEL, can elicit HLA-E-restricted T-cell responses [19, 20]. In addition, our group has recently demonstrated that an HLA-E-restricted CD8⁺ T cell subset represents a significant component of the adaptive immune response to CMV in genetically predisposed individuals [36, 37]. Recently, other authors demonstrated that chronic hepatitis C is associated with increased intrahepatic HLA-E expression and showed that HCV gives rise to a peptide that binds to HLA-E and can be recognized by CD8⁺ T cells via their TCR [38].

4. Role of HLA-E-Restricted CD8⁺ T Cells in Infections

4.1. Nonclassically Restricted CD8⁺ T Cells in the Host Response to Mtb. Mtb represents a leading cause of infectious disease morbidity and mortality worldwide. In addition to CD4⁺ T-cells, also CD8⁺ T lymphocytes appear essential for the containment of mycobacterial infection. The importance of HLA-E-restricted T cells in the host response to infection with Mtb has been defined by Heinzl and coworkers [19]. In particular, they found that these cells comprise the dominant CD8⁺ T cell response in latently infected individuals. Remarkably, this finding represents the first compelling evidence of the ability of HLA-E to present pathogen-derived antigens and extended the function of HLA-E beyond its well-known role as an NK inhibitor through the interaction with the CD94/NKG2A heterodimer. Although, the recognized Mtb-derived peptides have not been identified so far, Mtb-derived antigen presentation was found to require proteasomal processing, but not TAP-mediated peptide transport.

4.2. HLA-E Restricted CD8⁺ T Cells in the Host Response to HCV Infection. HCV is a single-stranded RNA virus belonging to the flaviviridae family. HCV infection is a common cause of liver disease worldwide. In most instances, HCV is not eliminated by the host and results in chronic infection, which may develop into cirrhosis and hepatocarcinoma. The mechanisms favoring persistent infection are still poorly understood. However, numerous studies clearly indicate an association between an impaired immune response and clinical outcome. Patients who spontaneously recover from HCV infection typically mount vigorous multi-epitope-specific CD4⁺ and CD8⁺ T-cell responses that are readily detectable in blood samples. By contrast, patients with chronic hepatitis C tend to have late, transient and narrowly focused T-cell responses [39]. Recently, some authors suggested an important role for HLA-E in regulating antiviral immunity. With respect to HCV infection they demonstrated that chronic hepatitis C is associated with enhanced intrahepatic HLA-E expression [25]. In particular, they showed that HCV gives rise to the YLLPRRGPRLL peptide (HCV core amino acid 35-44) that binds to, stabilizes HLA-E surface expression and protects cells from the NK cell-mediated cytotoxicity [25]. More importantly, they showed that, beyond its interaction with the CD94/NKG2A receptor, peptide-loaded HLA-E molecules can also be recognized by CD8⁺ T-cells via their TCR [38]. Patients with chronic HCV infection were analyzed for their nonclassical CD8⁺ T cell responses by using HLA-E transfected K562 cells, loaded with the appropriate HCV peptide, as antigen-presenting cells in an interferon (IFN)- γ ELISPOT assay. Interestingly, by this approach, they found that in nearly half of the HCV patients analyzed an HLA-E-restricted HCV-specific CD8⁺ T-cell response could be detected. It is of note, that HLA-E –restricted IFN- γ secretion was associated with low viral load. Importantly, no IFN- γ production was found in CD8⁺ T lymphocytes from healthy controls, confirming the specificity of this finding. Finally, they provided the first evidence for different

functional roles of the 2 confirmed HLA-E allelic variants (i.e., HLA-E^{107R} and HLA-E^{107G}) in hepatitis C infection. Thus, when patients were stratified according to HLA-E genotype, they found that the frequency of HLA-E-restricted responses was higher in patients homozygous for the HLA-E^{107R} allele as compared to carriers of other HLA-E genotypes [38].

4.3. HLA-E-Restricted CD8⁺ T Cells in the Host Response to CMV Infection. Human CMV is a β -herpesvirus that largely infects the human population, resulting in life long persistent asymptomatic infection, which, however, may cause severe morbidity in immunocompromised individuals. CMV infection is the most common viral complication following allogeneic hematopoietic stem cell transplantation and solid organ transplantation. Specific cytotoxic T-cell immunity represents a key factor to contain CMV. Human CMV has evolved an impressive variety of strategies to escape from the recognition mediated by conventional (i.e., MHC class Ia-restricted) CTLs. Different CMV viral proteins (i.e., the unique short, US, proteins) are well known to inhibit the MHC class I expression in infected cells. In particular, the human CMV gene products US2 and US11 bind to nascent HLA class I chains resulting in their shuttle from the ER to the cytosol, where they undergo proteasome-dependent degradation. US3 binds to HLA class I molecules and retains them in the ER. Finally, the TAP-mediated transport of antigenic peptides to the ER is blocked by US6 [40]. As a consequence, conventional HLA class Ia-restricted CTLs may result, at least in part, inefficient in counteracting CMV infection. Interestingly, human CMV increases the surface expression of HLA-E, while downregulating the expression of many other MHC class I molecules [41]. Thus, human CMV itself, through the expression of gpUL40 protein, can supply peptides, which bind HLA-E in a TAP-independent fashion. This results in surface expression of HLA-E at even higher concentrations than in uninfected cells [22, 41, 42]. Notably, the upregulation of HLA-E in CMV-infected cells has been interpreted as a mechanism of viral escape from NK cells expressing the inhibitory CD94/NKG2A receptor.

Studies performed in our lab provided evidence that some CD8⁺ T cells (via their TCR) recognize HLA-E when loaded with peptides (i.e., VMAPRTLIL and VMAPRTLVL) derived either from the gpUL40 proteins of different human CMV strains (e.g., AD169 and Toledo) or from the leader sequences of various classical HLA class I alleles [36]. In addition, we also obtained direct evidence *in vitro* that HLA-E-restricted CD8⁺ T-cells can recognize and kill fibroblasts infected with human CMV strain AD169 [37]. These finding may have particular relevance in the immune defenses against CMV. Thus, HLA-E-restricted CD8⁺ T cells could represent an additional type of effector cells playing a role in defense against a virus which can escape recognition mediated both by CTLs restricted by classical HLA class I molecules and by NK cells. Indeed, HLA-E-restricted CD8⁺ T cells are able to kill infected cells despite the sharp downregulation of HLA class Ia, by recognizing the gpUL40-derived VMAPRTLIL peptide presented in the context of HLA-E [37].

It is of note that, different CMV strains have a mutated form of gpUL40 from which different HLA-E-binding peptides can be generated [22, 43]. For example, the open reading frame *UL40* encoded by the CMV Toledo strain contains the sequence VMAPRTLVL in its predicted leader peptide. This nonamer is identical to the HLA-E-binding peptide present in the signal sequence of the HLA-A*02 allele [22]. In agreement with these findings, also HLA-E-restricted CD8⁺ T cells specific for the VMAPRTLVL peptide have been identified [36].

Donors that are able to develop an anti-CMV HLA-E-restricted CD8⁺ T-cell response can be divided into two different groups according to their HLA-A and HLA-Cw haplotype (Table 1). In particular, “group 2” donors, from which HLA-E-restricted CD8⁺ T cells specific for the VMAPRTLIL peptide could be derived, are characterized by a particular HLA class I haplotype that does not contain any VMAPRTLIL-bearing HLA-Cw alleles [36]. Thus, in these individuals this peptide represents a foreign antigen. On the other hand, donors who express HLA-Cw alleles carrying the VMAPRTLIL peptide, fail to generate HLA-E-restricted CD8⁺ T cells specific for this self-peptide. Thus, in these donors, HLA-E-restricted CD8⁺ T cell precursors may have been negatively selected in the thymus by HLA-E/VMAPRTLIL self-complexes. Along this line, “group 1” donors, from which HLA-E-restricted CD8⁺ T cells specific for both VMAPRTLIL and VMAPRTLVL peptides could be derived, do express in their haplotype neither VMAPRTLIL-bearing HLA-Cw alleles nor VMAPRTLVL-bearing HLA-A alleles [36]. Thus, these peptides represent foreign antigens for these donors. In summary, VMAPRTLIL represents a nonself peptide for “group 1” and “group 2” donors, and it is recognized with high avidity by HLA-E-restricted CD8⁺ T cells from both groups. On the other hand, VMAPRTLVL represents a self-peptide for “group 2” donors, and is therefore recognized only by “group 1” donors. Taken together, these data suggest that the HLA class I host genotype, as well as the infecting CMV strain, may deeply affect the ability of different individuals to exploit HLA-E-restricted CD8⁺ T cell-mediated defenses against CMV infection. Along this line, it is possible to speculate that only individuals carrying a particular MHC class I haplotype that lacks the VMAPRTLIL peptide (i.e., HLA-Cw*02 and/or HLA-Cw*07) are more resistant to severe CMV infection and/or reactivation occurring under certain pathological conditions. Because CMV UL40-derived VMAPRTLIL and VMAPRTLVL peptides are identical to those derived from the leader sequences of various HLA class I alleles, HLA-E-restricted CTLs may display a broad cytolytic activity against various HLA-E⁺ allogeneic tumor cell lines belonging to different histotypes (a function referred to as NK-like activity) [33]. In addition, these effectors cells are characterized by the surface expression of HLA class I-specific inhibitory NK receptors (iNKR) (i.e., KIR, CD94/NKG2A and ILT2/LIR1) [44, 45] and by mono-, oligoclonal TCR V β rearrangements, different in different donors [34]. Finally, by the use of HLA-E tetramers refolded with different peptides we also provided evidence that these cells are present *in vivo*, where they represent a sizeable fraction

of CD8 β ⁺T cells in some CMV seropositive individuals. These cells display an effector-memory surface phenotype (CD27⁻CD28⁻CD45RA⁺CCR7⁻) and express intracellular cytotoxic granules containing perforin and granzymes. In addition, functional analysis revealed that HLA-E-restricted CD8⁺ T cells are capable of prompt production of IFN- γ upon specific peptide stimulation [37]. All the various phenotypic and functional data illustrated above, together with the fact that HLA-E-restricted CD8⁺ T cells represent a oligo-, monoclonal expansion characterized by the expression of HLA class I-specific iNKRs, support the notion that they are effector-memory T cells possibly resulting from a chronic, antigen-driven stimulation [46]. The presence of these effector cells in the CD8⁺ T-cell memory pool of some CMV seropositive individuals, strongly suggests that HLA-E-restricted CD8⁺ T cells may actually play a relevant defensive role during CMV infection.

5. Possible Role of HLA-E-Restricted CD8⁺ T Cells in Immune Responses against Tumors

HLA-E expression by tumor cells has been recently reported in several types of human cancers. Notably, HLA-E may be overexpressed in fresh lymphomas [47], ovarian carcinomas [48], gliomas [49], colon cancer [50], and melanomas [12]. Because of its capacity to bind to the inhibitory CD94/NKG2A receptor expressed by NK cells and a subset of T cells, HLA-E expression by neoplastic cells might favor tumor cell escape from immunosurveillance. Thus, it has been reported that NK cell activity against glioma cell cultures could be greatly increased when HLA-E was downregulated by RNA interference [49]. To date, the potential role of HLA-E as antigen-presenting molecule for tumor-specific CD8⁺ T cells has been poorly addressed and tumor-derived peptides with binding specificity for HLA-E have not yet been identified. However, we recently showed that peptides specific for both HLA-E and classical HLA class I molecules (i.e., HLA-A2) could be generated by alternative splicing of the peroxiredoxin 5 (*Prdx5*) gene [29]. *Prdx5* splice isoforms show widespread expression in normal and neoplastic cell lines belonging to different histotypes. As a result of the splicing events, *Prdx5* isoforms encode two distinct nonapeptides (AMAPIKTHL or AMAPIKVRL) that bind to HLA-A2 and HLA-E molecules, stabilize HLA-A2 and HLA-E and allow their cell surface expression. Interestingly, we recently found that HLA-E⁺ targets, loaded with these peptides, are recognized (although with low avidity), by HLA-E-restricted CD8⁺ CTLs generated from healthy donors [29]. On the other hand, complexes composed by HLA-E and *Prdx5* splice peptides are not recognized by the CD94/NKG2A inhibitory receptor and thus do not downregulate the NK cell function. Increased levels of *Prdx5* can be detected in several diseases, such as osteoarthritis and tendon degeneration [51, 52]. Its upregulation has been reported during acute inflammation induced in rat lung by LPS, in chondrocytes by IL-1 α and TNF- α and in human tendon cells during H₂O₂ exposure. In melanoma cells, *Prdx5* expression can be upregulated by different

TABLE 1: Both the HLA class I host haplotype and the infecting CMV strain can affect the ability of different individuals to exploit an HLA-E-restricted CD8⁺ T-cell-mediated defence against CMV.

Donors	HLA-Cw haplotype	HLA-A haplotype	VMAPRTLIL ¹	VMAPRTLVL ²	HLA-E restricted CD8 ⁺ T cells against CMV
Group 1	HLA-Cw*02, -Cw*07, -Cw*0809, -Cw*15, -Cw*1701, Cw*1703, and -Cw*18 ³	HLA-A*01, -A*03, -A*11, -A*29, -A*30, -A*33, -A*3401, -A*36, -A*74, and -A*80 ⁴	nonsel self peptide	nonsel self peptide	YES (<i>against both AD169 and Toledo strains</i>)
Group 2	HLA-Cw*02, -Cw*07, -Cw*0809, -Cw*15, -Cw*1701, Cw*1703, and -Cw*18 ³	HLA-A*02, -A*23, -A*24, -A*25, -A*26, -A*3402, -A*43, -A*66, and -A*69 ⁵	nonsel self peptide	self peptide	YES (<i>against AD169 strain but not against Toledo strain</i>)

¹This peptide is contained in the UL40 open reading frame (ORF) from human CMV AD169 strain.

²This peptide is contained in the UL40 ORF from human CMV Toledo strain.

³These HLA-*Cw alleles *do not* contain in their leader sequences the VMAPRTLIL peptide.

⁴These HLA-*A alleles *do not* contain in their leader sequences the VMAPRTLVL peptide.

⁵These HLA-*A alleles *do* contain in their leader sequences the VMAPRTLVL peptide.

forms of cellular stress. Along this line, HLA-E-binding peptides such as those derived from alternatively spliced Prdx5 isoforms, may be induced under stress conditions and could contribute to HLA-E stabilization leading to CD8⁺-mediated recognition of tumor cells. It is therefore possible that when endogenous and environmental oxidative stress levels alter normal cellular processes and induce cell damage, as it might occur in cancer, peptides derived from Prdx5 variants may provide a signal that could activate MHC class Ib-restricted T cells, thus contributing to the elimination of stressed cells.

6. Concluding Remarks

While, the T-cell recognition of murine MHC class Ib molecules was described over a decade ago, the ability of human HLA class Ib molecules to stimulate T-cell responses is only now being recognized. Nevertheless, it is still unclear whether HLA-F and -G are involved in stimulating CD8⁺ T cells. HLA-G-restricted T cells that specifically recognize pp65-derived peptides and kill HCMV-infected astrocytoma cells, have been generated in transgenic mice [3]. However, further studies are needed to evaluate how and whether such induction of the HLA-G-restricted, anti-HCMV response exists in humans. In addition, although it has been reported that HLA-H (HFE) can be directly recognized by the $\alpha\beta$ TCR of CD8⁺ T-cells in a transgenic mouse model, it is usually unable to present antigenic peptides [53].

Conversely, during the past years a series of relevant discoveries unraveled several important functions of the poorly known MHC class Ib molecule HLA-E in humans. After the original report that HLA-E represents the ligand of CD94/NKG2 receptors, which are expressed by NK cells and CD8⁺ T cells, it became evident that HLA-E can represent a restriction element for some T cells characterized by cytolytic activity [17]. In most instances, they appear to represent oligoclonal or even monoclonal cell expansions. A relevant work pointed out how these cells may kill a number of allogeneic normal or tumor cells (the so-called NK-like activity) upon recognition of HLA-E/peptide complexes on

target cells [17]. This problem may represent a serious threat in transplantation [54, 55]. More recently, a number of studies revealed that HLA-E-restricted CTLs recognize a number of HLA-E-binding, pathogen-derived peptides, thus revealing the likely role exerted by these cells in host defenses. Remarkably, in certain viral infections (CMV and, possibly, HCV infections), HLA-E-restricted CTLs may play a role complementary to that of conventional (i.e., HLA class Ia-restricted) CTLs [37, 38, 56]. In particular, in human CMV infection, downregulation of HLA class Ia molecules and upregulation of HLA-E could mean that HLA-E-restricted CTLs might play a major role in antiviral defenses [37].

Finally, in view of the broad tissue expression of HLA-E, HLA-E-restricted CTLs should be investigated for their possible involvement in the rejection of HLA class Ia-deficient tumors. In this context, it should be stressed that frequently tumor cells may fail to express one or more HLA class Ia alleles or may display a general downregulation of HLA class Ia surface expression. Both these circumstances, might allow the induction of HLA-E-restricted CD8⁺ T-cell responses against cancer-associated antigens. Along this line, the increased expression of HLA-E in different tumors and the recent demonstration in mice that the nonclassical Qa-1^b molecule (i.e., the murine HLA-E homologue) plays a prominent role as a restriction element for CD8⁺ T cells in the adaptive immune response to TAP-deficient tumors [57], strongly suggest the need of intensive investigation in this field.

Abbreviations

CMV:	Cytomegalovirus
CTLs:	Cytotoxic T lymphocytes
EBV:	Epstein-Barr virus
ER:	Endoplasmic reticulum
HCV:	Hepatitis C virus
HIV:	Human immunodeficiency virus
HLA:	Human leukocyte antigen
iNKR:	Inhibitory NK receptors
Mtb:	<i>Mycobacterium tuberculosis</i>

MHC: Major histocompatibility complex
 NK: Natural killer
 Prdx5: Peroxiredoxin 5
 TAP: Transporter associated with antigen processing
 TCR: T cell receptor.

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