Molecular basis of human natural killer cell recognition of HLA-E (human leucocyte antigen-E) and its relevance to clearance of pathogen-infected and tumour cells*

Christopher A. O’CALLAGHAN
MRC Human Immunology Unit, Nuffield Department of Clinical Medicine, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, U.K.

ABSTRACT

HLA-E (human leucocyte antigen-E) is a conserved class I major histocompatibility molecule which has only limited polymorphism. It binds to the leader peptide derived from the polymorphic classical major histocompatibility molecules HLA-A, HLA-B and HLA-C. This peptide binding is highly specific and stabilizes the HLA-E protein, allowing it to migrate to the cell surface. A functioning TAP (transporter associated with antigen processing) molecule is required to transport these peptides into the endoplasmic reticulum, where they can interact with HLA-E. HLA-E then migrates to the cell surface, where it interacts with CD94/NKG2A receptors on natural killer cells. This interaction inhibits natural killer cell-mediated lysis of a cell displaying HLA-E. If the leader peptide is not present in the endoplasmic reticulum, HLA-E is unstable and is degraded before it reaches the cell surface. In damaged cells, such as virally infected or tumour cells, down-regulation of HLA-A, HLA-B and HLA-C production or inhibition of TAP prevents stabilization of HLA-E by the leader peptide. Under these circumstances, HLA-E does not reach the cell surface and the cell is then vulnerable to lysis by natural killer cells. The molecular mechanisms underlying this function of HLA-E have been revealed by crystallographic studies of the structure of HLA-E.

INTRODUCTION

The major histocompatibility complex (MHC) is the most polymorphic region of the human genome and is of great clinical and scientific interest. It encodes a range of molecules with central roles in the recognition process whereby the immune system distinguishes that which is to be destroyed from that which is to be tolerated. The veracity and reliability of this discriminatory recognition are essential to the survival of the individual, and thereby of the species, in the face of a range of pathogens. The molecular basis of immunological recognition leading to the destruction of pathogen-infected cells and tumour cells remains only partially understood. A fuller understanding of this recognition process should provide a more rational basis for therapy of diseases characterized by inadequate or excessive immune activity. Immune cells are capable of interacting with a range of molecules on the surface of other cells, and it is the outcome of these molecular interactions that determines whether an immune response is mounted. As a rational approach to investigating this immunological recognition, we are

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Key words: CD94, immune receptors, HLA-E, major histocompatibility complex, natural killer cell, NKG2, tetramer.

Abbreviations: HLA, human leucocyte antigen; MHC, major histocompatibility complex; NK, natural killer; TAP, transporter associated with antigen processing.

Correspondence: Dr C. A. O’Callaghan University of California San Francisco, Department of Microbiology and Immunology, Box 0414, 513 Parnassus Avenue, HSE 415–420, San Francisco, CA 94143-0414, U.S.A. (e-mail chrisoc@itsa.ucsf.edu).

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examine dominant individual interactions occurring at the cell surface, so that a progressively more complete molecular understanding of the entire recognition process can be developed.

NON-CLASSICAL HLA (HUMAN LEUCOCYTE ANTIGEN) MOLECULES

Within the MHC, the class Ia molecules HLA-A, HLA-B and HLA-C in humans (H-2K, H-2D and H-2L in mice) play the central role in immunological recognition. These molecules interact directly with T cell receptors and with the co-receptor molecule CD8 on T cells, and these interactions play the central role in determining whether the T cell will destroy the cell with which it is interacting. Class Ia molecules are highly polymorphic and their function has been extensively investigated [1]. They form a complex with a non-polymorphic protein, \( \beta_2 \)-microglobulin. This HLA–\( \beta_2 \)-microglobulin complex binds small intracellular peptide fragments inside the cell and then transports these fragments to the cell surface. At the cell surface, the T cell receptor interacts with both the HLA molecule and the bound peptide. The advantage of this system is that the HLA molecules bind peptides inside the cell and then display them at the cell surface, so conveying information to the T cell about intracellular activity and processes. Therefore, if a cell is infected by a virus, HLA molecules will pick up viral peptide fragments which are recognized as abnormal by the T cell, triggering T cell destruction of the virally infected cell.

Given the key role of these HLA molecules, we were interested in three HLA homologues, HLA-E, HLA-F and HLA-G, which have been identified within the human MHC. These molecules have only very limited polymorphism and are highly conserved. These features and their genomic location suggest a possible role in immunological recognition. However, their lack of polymorphism might indicate that their role involves surveillance of something more predictable than mutating pathogens, such as aberrant cellular growth. Our work initially focused on HLA-E, which is discussed here. Little is known about HLA-F, but HLA-G is expressed in the placenta and may play a role in reproductive immunology [2]. Expression of HLA-G within the placenta may protect foetal tissue at the foetal–maternal boundary from lysis by maternal natural killer (NK) cells [3,4]. In vitro, HLA-G expression can inhibit the lysis of target cells by decidual NK cells [5].

NK cells

NK cells are bone marrow-derived lymphocytes which can kill pathogen-infected or tumour cells and secrete soluble inflammatory mediators very early in infection. They account for 5–10% of human peripheral blood leucocytes and can kill a wide range of targets, including monocytes infected with Mycobacterium tuberculosis [6], erythrocytes infected with Plasmodium falciparum [7] and even extracellular trypanosomes [8]. Low NK activity in humans has been associated with severe recurrent viral infections and premature death (reviewed in [9]). Whereas T cell-derived cytotoxicity takes several days to develop, killing by NK cells is detectable within the first 24 h of viral infection [10] and may persist until the development of a T cell response (reviewed in [9]). As well as direct lysis, NK cells produce a range of soluble products which drive the subsequent immune response, including interferon \( \gamma \), tumour necrosis factor \( \alpha \), granulocyte/macrophage colony-stimulating factor and macrophage inflammatory protein 1 \( \alpha \). Interferon \( \gamma \) in particular is crucial to the development of protective immunity and has a wide range of effects, including the activation of macrophages with the promotion of intracellular anti-viral activity and up-regulation of MHC molecules [11]. Stimulation of NK cells enhances protective immunity during experimental vaccination in mice, and early NK cell responses can influence the development of subsequent protective immunity [12]. Manipulation of the NK response may be a key factor in the development of effective vaccine strategies.

Unlike antigen-specific T and B lymphocytes, there is no apparent receptor rearrangement in NK cells and no known depletion of autoreactive NK cells during maturation. However, as NK cells do not normally mediate self harm, they must be able to distinguish healthy from infected or tumour cells. NK cells kill targets deficient in MHC class I molecules, but this killing is inhibited by transfecting the target cells with MHC class I genes [13]. It appears that recognition by NK cells is a balance between positive and negative signals, with different NK cell receptors mediating inhibition or activation respectively [14,15]. Two major families of NK cell receptors have been identified in humans; in each family, some receptors inhibit and some receptors activate killing by NK cells. The first family identified in humans has extracellular immunoglobulin domains, and these receptors recognize HLA-B and HLA-C molecules [14]. Some members of this family of receptors are capable of inhibiting lysis by NK cells upon recognition of HLA-A, -B and -C molecules, and have been termed killer inhibitory receptors. However, it is now apparent that other members of the same family of molecules can activate NK cells [16]. The precise role of these receptors and how the different signals they transduce are integrated remains to be clarified. The second family of receptors are C-type lectin proteins, and are heterodimers of CD94 disulphide-bonded to one of NKG2A, B, C or E [17]. However, although these C-type lectin receptors were able to recognize cells expressing HLA-A, -B, -C or -G, it was not possible to
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demonstrate a direct interactions between the receptors and these ligands [18]. This was particularly curious, as the receptors could even distinguish between certain HLA-B alleles. In unravelling this paradox of recognition without a direct interaction, we identified, for the first time, a function for the human non-classical MHC molecule HLA-E [19].

CELL BIOLOGY OF HLA-E

Significant insights into the biology of HLA-E were gained from studies on the mouse homologue Qa-1b. The correct pairing of mouse and human MHC class I molecules which are true homologues is not straightforward as, by definition, all mouse and all human MHC class I molecules share some degree of similarity with each other. However, Qa-1b shares certain informative sequence similarities with HLA-E [20]. A threonine at position 143 and a tryptophan at position 147 form part of the peptide-binding groove and are present in almost all mouse and human class I molecules. However, both Qa-1b and HLA-E have serine residues at both of these positions. This shared and atypical amino acid usage suggested that HLA-E and Qa-1b might have similar functions.

In a series of elegant experiments in mice, Qa-1b has been shown to bind to the highly conserved hydrophobic leader peptide of the classical mouse MHC class I molecules [21,22]. The leader or signal peptide is at the N-terminus of a membrane protein and is the first part of the protein to be synthesized by the ribosome (Figure 1). The newly synthesized leader peptide interacts with a signal recognition particle, which brings it and the ribosome to the endoplasmic reticulum, and further interactions ensure that the synthesized protein is transported appropriately across the endoplasmic reticulum. When it has served its purpose, the signal peptide is cleaved from the mature protein by a signal peptidase.

Observations by Geraghty’s group confirmed that HLA-E behaved in a similar manner to Qa-1b [23]. The mutant B cell line 721.221 is deficient in class Ia molecules because the genes encoding these molecules are deleted in these cells. When HLA-E was transfected into 721.221 cells with its own leader sequence, there was no significant surface expression of HLA-E. Geraghty’s group engineered a hybrid HLA-E construct which contained the HLA-A2 leader sequence rather than the HLA-E leader sequence. When this hybrid construct was transfected into 721.221 cells there was a good level of expression of HLA-E at the cell surface, as detected with the anti-class I antibody w6/32. Moreover, co-transfection of the normal HLA-E construct into 721.221 cells together with a plasmid encoding another class Ia molecule also resulted in expression of HLA-E at the cell surface. These findings suggested that an HLA class Ia leader must be present in the cells for stable mature HLA-E protein to form and migrate to the cell surface.

We have directly confirmed the requirement for a leader peptide in vitro, showing that it is required to stabilize HLA-E in solution [24]. Recombinant HLA-E and β₂-microglobulin protein were produced in Escherichia coli as insoluble inclusion bodies. These proteins were then denatured in urea, and their ability to refold into a stable complex of the correct conformation was assayed. Using such an approach, we were able to show that the formation of a stable complex was energetically favourable in the presence, but not in the absence, of synthetic peptide corresponding to the HLA class Ia leader peptide [24]. Similarly, a modification of the class I assembly assay [25] has been used to demonstrate that newly synthesized HLA-E protein can only mature and form a correctly folded complex in the presence of the class Ia leader peptide [26]. Newly synthesized protein was metabolically labelled with $[^35]S$ methionine in 721.221 cells, and the antibody w6/32, which binds only to correctly folded class I complex, was used to immunoprecipitate correctly folded material. Using this approach it was shown that the class I leader peptide sequence

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could stabilize the formation of mature, correctly folded HLA-E complex. However, mutations at any of the positions of the nonamer peptide away from the canonical class I leader peptide sequence significantly reduced the binding of the peptide to HLA-E. The HLA-E leader sequence is slightly shorter than the other class I leader sequences and does not bind HLA-E itself.

The molecule TAP (transporter associated with antigen processing) is an ATP-binding cassette (ABC) transporter protein which translocates short peptides from the cytoplasm to the endoplasmic reticulum [27,28]. Efficient loading of Qa-1b and HLA-E with mouse or human class Ia leader sequence peptide respectively has only been observed in cells with a functioning TAP [21,23,29]. This suggests that the leader peptide enters the cytoplasm after it is cleaved from the mature protein by the signal peptidase (Figure 1). From the cytoplasm it is presumably transported by TAP into the endoplasmic reticulum, where it can interact with newly synthesized Qa-1b or HLA-E. This is not entirely expected, as leader peptides are inserted into the membrane of the endoplasmic reticulum during protein synthesis, and leader peptides from a range of cellular proteins can be eluted from class Ia molecules, even in the antigen processing mutant cell line T2 [30]. However, although leader sequences can enter the endoplasmic reticulum directly following cleavage of the leader sequence [30], this pathway may be relatively inefficient and does not result in significant levels of peptide-loaded Qa-1b or HLA-E able to migrate to the cell surface.

**CLASS Ia LEADER PEPTIDES**

A standard leader sequence is conserved throughout the class I molecules, and is of the form MAVMARTVLLSALALTQTWA. Using synthetic peptides, it has been shown that it is the peptide VMAPRTVLL derived from this leader sequence which binds HLA-E both in vitro in refolding assays and in cells using a modified assembly assay [24,26]. This portion of the leader sequence is highly conserved, with only minor variations occurring at positions 2, 7 and 8 [2]. The only functionally significant polymorphism in this peptide is the presence of a threonine at position 2 of the nonamer peptide combination. The only functional polymorphism which can stabilize the formation of mature, correctly folded HLA-E complex. However, mutations at any of the positions of the nonamer peptide away from the canonical class I leader peptide sequence significantly reduced the binding of the peptide to HLA-E. The HLA-E leader sequence is slightly shorter than the other class I leader sequences and does not bind HLA-E itself.

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IDENTIFICATION OF RECEPTORS FOR HLA-E

A number of observations suggested that HLA-E might be involved in immunological recognition. The genomic location of HLA-E in the MHC suggests an immunological role, and the homologous polymorphic MHC class Ia molecules, HLA-A, HLA-B and HLA-C, are known to be involved in T cell recognition. Furthermore, HLA-E was expressed at the cell surface and might be expected to interact with receptors on the surfaces of other cells. Recombinant protein technology was pivotal in identifying receptors for HLA-E.

We have recently developed a method for identifying antigen-specific T cells using tetramers of MHC class I molecules [31–33]. Recombinant HLA class I and β2-microglobulin protein made in E. coli can be refolded in the presence of synthetic peptide to produce a stable tertiary complex. It is possible to engineer on to the C-terminus of the HLA protein a tag which encodes a recognition sequence for the bacterial enzyme BirA. This enzyme specifically attaches a biotin molecule to a defined lysine residue within the tag. The protein avidin and its derivatives contain four biotin-binding sites, and so can bind four biotinylated HLA molecules. When avidin interacts with the biotinylated HLA complex the result is a tetramer containing four HLA molecules bound at the C-termini of the HLA proteins to an avidin core (Figure 2). The orientation of each recombinant HLA molecule with respect to the avidin is equivalent to the orientation of native HLA molecules with respect to the cell membrane. These tetramers bind with high avidity to cells which display the cognate T cell receptor for a given peptide/HLA molecule combination. If the avidin derivative is labelled with a fluorescent molecule such as phycoerythrin or fluorescein, these HMC tetramers can be used in flow cytometry to label and identify T cells displaying receptors for a specific class I MHC/peptide combination.

We applied a similar approach by producing tetramers of HLA-E to identify cells displaying receptors for HLA-E (Figure 2). Recombinant HLA-E was refolded around a synthetic peptide corresponding to the HLA class Ia leader sequence (VMAPRTVLL) in the presence of β2-microglobulin, as described [24]. The HLA-E protein was engineered to have the BirA recognition tag at its C-terminus and biotinylated using recombinant BirA [32]. Tetramers of HLA-E were used in a manner analogous to antibodies for flow cytometry of human
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Figure 2  Tetramer of HLA-E proteins
A structural model of the HLA-E tetramer is shown, based on the crystal structure of avidin, shown at the centre of the image, and the crystal structure of HLA-E, which we have solved. The avidin core can be labelled with a fluorochrome and has four biotin-binding sites, allowing it to bind to four biotinylated HLA-E molecules to produce a tetrameric complex. Tetramers of HLA-E protein had high enough avidity to allow them to be used for flow cytometry in a manner analogous to an antibody.

Peripheral blood leucocytes. The HLA-E tetramers bound to a significant population of peripheral blood leucocytes. Two-colour flow cytometry with phycoerythrin-labelled HLA-E tetramers and fluorescein- or tricolour-labelled antibodies demonstrated that the cells binding HLA-E expressed CD56 and CD16, both of which are markers for NK cells [19].

To identify the receptors, a panel of antibodies against known NK cell surface molecules was screened for antibodies with the ability to block the binding of the HLA-E tetramer to the NK cells. We showed that antibodies to the heterodimeric CD94/NKG2A receptors had this property. In addition, co-transfection of CD94 and NKG2 conveyed the ability to bind HLA-E tetramers to cells which did not otherwise bind these tetramers. The interaction of HLA-E with the CD94/NKG2A receptor produced an inhibitory signal in the NK cell which inhibited lysis of the cell displaying HLA-E by the NK cell. The HLA-E tetramers are made in E. coli and are not, therefore, glycosylated, which suggests that the CD94/NKG2A–HLA-E interaction is not dependent on glycosylation of HLA-E.

The finding that the CD94/NKG2 receptors can recognize HLA-E has been confirmed by the work of several other groups [34–37]. Although 721.221 cells do express HLA-E protein in the endoplasmic reticulum, they do not express HLA-A, HLA-B or HLA-C molecules, and so there is no significant expression of HLA-E at the cell surface. As 721.221 cells do not express HLA-E at the cell surface they are vulnerable to lysis by NK cells as a result of failure of recognition by CD94/NKG2A receptors. Borrego et al. [34] showed that, if these cells were incubated at 26 °C with synthetic peptide corresponding to the class I leader peptide (VMAPRTVLL from HLA-B0702 or HLA-B0801, or VMAPRTLIL from HLA-Cw0304, HLA-Cw0401 or HLA-Cw0801), then they became resistant to this lysis by NK cells. Similarly, if 721.221 cells were transformed with the class Ia molecule HLA-Cw0304 this also conferred protection from NK cells. Various antibodies were tested for their ability to block this protection from lysis by NK cells of 721.221 cells preincubated with leader peptide. Antibodies to the CD94/NKG2A receptors prevented this protection, confirming that this is the inhibitory receptor for HLA-E on NK cells.

A monoclonal antibody to HLA-E enabled Lee et al. [35] to establish that CD94/NKG2 molecules are receptors for HLA-E. Although normal 721.221 cells were vulnerable to NK cell-mediated lysis, 721.221 cells expressing the hybrid HLA-E construct with an HLA-A2 leader sequence were protected from this lysis. The protection from lysis was, therefore, associated with the cell surface expression of HLA-E promoted by the HLA-A2 leader peptide. This protection was abolished by anti-pan class I antibody, anti-HLA-E antibody or anti-CD94 antibody, and these observations are consistent with a CD94/NKG2–HLA-E interaction. In a rigorous study, Vales-Gomez et al. [37] have now demonstrated binding of soluble HLA-E to soluble CD94/NKG2 protein and measured the binding characteristics of this interaction. The binding of HLA-E to CD94/NKG2A has fast kinetics, and the affinity of the interaction was reduced if the bound peptide contained a threonine residue at position 2.

The role of Qa-1b appears to be similar to that of HLA-E. Tetramers of Qa-1b bind to a subpopulation of mouse NK cells independently of the Ly49 inhibitory receptors present on many mouse NK cells [38]. Qa-1b tetramers bind to mouse CD94/NKG2A receptors in both transfected cells and normal murine NK cells, and can mediate inhibition of lysis of vulnerable cells by the NK cells [39].

CD94/NKG2 RECEPTORS

This understanding of the role of HLA-E was consistent with earlier work from Lanier’s group on the CD94/NKG2 receptors [17,18]. These receptors consist of two C-lectin molecules which are disulphide-linked as heterodimers. CD94 can associate separately with NKG2A, NKG2B or NKG2C. These C-lectin molecules are all...
encoded on chromosome 12p12.3–p13.1, a region syntenic to that of mouse chromosome 6 encoding the Ly49 genes. This region of the genome encodes a number of NK cell surface molecules and has been termed the NK complex. CD94 does not have a proper cytoplasmic domain, but is required for the transport and membrane expression of NKG2 protein. There are several NKG2 proteins. NKG2A and its splice variant NKG2B possess an ITIM (immunoreceptor tyrosine inhibitory motif) in their cytoplasmic domains. Engagement of the CD94/NKG2A receptor by HLA-E induces recruitment of the phosphatase SHP-1 to tyrosine-phosphorylated NKG2A [40].

The dominant interaction of HLA-E with NK cells appears to result in inhibition of NK cell-dependent lysis, mediated by the inhibitory CD94/NKG2A receptors. However, we have also shown that CD94/NKG2C receptors, which activate NK cells [41], can also interact with HLA-E [19]. CD94/NKG2C receptors interact with DAP12, a surface molecule which contains an ITAM (immunoreceptor tyrosine-based activatory motif) [42]. DAP12 and NKG2C interact via charged residues in their transmembrane domains [42]. The functional significance of the interaction of HLA-E with these activatory receptors is currently unclear.

**STRUCTURAL STUDIES OF HLA-E**

If the appropriate leader peptide is absent, then HLA-E is degraded in the endoplasmic reticulum and does not reach the cell surface [23,29]. As human class I leader sequences are almost identical, HLA-E binding is highly specific for this effectively single peptide species, which is in stark contrast with the very promiscuous peptide binding of the classical HLA-A, -B and -C molecules. Alanine substitution studies suggested that each amino acid is required for optimal binding [26]. The evolution of a molecule with such specific binding to leader peptides of class I MHC molecules suggested that HLA-E plays a central immunological role. A key question was whether the peptide specificity of HLA-E resulted from intrinsic properties of HLA-E or from the specific delivery of peptide to HLA-E by a distinct processing pathway. To address these issues directly, we prepared recombinant HLA-E protein and crystallized it for structural studies [24].

Crystals of HLA-E in complex with β2-microglobulin and the HLA-B8 leader peptide (VMAPRTVLL) were formed over 4–6 months, and X-ray diffraction data were collected using synchrotron X-radiation at the European Synchrotron Research Facility at Grenoble, France (Figure 3). Overall, the structural framework of HLA-E is similar to that of the other human class I molecules that have been solved, such as HLA-A2 [43]. The peptide is bound in a groove with sides formed by two α-helices and a floor formed by a β-pleated sheet (Figure 3). Beneath this β-sheet, the α3 domain and the interaction with β2-microglobulin are conserved. However, the peptide-binding groove is highly specialized to provide an exquisitely specific binding site for the class I leader peptide. This contrasts with the highly promiscuous peptide-binding grooves of the classical HLA-A, HLA-B and HLA-C molecules.
MOLECULAR MECHANISMS OF PEPTIDE-BINDING SPECIFICITY IN HLA-E

The peptide-binding groove of HLA-E has similar dimensions to that of the promiscuous classical MHC class I molecule peptide-binding grooves, but its surface features are distinctive. The groove is highly hydrophobic, which favours binding of the hydrophobic leader peptide. This interaction buries a substantial hydrophobic surface (1333 Å²; 1 Å = 10⁻¹ nm) which, in energetic terms, is strongly favourable in the aqueous intracellular environment.

Along the peptide at each amino acid position there are structurally defined constraints on which amino acids can be tolerated. Pockets occur along the length of the groove and are well adapted for binding specific amino acids. Three deep hydrophobic pocket-like pockets at peptide positions 2, 7 and 9 bind methionine, valine and leucine respectively. There are also two shallow pockets at positions 3 and 6 which bind alanine and threonine respectively. A valine at position 1 and a leucine at position 8 point up out of the groove and form a stable interaction with the hydrophobic walls of the groove. This interaction with the hydrophobic walls minimizes the overall exposure of hydrophobic surface in an energetically favourable manner. In the mid-portion of the peptide a kink in the main chain lifts residues 4 and 5 up from the floor of the groove. The proline residue at position 4 stabilizes this kink, which makes a favourable entropic contribution to peptide binding. At position 5, the arginine side chain projects from the peptide over the α2 helix of HLA-E. From the crystal structure it appears that this arginine residue is able to form a salt-bridge interaction with a glutamic acid residue at position 152 of HLA-E.

At the C-terminal end of the peptide there are three highly hydrophobic aliphatic residues, and the groove is especially suitable for binding to such residues. The floor of the groove forms a stable hydrophobic stack, with a number of aromatic residues aligned with their planar faces in a parallel fashion (Figure 3). Underneath the N-terminal half of the peptide, two histidine residues form a hydrogen bond between their imidazole groups (Figure 3). Because the pKₐ of histidine is close to neutrality, this hydrogen bond will be easily disrupted by changes in the local environment. Such changes could occur during protein folding if an unfavourable amino acid residue was present in a potential ligand peptide. Disruption of this bond could interfere with the stable conformation of the floor of the groove, making the molecule unstable and vulnerable to degradation in the endoplasmic reticulum.

As well as these features, a number of hydrogen bonds are formed along the length of the peptide between residues in the groove and atoms of the peptide main chain. The requirement for specific interactions with pockets or surface features along the length of the peptide, coupled with the requirement for the peptide main chain to be in the correct conformation to form these hydrogen bonds, imposes very tight constraints on the combination of residues that is capable of binding to and stabilizing HLA-E. Overall, the crystal structure of HLA-E demonstrates that leader peptide specificity is a highly developed intrinsic property of HLA-E.

Although the structure of CD94 homodimers has been solved [44], the exact nature of the interaction between the CD94/NKG2 receptor and HLA-E is not known. If the binding interaction is similar to that of the T cell receptor with type I MHC molecules, then recent data on the fine specificity of peptide binding discussed above may have a straightforward structural explanation [36,37,45]. The substitution of the small alanine residue at position 3 with a charged glutamic acid residue will prevent that residue interacting with the small D-pocket and could interact with the arginine at position 5, possibly interfering with the receptor interaction. Similarly, substitution of the threonine at position 6 with an alanine would remove a polar hydroxy group from this portion of the interface. In addition, the alanine methyl group could form a close interaction with the C-pocket, favouring some movement of the main chain towards this pocket. In previous studies of T cell recognition, we have shown that, in structures of class I molecules with different peptides, subtle changes in the class I complex can induce substantial changes in cellular responses mediated by T cell receptors [46].

CONCLUSIONS – FUNCTIONAL AND DISEASE IMPLICATIONS OF CELL SURFACE EXPRESSION OF HLA-E

The model that we propose for NK recognition via CD94/NKG2 receptors is as follows (Figure 4). The specificity of the HLA-E binding groove allows it to function as a receptor in the endoplasmic reticulum for MHC class I signal peptides. In the absence of such peptides, HLA-E is degraded in the endoplasmic reticulum and is not present at the cell surface. NK cells will then receive no inhibitory signal from their CD94/NKG2A receptors and will lyse the cell. This lysis will occur in a tumour cell or in a virally infected cell if class I molecules have been down-regulated or if TAP has been inhibited, as can occur [47,48]. However, in a healthy cell, HLA-E binds signal peptides, reaches the surface and inhibits NK-cell-mediated lysis by interacting with CD94/NKG2A receptors. Thus HLA-E acts as a surrogate marker at the cell surface for the integrity of key intracellular processes essential to healthy antigen presentation, i.e. the expression of class I molecules and TAP function. If such a system is to operate reliably, it depends critically upon the specificity of HLA-E for the leader peptide, or otherwise any other similar peptide.
evading NK-cell-dependent lysis by circumventing HLA-E expression at the cell surface. It remains to be seen whether pathogens have evolved means of down-regulating HLA-E expression at the cell surface is a reliable marker for the availability of leader peptide available to stabilize HLA-E. The amount of HLA-E reaching the cell surface is, therefore, reduced, causing loss of the inhibitory signal to the NK cell. These changes may render virally infected or tumour cells vulnerable to NK-cell-mediated lysis.

 Figure 4  Biology of HLA-E

Synthesis of the classical HLA-A, HLA-B and HLA-C molecules produces leader peptide which enters the cytosol. These leader peptides are transported into the endoplasmic reticulum (ER) by the TAP transporter. Binding of these leader peptides to HLA-E stabilizes HLA-E, which then migrates to the cell surface. At the cell surface, HLA-E interacts with CD94/NKG2 receptors on NK cells, causing an inhibitory signal to be generated in the NK cell. This protects the cell displaying HLA-E from lysis by the NK cell. In virally infected or tumour cells, interference with the normal mechanisms of antigen processing, such as inhibition of HLA-A, HLA-B and HLA-E synthesis or inhibition of TAP transporter activity, reduces the supply of leader peptide available to stabilize HLA-E. The amount of HLA-E reaching the cell surface is, therefore, reduced, causing loss of the inhibitory signal to the NK cell. These changes may render virally infected or tumour cells vulnerable to NK-cell-mediated lysis.

generated in the cell might promote the surface expression of HLA-E.

Structural studies of HLA-E show that it is indeed highly evolved to bind specifically to the class I leader peptide, and the molecular basis for this specificity is apparent from the crystal structure of HLA-E. The result of this specificity is that the presence of HLA-E at the cell surface is a reliable marker for the availability of the leader peptide in the endoplasmic reticulum. Tumour and virally infected cells can down-regulate the expression of class I molecules [47] and viruses can inhibit TAP function [48]. Such interference with the normal antigen-processing pathways would interfere with T cell recognition of the cell, and would render the cell vulnerable to NK-cell-mediated lysis by down-regulating HLA-E expression at the cell surface. It remains to be seen whether pathogens have evolved means of evading NK-cell-dependent lysis by circumventing HLA-E recognition. It will be of great interest to determine the precise function of the other non-classical HLA molecules and of various novel C-lectin receptors, which are currently under investigation.

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