Alternative antigen processing and presentation pathways by tumors

Cunha, Oliveira C. da

6

THE OTHER JANUS FACE OF Qa-1 AND HLA-E: DIVERSE PEPTIDE REPERTOIRES IN TIMES OF STRESS

Thorbald van Hall[1], Cláudia C. Oliveira[1,3], Simone A. Joosten[2] and Tom H. M. Ottenhoff[2]

[1]Department of Clinical Oncology
and [2]Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands;
[3]Graduate Program in Areas of Basic and Applied Biology, Porto, Portugal.

Published in:
Microbes and Infection 2010, 12(12-13): 910-918.

| ABSTRACT

The non-polymorphic MHC molecule Qa-1 and its human counterpart HLA-E present monomorphic signal peptides to innate receptors and thereby regulate lymphocyte activity. Under stress, this peptide content is replaced with a surprisingly diverse repertoire of novel peptides that are associated with heat shock proteins, infectious agents or antigen processing defects.

| INTRODUCTION

MHC class I molecules serve to pickup and present intracellular antigens to circulating lymphocytes, which recognize these target structures after physical interaction with their T cell receptors (TCR). The immune system is thereby enabled to recognize and remove pathogens and transformed cells from the body. MHC class I molecules can be divided into classical Ia and non-classical Ib. The most striking feature of MHC class Ib molecules is their highly conserved nature. Whereas MHC class Ia genes display extensive polymorphism among their family members, the prototypic mouse MHC class Ib Qa-1 and its direct human homolog HLA-E are essentially nonpolymorphic. In this review we will refer to Qa-1 and HLA-E molecules as Qa-1/HLA-E. It is of interest that the Qa-1/HLA-E bound peptide content is normally highly restricted, comprising mostly of signal peptides derived from MHC Ia proteins. The resulting non variable peptide/MHC-Ib complexes constitute a ligand for the germ line encoded receptors CD94/NKG2. This function of Qa-1/HLA-E molecules is well established. However, in times of cellular stress, Qa-1/HLA-E shows its other face, like the Roman god Janus, typically portrayed with two faces in opposite directions (figure 1). This alternative face of Qa-1/HLA-E is associated with the replacement of bound signal peptides by a novel much more diverse repertoire of peptides, which can be sensed by αβ TCRs. We have recently described two such novel peptide repertoires that were presented by Qa-1/HLA-E molecules to specific $CD8^+$ T cells, which were related to sensing intracellular infection with mycobacteria, or to antigen processing defects in tumors[1,2]. These two situations of intracellular stress illustrate that Qa-1/HLA-E probably serve a much broader function in adaptive immunity than thus far anticipated.

Figure 1. The Roman god Janus is always portrayed with two faces in opposite directions. Thereby, he represented an intermediary between two worlds. Qa-1/HLA-E molecules have an important function in innate immunity through interacting with innate, invariable CD94/NKG2 receptors. In addition, these conserved MHC class I molecules have an novel function in adaptive immunity since they can present diverse intracellular stress related peptide repertoires to hypervariable T cell receptors.

| Qa-1/HLA-E NORMALLY PRESENT MONOMORPHIC PEPTIDES IN THEIR BINDING GROOVES

The mouse Qa-1 protein is a nonclassical (class Ib) MHC molecule, encoded by the T23 locus and constitutes the mouse homolog of HLA-E. Like its human counterpart, Qa-1 is virtually nonpolymorphic, as sequencing of multiple mouse strains and even outbred mice revealed only two dominant allelic variants: Qa-1^{a} and Qa-1^{b} [3]. Strikingly, while human HLA-E and mouse Qa-1 share 73% homology at the protein level, their function and the non-variable peptides that they bind are surprisingly similar[4, 5]. Both MHC class Ib molecules accommodate signal peptides in their hydrophobic peptide binding groove, mostly derived from the leader sequences of classical MHC class Ia molecules. In the mouse, this peptide comprises the amino acid sequence AMAPRTLLL and has been called Qdm for 'Qa-1-determinant-modifier', since this factor had a strong influence on Qa-1 stability. It was a surprise-discovery that the Qa-1 bound signal peptide appeared to be encoded within the MHC I region[6] and was derived from the classical class Ia molecules H-2D and H-2L. The other MHC class Ia protein in the mouse, H-2K, does not encode Qdm signal peptides and thus does not contribute to stabilization of Qa-1. Peptide elution studies of Qa-1 revealed that this Qdm peptide represented up to 70% of the total peptide content of Qa-1[7]. We recently confirmed the dominance of this one particular peptide using state-of-the-art mass-spectrometry[2]. Although we also found other peptide sequences in Qa-1, these represented only minor amounts compared to Qdm. Not surprisingly, the AMAPRTLLL peptide seems to have an amino acid sequence optimal for binding and stabilization in the hydrophobic groove of Qa-1. Changes in amino acid residues in this 9-mer peptide invariably lead to decreased binding affinity[8, 9]. Compared to classical peptide/MHC complexes, though, the Qdm/Qa-1 complex is relatively unstable at the cell surface, and its expression therefore probably reflects the actual Qdm availability in the cell[10].

As mentioned, humans share a nearly identical Qa-1 like MHC-Ib molecule, HLA-E. Only one single amino acid difference has been identified between the two HLA-E subtypes; this coding variation is located at position 107 (HLA-E*0101 has an arginine and E*0103 carries a glycine) on the loop between β-strands in the α2 domain of the heavy chain, outside the peptide binding groove[11, 12]. Both variants are indistinguishable in their structure and peptide binding features, although HLA-E^{G} homozygous cells seem to express higher levels of HLA-E at the cell surface[5, 12, 13]. The frequency of both variants is equal amongst different populations suggesting balanced selection at the population level[14]. HLA-E molecules are filled with peptides with striking similarity to the mouse Qdm: some classical HLA class Ia proteins comprise signal peptides with the sequence VMAPRTLLL, differing at only one amino acid position from Qdm. Due to the extensive repertoire of human HLA class I, which comprises more than hundred allelic variants, the exact sequence of the human Qdm counterpart differs somewhat between different HLA class Ia backgrounds, especially at positions 2, 7 and 8 (Table I). Most of these signal peptides indeed efficiently

Table I. Qdm peptide sequences from HLA class I molecules

Locus	sequence	examples of HLA types	HLA-E binding
HLA-A	VMAPRTLLL	A*01, A*03	yes
	VMAPRTLVL	A*02, A*24	yes
HLA-B	VMAPRTVLL	B*07, B*08	yes
	VTAPRTLLL	B*13, B*27	no
HLA-C	VMAPRTLLL	Cw*02, Cw*15	yes
	VMAPRTLIL	Cw*03, Cw*04[a]	yes
HLA-E	MVDGTLLL	E*01	no
HLA-F	MAPRSLLL	F*01	no
HLA-G	VMAPRTLFL	G*01	yes

[a] except Cw*0402 subtype

bind to HLA-E, implying that they all support cell surface display of HLA-E. Exceptions are Qdm-like peptides from some HLA-B alleles, which carry a threonine amino acid at position 2, and HLA-E itself, which is too short and, in contrast to HLA-Ia molecules, does not contain the Qdm motif (Table I). A direct consequence is that HLA-E can not support its own stabilization at the cell surface, but is dependent on the *de novo* production of classical HLA Ia proteins[15]. All individuals in the human population carry at least one allele that provides a HLA-E-binding Qdm peptide. Interestingly, the leader sequence of the class Ib molecule HLA-G, which shows a highly restricted expression in placenta tissue, is one of the most optimal binding peptides[16]. HLA-G and HLA-E are amongst the few HLA molecules expressed in human throphoblasts, which invades the maternal part (the decidua) of the placenta[17]. Increased regulatory T cell numbers are observed in the placenta, mostly at this site of feto-maternal contact, maybe as a result of HLA-E. In any case, expression of HLA-G and HLA-E is associated with immunosuppression, needed to prevent rejection of the immunologically 'foreign' fetus[17].

Another interesting aspect of the variations of Qdm-like peptides in the human population is the finding that these peptides behave as minor histocompatibility antigens (mHAGs). In mixed lymphocyte cultures, in which alloreactive T cell responses are induced, HLA-E restricted T cells can be detected that are specific for a 'non-self' Qdm peptide variant[18-20]. These T cells might have been induced by viruses, as CMV has been shown to encode exactly the same Qdm-like peptide variants[5].

| Qa-1/HLA-E AS REMOTE SENSORS OF THE INTRACELLULAR ANTIGEN PROCESSING MACHINERY

Qdm-like peptides are encoded by the N-terminal regions of MHC I molecules, which serve as hydrophilic segments to co-translationally target the newly synthesized proteins to the ER. Once in the ER, the hydrophilic N-terminus is released from the class I proteins by the dedicated enzyme signal peptidase (SPase)[21]. The native class I protein is further

chaperoned by calnexin and incorporated into a protein complex responsible for peptide loading, the so-called peptide loading complex (PLC)[22]. The chopped signal peptide of MHC I molecules, which is in most cases 24 amino acids long, remains in the ER membrane due to its hydrophobicity and needs liberation from this location by a second cleavage mediated by the signal peptide peptidase (SPPase), an aspartic protease, which acts within the ER membrane[23]. This SPPase mediated cleavage results in release of the Qdm-containing part of the leader into the cytosol[21]. This cytosolic part is not just degraded, but gets a second life as peptide ligand of Qa-1/HLA-E. For this loading process, the precursor peptide is further sculpted by the proteasome to make it suitable for transport back into the ER by the peptide transporter TAP[24]. In the ER, the PLC facilitates loading of the signal peptide into the groove of Qa-1/HLA-E, followed by transport to the cell surface[15, 25, 26]. This complicated intracellular processing pathway of MHC class I leader peptides thus implicates a large set of proteins that is necessary for proper display of the class Ia-derived peptides in Qa-1/HLA-E: classical HLA Ia molecules, SPase, SPPase, proteasome, TAP and tapasin (figure 2). Deficiencies in each of these enzymes and chaperones have been demonstrated to result in failure to load and bind Qdm-like peptides. In other words, if one of these components is absent or disabled, the Qdm peptides are not presented anymore at the cell surface by Qa-1/HLA-E. This way, the Qa-1/HLA-E system elegantly reveals the integrity of the whole MHC I antigen processing pathway and is therefore often called 'remote sensors of proper class I presentation'. Malfunctioning cells, in cases of stress,

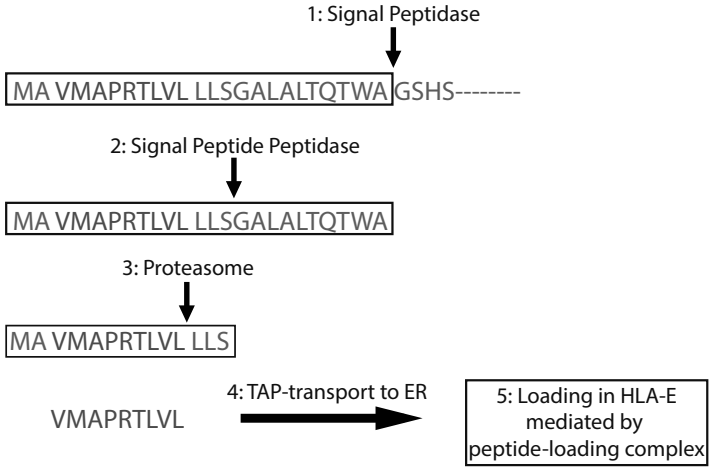

Figure 2. The processing requirements for proper presentation of Qdm peptides by Qa-1/HLA-E. The signal sequence of HLA-A2 molecules is taken as an example in this figure. The liberation of the Qdm peptide from its protein context needs at least three distinct proteolytic enzymes: signal peptidase (1) to remove the leader from HLA-A2 proteins, the signal peptide peptidase (2) to release the membrane associated peptide from the ER membrane and the proteasome (3) to generate the right C-terminus. It is unknown if the two N-terminal amino acids are removed by the proteasome or by aminopeptidases in the ER. The peptide loading complex involves at least tapasin, ERp57 and calreticulin.

infection or transformation, are thus marked for detection by immune cells, due to the lack of Qdm presentation. Intracellular pathogens are, however, able to sabotage this system and escape immune detection. Intriguingly, processing defects can lead to replacement of Qdm-like peptides with an alternative, surprisingly broad repertoire of peptides[2], supporting earlier indications that the binding motif of Qa-1/HLA-E molecules is much less stringent as generally appreciated[27]. This novel repertoire is a clear witness of the other face of Qa-1, which will be discussed later.

| PRESENTATION OF Qdm PEPTIDES BY Qa-1/HLA-E IS SENSED BY CD94/NKG2 RECEPTORS

The importance of Qa-1/HLA-E is illustrated by the fact that Qdm-like peptides have been broadly determined across mammalian species, including mouse, rat, dog, cat, cow, gorilla, chimpanzee and human[28]. The sequences of all these peptides are strikingly conserved and actually all efficiently bind HLA-E. The identification of the cognate receptor for this non-variable Qdm/MHC-Ib complex was a breakthrough and strongly boosted the unraveling of biological functions of this receptor-ligand pair. Tetramerized peptide/HLA-E complexes revealed the heterodimeric CD94/NKG2 surface molecules as the specific and selective receptors for HLA-E when filled with Qdm-like peptide[29,30]. These receptors are invariant transmembrane molecules that are expressed by approximately half of all natural killer (NK) cells and activated $CD8^+$ T cells[31]. The CD94 subunit makes most contact with peptide residues and the upward pointing alpha helices of HLA-E[32-34]. The NKG2 subunit hardly interacts with peptide residues, but contains the signal transduction domain[32-34]. NKG2 receptor genes are clustered in the NK-complex locus, and come in different flavors: NKG2A, NKG2C, and NKG2E. These three variants differ from each other only at few amino acids, which determine their affinity for CD94 and whether the specific CD94/NGK2 heterodimer acts as an inhibitory or activating receptor. NKG2C and NKG2E are less frequently expressed on lymphocytes, lack an ITIM motif in their cytoplasmic domains and associate with the activating adaptor molecule DAP12, whereas the inhibitory ITIM-bearing NKG2A recruits phosphatases, like SHP-1 to the signal transducing synapse[5,31]. The overall structures of the inhibiting and activating receptors are quite similar and their 'footprint' on the peptide/HLA-E complex is also conserved[32-34]. However, the binding affinity of the activating receptors is considerably lower than that of the inhibitory ones.

Reactivity of NK cells is governed by the balance between incoming activating and inhibiting signals from surface receptors; the CD94/NKG2A receptor behaves like a strong silencer on NK cells. We have to keep in mind that the non-variable Qdm/HLA-E molecules are widely expressed by most nucleated cells of the body, so that NK cells, which constitute approximately 10% of lymphocytes in the blood continuously sense the presence of HLA-E. NK cells have to overcome this input of inhibitory signals by ligation of activating receptors. On the other hand, the absence of this Qdm/HLA-E complex on

target cells increases their susceptibility to killing. Hence, CD94/NKG2A enables NK cells to sense the absence of 'self' MHC on target cells in an indirect way and behaves like a remote sensor for NK cells to select target cells for lysis[31]. The discovery of such HLA-interacting receptors forms the molecular basis of the 'missing self' hypothesis that was postulated by Ljunggren and Kärre in the 1980's[35], which formulated that NK cells exhibit preference for tumor cells with low MHC class Ia expression. More recently, the important role of Qa-1 to protect normal cells from NK mediated lysis was illustrated by using genetically modified mice completely lacking Qa-1, or expressing Qa-1 molecules that failed to interact with CD94/NKG2A receptors[36]. T lymphocytes from mice carrying an aberrant Qa-1 molecule failed to expand *in vivo* and were very sensitive for NK attack. Depletion of NK cells completely restored the clonal expansion upon antigen encounter[36]. Furthermore, the Qdm peptide could be exploited to protect adoptively transferred cells[37, 38]. Collectively, these studies indicate that immunosurveillance of NK cells is particularly efficient in the hematopoietic system and that sensing the Qdm presentation by Qa-1/HLA-E plays a key role in the regulating NK reactivity.

During the last decade it has become evident that also activating receptors contribute to the establishment of NK cell reactivity[31]. The activating partners of CD94 (NKG2C and NKG2E) engage the same Qdm/HLA-E complex as the inhibiting receptor NKG2A, although with lower affinity[39]. It is still puzzling how two receptors recognizing the same ligand can have opposing functions. Nevertheless, the increase of NKG2C positive NK cells after CMV infection[40] eludes to an adaptation of the NK repertoire favoring protection against infectious agents.

| CD94/NKG2A RECEPTORS ARE ALSO EXPRESSED BY LYMPHOCYTES IN ADAPTIVE IMMUNITY

In addition to being expressed on NK cells, CD94/NKG2A receptors are also expressed on $CD8^+$ T cells. Approximately 5% of circulating $CD8^+$ T cells express both CD94 and NKG2A. The expression of these NK receptors is found on conventional antigen-specific T cells with classically rearranged TCR$\alpha\beta$ chains, and it has created considerable confusion to designate these cells as a separate subpopulation called NKT cells. The currently emerging picture instead is that this subpopulation of $CD8^+$ T cells belongs to the antigen-experienced memory pool, and that the CD94/NKG2A receptors are induced by activation at T cell priming or by the cytokines IL-12, IL-15 or TGFβ[31]. These receptors might even directly promote the formation of memory cells, since CD94/NKG2A positive T cells seem to be less prone to activation-induced cell death[41]. Viruses are again likely involved in inducing CD94/NKG2A receptor expression on T cells: examples include the strong increase of this receptor on antigen-specific T cells after polyoma virus infection in a mouse model; the regulating effect of CD94/NKG2A on the cytolytic function of T cells in a Herpes Simplex virus model; and the steep increase of T cell subpopulations carrying this receptor after CMV seroconversion in

transplant patients[42-44]. In general, the frequencies of CD94/NKG2A expressing T cells are much higher than those expressing other NK-related receptors, e.g. NK1.1, DX5, Ly49 and KIR. Considering the large pool of T cells that expresses the Qdm/HLA-E-recognizing receptor in a given individual, these receptors might be regarded in fact as 'T cell associated receptors' instead of 'NK receptors'. Anyhow, the prevailing conclusion seems that expression of CD94/NKG2A on activated CD8+ T cells is common, and that QA-1/HLA-E detection by these adaptive lymphocytes might dampen overt cytotoxicity. Pathogens are apparently able to exploit this system to prevent total eradication.

| HCMV BRINGS ALONG ITS OWN Qdm PEPTIDE

Viruses and especially herpes viruses are able to inhibit MHC class I presentation as a strategy to hide from adaptive T cell immunity. For most herpes viruses, which induce live long persistent infections, this strategy is quite successful. The host is not capable of clearing herpes virus family members like CMV, EBV and HSV, and a high percentage of the human population is latently infected. In situations of immune suppression, such as post transplant patients and the elderly, herpes viruses can reactivate from latency into a productive phase and pathology. A multitude of dedicated viral proteins cooperate in this stealth technology[45]. The important bottleneck of the intracellular processing machinery is the peptide transporter TAP: several herpes virus proteins have been identified with a TAP-destabilizing function. TAP deficiency will directly block the presentation of Qdm-like peptides by HLA-E, since its loading is TAP dependent. This poses the question as to why do such infected cells in the body survive NK controlled immunosurveillance, since an effective shutdown of MHC class Ia presentation should sensitize these cells for NK attack. Part of the answer was provided by studying the HLA-E cell surface display on human CMV infected cells. Infection with CMV indeed led to the decrease of Qdm-like peptides in HLA-E, due to the action of immune evasion molecules that target the display of classical HLA. However, HLA-E cell surface presentation was retained by loading a peptide from one of the viral proteins, UL40[46, 47]. The signal peptide from UL40 contains an exact Qdm-like sequence and the supply of this viral peptide is able to substitute for the lack of natural leader peptides from classical MHC class Ia. Of course, loading of the UL40 leader peptide needed to be TAP-independent since this transporter is blocked by US6 in CMV infected cells[45]. Intriguingly, the flanking amino acids of the UL40 signal peptide differs from that of the class Ia and it is exactly this segment that specifies the SPPase cleavage site[48]. The final result is that the processed UL40 leader directly falls back in the lumen of the ER, instead of arriving in the cytosol where it would need to regain access to the ER via TAP. In this manner, CMV bypasses the normal HLA-E loading system by developing a shortcut which is necessary because of its own obstruction of the classical peptide presentation pathway. HCMV is not the only virus that brings along an own Qdm peptide, but this example clearly illustrates that viral pathogens have developed dedicated mechanisms to protect themselves for NK recognition via the HLA-E pathway.

| CELLULAR STRESS REMOVES Qdm PEPTIDES FROM Qa-1/HLA-E MOLECULES

Thus far, we discussed Qa-1/HLA-E molecules carrying signal peptides derived from classical MHC Ia molecules, which are the dominant constituents of the HLA-E binding groove. These Qdm-like peptides bind with relatively high affinity to HLA-E and render this molecule an essentially non-variable structure, in contrast to other MHC class I a/peptide molecules. On this side of the Janus face Qa-1/HLA-E looks and acts like an innate molecule with an important role in NK- and T-cell reactivity via interacting with the conserved receptors CD94/NKG2. However, in situations of cellular stress and induced expression of heat-shock proteins, HLA-E replaces its Qdm-like peptides by alternative peptides such as heat-shock protein-derived peptides[49, 50]. These peptides share some sequence homology with Qdm, but are clearly distinct. The most interesting aspect of this finding was that the CD94/NKG2A receptors failed to recognize HLA-E molecules binding an hsp60 peptide. As a direct consequence, the stressed cells were no longer protected from NK cell lysis[50]. The CD94/NKG2A receptor was indeed shown to directly interact with side residues of the Qdm peptide and changes at certain amino acid positions, especially p5 and p8, results in failure to bind Qdm/MHC complexes[8, 33, 34]. Thus, this NK receptor is truly Qdm peptide-specific. Interestingly, the crystal structure of a Qdm-specific alloreactive TCR showed an interface with the Qdm/HLA-E ligand that was quite similar to that of CD94/NKG2A[51]. In that sense, the heterodimer CD94/NKG2A shares unique characteristics with the peptide-sensing TCRαβ chains of T lymphocytes.

| Qa-1/HLA-E AS THE RECOGNITION ELEMENT FOR T CELL RECEPTORS: SELF PEPTIDES

The other side of the Janus face of Qa-1/HLA-E shows a MHC class I molecule presenting a surprisingly diverse repertoire of peptides. At first glance, the extensive variability of TCRs and the high specificity for their peptide targets do not associate well with the knowledge we have on the nonpolymorphic QA-1/HLA-E molecules. The fact that TCRs are able to recognize Qdm peptide variants in QA-1/HLA-E was evidently shown in alloreactive responses, as described above. Furthermore, one of the hsp60 peptides that can replace Qdm is also a target for TCRs[49, 52]. These Qa-1-restricted $CD8^+$ T cells cross-react to the *Salmonella* derived GroEl peptide, an example of molecular mimicry, and might explain autoimmune pathology in reactive arthritis[52]. Examples of other 'self' peptides presented by Qa-1/HLA-E are those that arise from TCR chains, multidrug resistance-associated protein and those that are associated with antigen processing defects[2, 53, 54]. The peptides that are derived from V-segments of TCRs are targeted by a population of $CD8^+$ regulatory T cells and mediate alleviation of autoimmune disorders via dampening the pathogenic CD4+ T cells[55].

Recently, we described a diverse repertoire of Qa-1-presented peptides that is associated with antigen processing defects[2, 56] As mentioned above, the presentation of Qdm is strictly dependent on the integrity of the antigen processing machinery (figure 2).

In cases were one of the processing components is downregulated or mutated, as found in a high frequency in tumor cells, Qdm does not emerge at the cell surface. Interestingly, the surface expression of Qa-1/HLA-E molecules is relatively preserved[10, 57, 58], most likely due to the replacement with an unique set of 'self' peptides. The novel peptides that replace Qdm in TAP-negative cells originated from normal proteins within the cell and constitute epitopes for $CD8^+$ T cells, since they represent neo-antigens that are normally not displayed by Qa-1[2, 56]. This T cell subpopulation was reactive against tumors with processing deficiencies like TAP defects, and our findings strongly suggested that the novel Qa-1 peptides are suitable for vaccination-based therapies, benefiting from the selective activation of the Qa-1-restricted effector $CD8^+$ T cells. Interestingly, multiple tumors of different MHC haplotypes were recognized by this T cell subset, due to the conserved nature of Qa-1. Moreover, tumors of different tissue origin were targets, because Qa-1 is expressed by most nucleated cells of the body. In contrast to what is sometimes thought, Qa-1/HLA-E proteins are not restricted to the hematopoietic system, but can be detected ubiquitously. Together with the organization of the Human Protein Atlas (www.proteinatlas.org), we charted all human tissues for HLA-E expression and observed an expression pattern quite similar to that of HLA class Ia molecules.

Importantly, the novel peptide repertoire that we determined in Qa-1 did not fulfill the binding motif as previously described for Qa-1 and HLA-E[8, 9, 59]. The major anchor binding pockets for the peptides, positions 2 and 9, are optimally accommodating leucine/methionine and aliphatic amino acids, respectively. This binding motif is not unique for Qa-1/HLA-E and is actually similar to the binding motif for the classical HLA-A2 allele[60, 61]. Our study seems to indicate that binding requirements of Qa-1/HLA-E are not so strict, as suggested before[27]. Together, these findings portrait Qa-1/HLA-E as a highly flexible MHC class I molecule with an important function to present a broad peptide repertoire to αβ T cells, completely different from the non-variable Qdm structure described above.

| HLA-E AS THE RECOGNITION ELEMENT FOR T CELL RECEPTORS: PATHOGEN-DERIVED PEPTIDES

The adaptive face of Qa-1/HLA-E is also revealed in studying infectious pathogens. Several pathogens have been reported to encode HLA-E binding peptides: *Salmonella, Listeria monocytogenes*, LCMV, HIV, HBV, EBV, HCV and *Mycobacterium tuberculosis* (*Mtb*)[4, 5]. This long list points at the existence of a subset of $CD8^+$ T cells that sense pathogen derived antigens in Qa-1/HLA-E molecules with their TCRs. How large this population of non-classically restricted cells is, remains elusive and in addition to Qa-1/HLA-E, also other non-classical presentation molecules have been associated with T cell recognition. The T cell repertoire in mice that lack classical MHC class Ia molecules suggests that approximately 10% of the $CD8^+$ pool is restricted by non-classical MHC class Ib molecules[62].

First indications that *Mtb* antigens are presented by the conserved HLA-E molecule date back to 2002[63]. The identity of the peptide-epitopes recognized by the human HLA-E-

restricted CD8+ T-cell clones, however, remained elusive. Recently, we studied CD8+ T cell recognition of peptides that contained *in silico* predicted HLA-E epitopes for recognition by human T cells[1]. Newly identified *Mtb* peptide epitopes were found by measuring proliferation of human CD8+ T-cells from PPD-responsive adults and BCG-vaccinated infants. The responding cells had cytotoxic activity, and lysed target cells in a peptide-specific and HLA-E dependent fashion, strongly suggesting peptide/HLA-E engagement by the TCR. Live mycobacterium infected monocytes were killed by these effector T cells, demonstrating proper antigen presentation by infected target cells. Strikingly, in addition to their cytolytic capacity, these *Mtb*-reactive T-cell lines also exhibited strong immunosuppressive properties, as they inhibited proliferation of unrelated responder T-cells. This suppression was dose-dependent, required cell-cell contact and was mediated, at least in part, by membrane bound TGFβ1 (mTGFβ1)[1]. T cell lines and clones derived from limiting dilution assays demonstrated that the dual activity could be, but was not necessarily mediated by the same HLA-E/peptide induced T cells. So, both types of HLA-E mediated immune reactivity might play a role during infection. The relative contribution of these two activities to resolution and persistence of the mycobacteria remains to be studied. We need to decipher if HLA-E mediated antigen presentation in tuberculosis preferentially activates T-cells with regulatory properties as compared to T-cells with cytolytic activity. Interesting in this context is the finding that mycobacteria reside in phagosomes of antigen presenting cells, especially macrophages, and that these phagosomes are enriched in HLA-E. This at least suggests a relatively large presentation of mycobacterial peptides on human HLA-E[64]. Recent mouse experiments have indicated that regulatory T cells are "bad guys" in tuberculosis when it comes to control of bacterial growth, especially early during infection[65-67]. This balance between effector and regulatory immunity in the context of HLA-E might allow partial clearance of pathogen from the host, thus providing sufficient levels of protection while avoiding excessive inflammation and pathology, but at the expense of pathogen persistence and chronic infection.

In addition to its potential contribution to immune suppression via regulatory T cells, HLA-E might offer unique opportunities to embark on vaccination strategies for tuberculosis, due to the low level of variation in HLA-E in human populations. Mtb vaccination strategies that focus on HLA-E might be beneficial especially in settings with highly prevalent co-infection with HIV. In Southern Africa alone approximately 70% of TB-patients are also HIV-infected. HIV infection down-regulates expression of classical HLA-A and -B molecules through its Nef proteins, thus decreasing antigen presentation capacities[68]. In contrast, however, HLA-E is resistant to HIV-nef-mediated down-regulation due to a single amino acid substitution in the HLA-E cytoplasmic tail[69]. Thus, while HIV might inhibit antigen presentation by HLA class Ia molecules on monocytes and macrophages[70], HLA-E dependent antigen presentation is likely less affected by HIV co-infection. Thus, targeting *Mtb*-specific HLA-E-restricted immunity by vaccination may be a novel and advantageous approach.

| SUMMARY AND PROSPECTS

We have reviewed recent studies which demonstrate that Qa1/HLA-E molecules reveal different faces and phases in NK- and T-cell immunity, with important implications for immuno-surveillance and immune regulation: first, Qa1/HLA-E molecules interact in an 'innate' immune fashion, through one molecular face, with members of the heterodimeric CD94/NKG2 receptor family. The NKG2 member determines whether ligation leads to inhibiting (NKG2A) or stimulating (NKG2C) signals to NK- and T-cells. The second role of Qa1/HLA-E molecules has only more recently become appreciated, notably their capacity to present a highly variable array of antigenic peptides to T cell receptors on $CD8^+$ T cells. Under usual homeostatic conditions the peptide repertoire bound to Qa1/HLA-E is highly restricted, and largely confined to self MHC signal sequences. However, under cellular stress conditions such as malignant cell transformation or intracellular infection with chronic pathogens, a new peptide repertoire is loaded onto Qa1/HLA-E molecules and is presented to $CD8^+$ T cells with cytotoxic activity, able to kill tumor cells or infected cells. A brief overview of these different roles is summarized in Table II.

These new findings not only significantly extend the role of Qa1/HLA-E in NK and $CD8^+$ T cell surveillance, but due to the extremely limited genetic polymorphism of Qa1/HLA-E also offer interesting perspectives for vaccination with high affinity peptides in cancer and infectious disease. In both cases efforts should similarly evolve around identifying the relevant antigens, which potentially can be useful in therapeutic as well as prophylactic vaccination.

Clearly, more research is needed to dissect the role of Qa1/HLA-E bound peptides in cancer and infection. One question concerns their precise vaccine potential, that is the efficiency by which peptide-specific $CD8^+$ T cells are capable of detecting and eradicating MHC-class Ia downregulation on tumor cell escape variants. The potential adverse effects need to be assessed as well, such as induction of auto-immune like phenomena in the case that such peptides are also expressed during "normal" cellular stress during local inflammation.

Another key question is what functional roles Qa1/HLA-E peptide-specific $CD8^+$ T cells exactly play: next to CTL, it is clear that also Tregs are induced. What dictates the balance in immune effector vs. regulatory mechanism at this level? Are different $CD8^+$ T cell subsets involved with either unique CTL or Treg activity, or is dual functionality the common rule?

Table II. Peptides presented by Qa-1/HLA-E to innate and adaptive immune receptors

Immune receptor	peptide origin	self or non-self
CD94/NKG2	Qdm from self MHC Ia	self
	Qdm from HCMV	non-self
T cell receptor	Qdm from allo MHC Ia	non-self
	hsp60 and GroEl	self and non-self
	Vβ TCR	self
	associated with processing defects	induced self
	M. tuberculosis	non-self

How can we identify and manipulate these functional activities for therapeutic purposes? Clearly, the recent studies we have discussed here shed exciting new light on the potential new role of Qa1/HLA-E mediated antigen presentation in cancer and infection.

| ACKNOWLEDGMENTS

Dr. J van Bergen is acknowledged for critical reading the manuscript and Dr. I Doxiadis and Y Zoet for input on HLA sequences. Financial support was provided by FP7 NEWTBVAC (THMO), NWO-VENI (SAJ), Gisela Their foundation (SAJ), the Netherlands Leprosy Relief Foundation, the AICR (09-776, TvH) and Portuguese Foundation For Science and Technology (MCTES) Portugal (SFRH/BD/33539/2008 to CCO).

| REFERENCES

1. Joosten, S.A. 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* **6**, e1000782 (2010).
2. Oliveira, C.C. et al. The nonpolymorphic MHC Qa-1b mediates CD8+ T cell surveillance of antigen-processing defects. *J Exp Med* **207**, 207-21 (2010).
3. Hermel, E. et al. Polymorphism and conservation of the genes encoding Qa1 molecules. *Immunogenetics* **56**, 639-649 (2004).
4. Jensen, P.E., Sullivan, B.A., Reed-Loisel, L.M. & Weber, D.A. Qa-1, a nonclassical class I histocompatibility molecule with roles in innate and adaptive immunity. *Immunol. Res.* **29**, 81-92 (2004).
5. Rodgers, J.R. & Cook, R.G. MHC class Ib molecules bridge innate and acquired immunity. *Nat Rev Immunol* **5**, 459-471 (2005).
6. Aldrich, C.J. et al. Identification of a Tap-dependent leader peptide recognized by alloreactive T cells specific for a class Ib antigen. *Cell* **79**, 649-658 (1994).
7. DeCloux, A., Woods, A.S., Cotter, R.J., Soloski, M.J. & Forman, J. Dominance of a single peptide bound to the class Ib molecule, Qa-1b. *J. Immunol.* **158**, 2183-2191 (1997).
8. Kraft, J.R. et al. Analysis of Qa-1(b) peptide binding specificity and the capacity of CD94/NKG2A to discriminate between Qa-1-peptide complexes. *J. Exp. Med.* **192**, 613-624 (2000).
9. Miller, J.D. et al. Analysis of HLA-E peptide-binding specificity and contact residues in bound peptide required for recognition by CD94/NKG2. *J Immunol* **171**, 1369-1375. (2003).
10. Kambayashi, T. et al. The nonclassical MHC class I molecule Qa-1 forms unstable peptide complexes. *J. Immunol.* **172**, 1661-9 (2004).
11. Grimsley, C. et al. Definitive high resolution typing of HLA-E allelic polymorphisms: identifying potential error in existing allele data. *Tissue Anigens* **60**, 206-212 (2002).
12. Strong, R.K. et al. HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. *J. Biol. Chem.* **278**, 5082-90 (2003).
13. O'Callaghan, C.A. et al. Structural features impose tight peptide binding specificity in the nonclassical MHC molecule HLA-E. *Mol. Cell* **1**, 531-541 (1998).
14. Grimsley, C. & Ober, C. Population genetic studies of HLA-E: evidence for selection. *Hum Immunol* **52**, 33-40 (1997).
15. Braud, V.M., Allan, D.S.J., Wilson, D. & McMichael, A.J. TAP- and tapasin-dependent HLA-E surface expression correlates with the binding of an MHC class I leader peptide. *Curr. Biol.* **8**, 1-10 (1997).
16. Lee, N., Goodlett, D.R., Ishitani, A., Marquardt, H. & Geraghty, D.E. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. *J. Immunol.* **160**, 4951-4960 (1998).
17. Hunt, J.S. Stranger in a strange land. *Immunol Rev* **213**, 36-47 (2006).
18. Pietra, G. et al. HLA-E-restricted recognition of cytomegalovirus-derived peptides by human CD8+ cytolytic T lymphocytes. *Proc Natl Acad Sci U S A* **100**, 10896-901 (2003).
19. Salerno-Goncalves, R., Fernandez-Vina, M., Lewinsohn, D.M. & Sztein, M.B. 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* **173**, 5852-62 (2004).
20. Mazzarino, P. 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* **35**, 3240-7 (2005).
21. Martoglio, B. & Dobbertein, B. Signal sequences: more than just greasy peptides. *trends in Cell Biol.* **8**, 410-415 (1998).
22. Cresswell, P., Ackerman, A.L., Giodini, A., Peaper, D.R. & Wearsch, P.A. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol Rev* **207**, 145-57 (2005).
23. Lemberg, M.K., Bland, F.A., Weihofen, A., Braud, V.M. & Martoglio, B. Intramembrane proteolysis of signal peptides: an essential step in the generation of HLA-E epitopes. *J. Immunol.* **167**, 6441-6 (2001).
24. Bland, F.A., Lemberg, M.K., McMichael, A.J., Martoglio, B. & Braud, V.M. Requirement of the proteasome for the trimming of signal peptide-derived epitopes presented by the nonclassical major histocompatibility complex class I molecule HLA-E. *J. Biol. Chem.* **278**, 33747-52 (2003).
25. Bai, A., Broen, J. & Forman, J. The pathway for processing leader-derived peptides that regulate the maturation and expression of Qa-1. *Immunity* **9**, 413-421 (1998).
26. Li, L. et al. Differential requirement for tapasin in the presentation of leader- and insulin-derived peptide antigens to Qa-1b-restricted CTLs. *J. Immunol.* **173**, 3707-15 (2004).
27. Stevens, J., Joly, E., Trowsdale, J. & Butcher, G.W. Peptide binding characteristics of the non-classical class Ib MHC molecule HLA-E assessed by a recombinant random peptide approach. *BMC Immunol* **2**, 5 (2001).
28. Kurepa, Z., Hasemann, C.A. & Forman, J. Qa-1(b) binds conserved class I leader peptides derived from several mammalian species. *J. Exp. Med.* **188**, 973-978 (1998).
29. Braud, V.M. et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795-799 (1998).
30. Vance, R.E., Jamieson, A.M. & Raulet, D.H. Recognition of the class Ib molecule Qa-1b by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. *J. Exp. Med.* **190**, 1801-1812 (1999).
31. Lanier, L.L. NK cell recognition. *Annu. Rev. Immunol.* **23**, 225-274 (2005).
32. Sullivan, L.C. et al. The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition. *Immunity* **27**, 900-11 (2007).
33. Kaiser, B.K., Pizarro, J.C., Kerns, J. & Strong, R.K. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc Natl Acad Sci U S A* **105**, 6696-701 (2008).
34. Petrie, E.J. et al. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J Exp Med* **205**, 725-735 (2008).
35. Ljunggren, H.G. & Karre, K. In search of the 'missing self'. MHC molecules and NK cell recognition. *Immunol. Today* **11**, 237-244 (1990).
36. Lu, L. et al. Regulation of activated CD4+ T cells by NK cells via the Qa-1-NKG2A inhibitory pathway. *Immunity* **26**, 593-604 (2007).
37. Colmenero, P. et al. Qa-1(b)-dependent modulation of dendritic cell and NK cell cross-talk in vivo. *J Immunol* **179**, 4608-15 (2007).
38. Persson, C.M., Assarsson, E., Vahlne, G., Brodin, P. & Chambers, B.J. Critical role of Qa1b in the protection of mature dendritic cells from NK cell-mediated killing. *Scand J Immunol* **67**, 30-6 (2008).
39. Vales-Gomez, M., Reyburn, H.T., Erskine, R.A., Lopez-Botet, M. & Strominger, J.L. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2A and the activating receptor CD94/NKG2C to HLA-E. *EMBO J.* **18**, 4250-4260 (1999).
40. Guma, M. et al. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* **107**, 3624-31 (2006).
41. Gunturi, A., Berg, R.E. & Forman, J. Preferential survival of CD8 T and NK cells expressing high levels of CD94. *J Immunol* **170**, 1737-45 (2003).
42. Moser, J.M., Gibbs, J., Jensen, P.E. & Lukacher, A.E. CD94-NKG2A receptors regulate antiviral CD8+ T cell responses. *Nature immunol.* **3**, 189-195 (2002).
43. Suvas, S., Azkur, A.K. & Rouse, B.T. Qa-1b and CD94-NKG2a interaction regulate cytolytic activity of herpes simplex virus-specific memory CD8+ T cells in the latently infected trigeminal ganglia. *J Immunol* **176**, 1703-11 (2006).
44. van Stijn, A. et al. Human cytomegalovirus infection induces a rapid and sustained change in the expression of NK cell receptors on CD8+ T cells. *J Immunol* **180**, 4550-60 (2008).
45. Griffin, B.D., Verweij, M.C. & Wiertz, E.J. Herpesviruses and immunity: the art of evasion. *Vet Microbiol* **143**, 89-100 (2010).
46. Tomasec, P. et al. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by

human cytomegalovirus gpUL40. *Science* **287**, 1031 (2000).

47. Wang, E.C. et al. UL40-mediated NK evasion during productive infection with human cytomegalovirus. *Proc Natl Acad Sci U S A* **99**, 7570-5 (2002).
48. Lemberg, M.K. & Martoglio, B. Requirements for signal peptide peptidase-catalyzed intramembrane proteolysis. *Mol. Cell* **10**, 735-744 (2002).
49. Davies, A. et al. A peptide from heat shock protein 60 is the dominant peptide bound to Qa-1 in the absence of the MHC class Ia leader sequence peptide Qdm. *J. Immunol.* **170**, 5027-33 (2003).
50. Michaelsson, J. et al. A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition. *J. Exp. Med.* **196**, 1403-14 (2002).
51. Hoare, H.L. et al. Structural basis for a major histocompatibility complex class Ib-restricted T cell response. *Nat Immunol* **7**, 256-264 (2006).
52. Lo, W.-F. et al. Molecular mimicry mediated by MHC class Ib molecules after infection with Gram-negative pathogens. *Nat. Medicine* **6**, 215-218 (2000).
53. Jiang, H. & Chess, L. The specific regulation of immune responses by CD8+ T cells restricted by the MHC class Ib molecule, Qa-1. *Annu. Rev. Immunol.* **18**, 185-216 (2000).
54. Wooden, S.L., Kalb, S.R., Cotter, R.J. & Soloski, M.J. HLA-E binds a peptide derived from the ATP-binding cassette transporter multidrug resistance-associated protein 7 and inhibits NK cell-mediated lysis. *J. Immunol.* **175**, 1383-1387 (2005).
55. Lu, L., Werneck, M.B.F. & Cantor, H. The immunoregulatory effects of Qa-1. *Immunol Rev* **212**, 51-59 (2006).
56. Van Hall, T. et al. Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. *Nat. Medicine* **12**, 417-424 (2006).
57. Furukawa, H. et al. Cell surface expression of HLA-E molecules on PBMC from a TAP1-deficient patient. *Tissue Antigens* **53**, 292-5 (1999).
58. Palmisano, G.L. et al. HLA-E surface expression is independent of the availability of HLA class I signal sequence-derived peptides in human tumor cell lines. *Hum. Immunol.* **66**, 1-12 (2005).
59. Braud, V., Jones, E.Y. & McMichael, A. The human major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. *Eur J Immunol* **27**, 1164-9 (1997).
60. Sensi, M. et al. Peptides with dual binding specificity for HLA-A2 and HLA-E are encoded by alternatively spliced isoforms of the antioxidant enzyme peroxiredoxin 5. *Int Immunol* **21**, 257-68 (2009).
61. Nattermann, J. et al. The HLA-A2 restricted T cell epitope HCV core 35-44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. *Am J Pathol* **166**, 443-53 (2005).
62. Perarnau, B. et al. Single H2Kb, H2Db and double H2KbDb knockout mice: peripheral CD8+ T cell repertoire and anti-lymphocyte choriomeningitis virus cytolytic responses. *Eur. J. Immunol.* **29**, 1243-1252 (1999).
63. Heinzel, A.S. et al. HLA-E-dependent presentation of Mtb-derived antigen to human CD8+ T cells. *J. Exp. Med.* **196**, 1473-1481 (2002).
64. Grotzke, J.E. et al. The Mycobacterium tuberculosis phagosome is a HLA-I processing competent organelle. *PLoS Pathog* **5**, e1000374 (2009).
65. Kursar, M. et al. Cutting Edge: Regulatory T cells prevent efficient clearance of Mycobacterium tuberculosis. *J Immunol* **178**, 2661-5 (2007).
66. Scott-Browne, J.P. et al. Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. *J Exp Med* **204**, 2159-69 (2007).
67. Shafiani, S., Tucker-Heard, G., Kariyone, A., Takatsu, K. & Urdahl, K.B. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J Exp Med* **207**, 1409-20 (2010).
68. Collins, K.L., Chen, B.K., Kalams, S.A., Walker, B.D. & Baltimore, D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* **391**, 397-401 (1998).
69. Cohen, G.B. et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* **10**, 661-71 (1999).
70. Noursadeghi, M., Katz, D.R. & Miller, R.F. HIV-1 infection of mononuclear phagocytic cells: the case for bacterial innate immune deficiency in AIDS. *Lancet Infect Dis* **6**, 794-804 (2006).