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A Structural Basis for Antigen Presentation by the MHC Class Ib Molecule, Qa-1b

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The primary function of the monomorphic MHC class Ib molecule Qa-1b is to present peptides derived from the leader sequences of other MHC class I molecules for recognition by the CD94-NKG2 receptors expressed by NK and T cells. Whereas the mode of peptide presentation by its ortholog HLA-E, and subsequent recognition by CD94-NKG2A, is known, the molecular basis of Qa-1b function is unclear. We have assessed the interaction between Qa-1b and CD94-NKG2A and shown that they interact with an affinity of 17 μM. Furthermore, we have determined the structure of Qa-1b bound to the leader sequence peptide, Qdm (AMAPRTLLL), to a resolution of 1.9 Å and compared it with that of HLA-E. The crystal structure provided a basis for understanding the restricted peptide repertoire of Qa-1b. Whereas the Qa-1b-AMAPRTLLL complex was similar to that of HLA-E, significant sequence and structural differences were observed between the respective Ag-binding clefts. However, the conformation of the Qdm peptide bound by Qa-1b was very similar to that of peptide bound to HLA-E. Although a number of conserved innate receptors can recognize heterologous ligands from other species, the structural differences between Qa-1b and HLA-E manifested in CD94-NKG2A ligand recognition being species specific despite similarities in peptide sequence and conformation. Collectively, our data illustrate the structural homology between Qa-1b and HLA-E and provide a structural basis for understanding peptide repertoire selection and the specificity of the interaction of Qa-1b with CD94-NKG2A receptors. The Journal of Immunology, 2012, 188: 302–310.

1L.Z. and L.C.S. contributed equally to this work.
2C.S.C. and A.G.B. are joint senior authors.

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The coordinates presented in this article have been submitted to the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) under accession number 3VJ6.

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Abbreviations used in this article: hCD94-NKG2A, human CD94-NKG2A; mCD94-NKG2A, murine CD94-NKG2A; PDB, Protein Data Bank; Qdm, Qa-1 determinant modifier; r.m.s.d., root mean square deviation; SPR, surface plasmon resonance.

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CD94-NKG2 receptors (17, 18). The interaction between HLA-E and CD94-NKG2A represents a central innate mechanism by which NK cells indirectly monitor the expression of other MHC class I molecules within a cell. Recently, the structure of the CD94-NKG2A-HLA-EVMAPRTLLL complex provided a basis for understanding the specificity of this interaction as well as the relative contribution of the CD94 and NKG2A subunits in recognizing the HLA-EVMAPRTLLL complex (19, 20). In addition, HLA-E can also present peptides from pathogens such as CMV and Mycobacterium tuberculosis and stimulate cytotoxic T cell responses (21, 22).

Whereas the mode of HLA-E–mediated Ag presentation and recognition by innate and adaptive immune receptors is well established, much less is known regarding the murine Qa-1b molecule. Early studies suggested that Qa-1b is widely expressed (23) and involved in both innate and adaptive immune stimulation by both αβ (24, 25) and γδ (26) T cells. Recognition of Qa-1b by alloreactive CTL was dependent on the presence of a H-2D–linked gene referred to as the Qa-1 determinant modifier (Qdm) (23). Qdm was subsequently identified as a nonamer peptide (AMAPRTLLL) derived from residues 3–11 of the leader peptide present in most H-2D proteins (27). Peptide elution studies subsequently demonstrated that Qdm was the dominant peptide associated with Qa-1b (28).

Like HLA-E, Qa-1b is recognized by NK cells, specifically by CD94-NKG2A (29), in addition to the activating receptors CD94-NKG2C and CD94-NKG2E (30). Whereas initial studies suggested that HLA-E and Qa-1b are the result of convergent evolution (31), more recent analysis has found that because individual domains of the MHC undergo different rates of evolutionary change, HLA-E and Qa-1b are orthologs (32). As Qa-1b acts in an analogous role to HLA-E, acting as a checkpoint for class I expression within a cell. Recently, the structure of the HLA-EVMAPRTLLL complex provided a basis for understanding the specificity of this interaction as well as the relative contribution of the CD94 and NKG2A subunits in recognizing the HLA-EVMAPRTLLL complex (19, 20). In addition, HLA-E can also present peptides from pathogens such as CMV and Mycobacterium tuberculosis and stimulate cytotoxic T cell responses (21, 22).

Materials and Methods

Cloning and expression of Qa-1b and murine CD94-NKG2A

RNA from the cell line RMA-S was reverse transcribed and used to amplify a cDNA fragment encoding the extracellular domain of Qa-1b (residues 1–280) that was then cloned into a pET-expression vector. The Qa-1b H chain and murine β2-microglobulin were expressed separately in Escherichia coli, refolded, and purified, essentially as described previously (33, 34). Following reverse transcription of RNA prepared from C57BL/6 splenocytes, cDNAs encoding residues 43–179 and 119–224 of CD94 and NKG2A, respectively, were generated by PCR and cloned into a pET-30–based expression vector. The extracellular domains of murine CD94 and NKG2A were expressed individually in E. coli from which inclusion bodies were isolated. These were resuspended in 8 M urea, 0.5 mM Na-EDTA, and 1 mM DTT, and CD94-NKG2A was refolded by flash dilution in a solution containing 5 M urea, 100 mM Tris-HCl (pH 8.0), 400 mM l-arginine-HCl, 5 mM reduced glutathione, and 0.5 mM oxidized glutathione. The refolding solution was then dialyzed against 1 M urea and 10 mM Tris-HCl (pH 8.0), followed by dialysis against 10 mM Tris-HCl (pH 8.0). The refolded CD94-NKG2A heterodimer was purified by ion exchange and size exclusion chromatography, followed by an additional anion–exchange chromatography step. HLA-E and human CD94-NKG2A (hCD94-NKG2A) were prepared, as described previously (20).

Surface plasmon resonance

The interaction between soluble murine and human CD94-NKG2A with Qa-1b or HLA-E was analyzed by surface plasmon resonance (SPR) using a Bio-Rad ProteOn XPR36 instrument (Hercules, CA). All experiments were performed at 25°C in a buffer containing 10 mM HEPES (pH 7.4), 150 mM NaCl, and 0.05% Tween 20 (HBS-T). hCD94-NKG2A and murine CD94-NKG2A (mCD94-NKG2A) were diluted into 10 mM sodium acetate (pH 5) and immobilized on adjacent flow cells of a GLC Sensorchip (Bio-Rad) by amine coupling (~300 response units each). An adjacent flow cell to which CD94-NKG2A was not added was activated and quenched in the same manner and served as a control cell. Recombinant Qa-1b and HLA-E were purified using size exclusion chromatography within 24 h of analysis, and the concentration of purified protein was estimated by measuring the absorbance at 280 nm. Qa-1b or HLA-E was then serially diluted in HBS-T (0.03–100 μM) and injected simultaneously over the test and control surfaces at a flow rate of 30 μl/min, and measurements were taken in duplicate. Following subtraction of data from control flow cells, the equilibrium K_D values were analyzed using ProteOn Manager software version 3.0.1 (Bio-Rad) and Prism (GraphPad).

Results

Qa-1b-AMAPRTLLL binds CD94-NKG2A with low affinity

Although the interaction between HLA-E and hCD94-NKG2A has been well characterized (19, 20, 42, 43), there has been no measurement of the affinity of the interaction between Qa-1b and mCD94-NKG2 receptors. Consequently, cDNAs encoding the extracellular domains of murine CD94 and NKG2A were cloned and expressed in E. coli, and the proteins refolded to generate soluble recombinant mCD94-NKG2A. Similarly, the extracellular domain of Qa-1b was also expressed in E. coli and refolded with β2-microglobulin and the Qdm peptide, and the interaction between refolded Qa-1b and mCD94-NKG2A was assessed by SPR. mCD94-NKG2A bound Qa-1b-AMAPRTLLL with rapid binding kinetics that precluded the accurate determination of both on and off rates, similar to the interaction between HLA-E and HLA-EVMAPRTLLL (Fig. 1) (43). Equilibrium-binding analyses yielded a K_D of ~17 μM. Given the capacity of both Qa-1b and HLA-E to bind peptides derived from the leader sequences of MHC-I molecules and the extensive sequence conservation between the mCD94-NKG2A and hCD94-NKG2A receptors, the extent to which Qa-1b and HLA-E could interact with either mCD94-NKG2A or hCD94-NKG2A was also assessed by SPR. hCD94-NKG2A bound HLA-EVMAPRTLLL with an affinity of ~5 μM, an affinity similar to previously published data (Fig. 1) (20). Comparable affinities were obtained with lower amounts of receptor immobilized (~65 response units; data not shown). Strikingly, there was no detectable interaction between hCD94-NKG2A and Qa-1b-AMAPRTLLL or between mCD94-NKG2A and HLA-EVMAPRTLLL (Fig. 1B, 1D).

Structural overview of Qa-1b

To address the structural basis of peptide binding to Qa-1b and the strict species specificity of the interactions between hCD94-NKG2A and Qa-1b-AMAPRTLLL, we carried out systematic alanine-substitution mutagenesis that precluded the accurate determination of both on and off rates, similar to the interaction between HLA-E and HLA-EVMAPRTLLL (Fig. 1) (43). Equilibrium-binding analyses yielded a K_D of ~17 μM. Given the capacity of both Qa-1b and HLA-E to bind peptides derived from the leader sequences of MHC-I molecules and the extensive sequence conservation between the mCD94-NKG2A and hCD94-NKG2A receptors, the extent to which Qa-1b and HLA-E could interact with either mCD94-NKG2A or hCD94-NKG2A was also assessed by SPR. hCD94-NKG2A bound HLA-EVMAPRTLLL with an affinity of ~5 μM, an affinity similar to previously published data (Fig. 1) (20). Comparable affinities were obtained with lower amounts of receptor immobilized (~65 response units; data not shown). Strikingly, there was no detectable interaction between hCD94-NKG2A and Qa-1b-AMAPRTLLL or between mCD94-NKG2A and HLA-EVMAPRTLLL (Fig. 1B, 1D).
NKG2A and mCD94-NKG2A with their MHC-Ib ligands, the crystal structure of Qa-1b in complex with the Qdm (AMAPRTLLL) peptide was determined. Crystals of the refolded Qa-1b-AMAPRTLLL complex formed in the space group P2_12_1 with unit cell dimensions of a = 59.3, b = 72.8, and c = 95.8 Å, and diffracted to a resolution of 1.9 Å. The asymmetric unit comprised one heterodimeric interface with 3 domain that forms the heterodimeric interface with 2 domains that form the peptide binding cleft and 1 domain colored dark blue, allowing for ready interpretation of the interactions between the peptide and the Qa-1b H chain (Fig. 2B).

The overall structure of Qa-1b is similar to that of other MHC-I peptide complexes (Fig. 2A). The H chain contains three domains, the α1 and α2 domains that form the peptide binding cleft and the α3 domain that forms the heterodimeric interface with β2-microglobulin. Sequence comparison of Qa-1b with the MHC-Ia molecules H-2Kβ (44) and H-2Dβ (45) and with the murine MHC-Ib molecules Qa-2 (46) and H2-M3 (47) shows significant similarities with sequence identities of 79, 76, 77, and 68%, respectively. The root mean square deviations (r.m.s.d.) upon superposition of Qa-1b and H2-Kβ, Qa-2, H2-M3, and H-2Dβ are 1.0, 1.1, 1.2, and 1.4 Å, respectively (over all main-chain atoms). The majority of the structural differences between Qa-1b and the other murine MHC-I involved side chains clustered around the peptide-binding cleft, highlighting that subtle changes in this region reflect differences in Ag presentation and recognition by receptors expressed primarily by NK cells and T cells.

Peptide-binding specificity

The Qdm peptide is bound in an extended conformation, tethered by an extensive network of hydrogen bonds (Fig. 3, Table II). Contacts between Qa-1b and Qdm include 14 direct hydrogen bonds distributed across the length of the peptide and 8 water-mediated hydrogen bonds centered on the 4 C-terminal peptide residues P6–P9 (Table II). All of the Qa-1b residues involved in hydrogen bonding to Qdm were also conserved across Qa-1c, Qa-1d, and thus it is anticipated the mode of peptide recognition is representative of at least four independent experiments.
binding will be shared across Qa-1 allotypes. With the exception of the P4-Pro and the P6-Thr, the main chain of each peptide residue is stabilized by a direct hydrogen bond with Qa-1β. For example, the amide group of P2-Met forms a hydrogen bond, which is conserved in most MHC-Ia structures, to the terminal carboxyl oxygen of Glu63, whereas Lys66 in Qa-1β forms a hydrogen bond to the P2-Met carbonyl group. The N terminus of the peptide is anchored by hydrogen bonds between the P1-Ala and the side-chain hydroxyl groups of Tyr7 and Tyr171 (Fig. 3, Table II).

In addition to the hydrogen bond tethering of the termini of the Qdm peptide, Qa-1β also has five, primarily hydrophobic, binding pockets that also serve to anchor the peptide. These pockets are conserved in all MHC class I–peptide structures (Figs. 3, 4A) (48). Similarly, the C terminus of P9-Leu is anchored by a network of hydrogen bonds to the side-chain hydroxy groups of Tyr34 and Ser143 and to the side-chain amino group of Lys146, as well as forming water-mediated hydrogen bonds to the side chains of Asn77 and Thr80 (Fig. 3, Table II).

In addition to the hydrogen bond tethering of the termini of the Qdm peptide, Qa-1β also has five, primarily hydrophobic, binding pockets that also serve to anchor the peptide. These pockets ac-

Table II. Peptide/Qa-1β contact residues

<table>
<thead>
<tr>
<th>Peptide Residue</th>
<th>Qa-1β Residue</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala1</td>
<td>Tyr7, Glu63, Tyr159, Trp167, Tyr171</td>
<td>van der Waals</td>
</tr>
<tr>
<td>AlaN</td>
<td>Tyr7 OH</td>
<td>H-bond</td>
</tr>
<tr>
<td>AlaO</td>
<td>Trp167 OH</td>
<td>H-bond</td>
</tr>
<tr>
<td>Met</td>
<td>Tyr7, Ile24, Met45, Glu63, Lys66, Met70, Tyr99, Tyr159</td>
<td>van der Waals</td>
</tr>
<tr>
<td>MetN</td>
<td>Glu63 O2</td>
<td>H-bond</td>
</tr>
<tr>
<td>MetO</td>
<td>Lys66 Nε</td>
<td>H-bond</td>
</tr>
<tr>
<td>Ala1</td>
<td>Met30, Tyr99, Tyr159</td>
<td>van der Waals</td>
</tr>
<tr>
<td>AlaN</td>
<td>Tyr99 OH</td>
<td>H-bond</td>
</tr>
<tr>
<td>AlaO</td>
<td>Met30, Arg150</td>
<td>van der Waals</td>
</tr>
<tr>
<td>Pro</td>
<td>Trp97, Val150, Glu152, Gln155, Gln156</td>
<td>van der Waals</td>
</tr>
<tr>
<td>Arg</td>
<td>Glu152 Oε1</td>
<td>H-bond</td>
</tr>
<tr>
<td>ArgN</td>
<td>Glu152 Oε2</td>
<td>H-bond</td>
</tr>
<tr>
<td>ArgO1</td>
<td>Glu156 Nε2</td>
<td>H-bond</td>
</tr>
<tr>
<td>Thr</td>
<td>Asn77, Phe64, Trp97, Glu116, Glu152</td>
<td>van der Waals</td>
</tr>
<tr>
<td>ThrO2</td>
<td>Asn77 Nε2</td>
<td>Water-mediated H-bond</td>
</tr>
<tr>
<td>Leu</td>
<td>Glu116 Oε2</td>
<td>H-bond</td>
</tr>
<tr>
<td>LeuO</td>
<td>Asn77 Nε2</td>
<td>Water-mediated H-bond</td>
</tr>
<tr>
<td>Leu1</td>
<td>Asn77, Lys146, Ser147 Oγ, Glu152 Oε1, Ser143 O, Ser147 Oγ</td>
<td>van der Waals</td>
</tr>
<tr>
<td>LeuN</td>
<td>Asn77, Lys146, Ser147 Oγ, Glu152 Oε1, Ser143 O, Ser147 Oγ</td>
<td>Water-mediated H-bond</td>
</tr>
<tr>
<td>LeuO</td>
<td>Asn77, Lys146, Ser147 Oγ, Glu152 Oε1, Ser143 O, Ser147 Oγ</td>
<td>Water-mediated H-bond</td>
</tr>
<tr>
<td>LeuOCT</td>
<td>Asn77, Lys146, Ser147 Oγ, Glu152 Oε1, Ser143 O, Ser147 Oγ</td>
<td>Water-mediated H-bond</td>
</tr>
</tbody>
</table>
 FIGURE 4. Comparison of the contacts mediated by the binding pockets of Qa-1b and HLA-E. Comparison of the peptide conformation within the Qa-1b– and HLA-E–binding groove (PDB accession number: 3BZE). The Qdm peptide is shown in stick representation, with carbons colored green, oxygens red, nitrogen blue, and sulfur yellow. The peptide bound to HLA-E is similarly represented, with carbons colored magenta. The position of the amino acid constituents of the peptides is labeled. The residues of Qa-1b and HLA-E are represented as sticks. Qa-1b carbons are colored in cyan, with HLA-E carbon atoms colored in pink. The residue number and type are labeled. A. View of the P1 amino acid in the binding cleft. B. The binding pocket of the P2 position. C. The binding pocket of the P3 position. D. The binding pocket of the P6 position and the salt-bridge interaction between the P5-Arg and Glu152. E. The binding pocket of the P7 position. F. The binding pocket of the P9 position.

FIGURE 5. Comparison of the anchoring pockets in Qa-1b and HLA-E. Space-filling model showing peptide binding to Qa-1b (A) and HLA-E (B). The Qdm peptide in Qa-1b is colored green and the VMAPRTLFL peptide in HLA-E (PDB accession number: 3BZE) is colored magenta. The pockets of the MHCs are contoured about the side-chain atoms of the anchoring residues with a probe radius of 1.4 A. Positions were not confined to Qa-1b–binding pockets, but were solvent exposed (Figs. 2B, 5). Interestingly, the P4-Pro made no significant interactions with Qa-1b, suggesting it may play a role in constraining the conformation of the peptide, which is consistent with the importance of intramolecular constraints in MHC-I peptide presentation (50). Similarly, whereas the side chain of P8-Leu made no significant contacts with Qa-1b, its main chain bound to Qa-1b by a series of water-mediated hydrogen bonds. Interestingly, whereas the P5-Arg side chain was largely solvent exposed, substitution of this residue to Ala impairs peptide binding to Qa-1b (49). This effect appears attributable to the disruption of a salt bridge between P5-Arg and Glu152 on the α2 helix of Qa-1b (Fig. 4D). Collectively, the structure of the Qa-1b–AMAPRTLFL complex confirmed the role of the P2, P3, P6, P7, and P9 positions as anchor residues, which together act to impose considerable constraints on diversity of the peptide repertoire associated with Qa-1b. In contrast, the P4, P5, and P8 side chains are solvent exposed, thus potentially forming part of the ligand recognized by NK and T cells.

Qa-1b is a structural homolog of HLA-E

The H chains of Qa-1b and HLA-E share 74% overall identity, and 71% identity in the peptide-binding region (α1 and α2 domains) (Figs. 6, 7). The overall architecture of this region is structurally well conserved with r.m.s.d. of 0.90 Å over main-chain atoms (residues 2–182), a point reflected in the ability of Qa-1b to bind human MHC-I leader sequences presented by HLA-E (51) (Fig. 6). Similarly, this structural conservation is reflected in the conformation of the Qdm peptide, which shows remarkable similarity to the MHC-I–derived peptides presented by HLA-E with backbone r.m.s.d. of 0.6 Å over main-chain atoms (Fig. 6).

The network of charged interactions between the H chain and the peptide is well conserved between Qa-1b and HLA-E. For example, P5-Arg forms a salt bridge to Glu152 of Qa-1b, a feature also observed in HLA-E/peptide interactions (Fig. 4D) (11). Likewise, with the exception of positions 66 and 99, all residues involved in direct hydrogen bonds with the peptide main chain are conserved (Fig. 4). Lys66 in Qa-1b forms a hydrogen bond with the backbone carbonyl of the P2-Met (Fig. 4B). In HLA-E, position 66 is a Ser and also forms a stabilizing contact to P2, albeit through a bridging water molecule that contacts Glu63. By contrast, the hydrogen bond from Tyr99 of Qa-1b to the peptidyl amide of P3 is not maintained by the His99 substitution in HLA-E (10) (Fig. 4C). Conceivably, this may allow greater flexibility in the positioning of...
The dimensions of the anchor residue-binding pockets are well conserved between Qa-1b and HLA-E structures, with subtle differences underlying small variations in peptide conformation. The orientation of residues Tyr1, Met45, Glu63, and Tyr159 that line the deep hydrophobic cavity of the P2-binding pocket is similar between both Qa-1b and HLA-E (Fig. 4B). However, substitution at position 24 of Ile in Qa-1b for the polar Ser in HLA-E and at position 70 of the hydrophobic Met for the polar Thr results in a shallower cavity in Qa-1b and an altered conformation of the P2-Met side chain. The deeper cavity of HLA-E allows the P2-Met to adopt two conformations, as observed in the crystal structures determined to date (10) (3BZE versus 3BZF). The steric hindrance imposed by Met70 in Qa-1b prevents the P2-Met from adopting either of the conformations observed in HLA-E. Instead, the side chain is shifted 1.1 Å toward the face of the pocket lined by residues Tyr7, Ile24, and Met45 (Figs. 4B, 5). Furthermore, the substitution of Tyr99 and Lys66 in Qa-1b for His and Ser, respectively, in HLA-E not only affects hydrogen-bonding interactions with P2 and P3, but also narrows the binding groove about the Cα atom of P2. These substitutions are coincident with the P2-Met being shifted 0.8 Å toward the α1 helix relative to its HLA-E counterpart (Fig. 4B).

Underscoring the strict conservation of Leu at P9 in peptides presented by both Qa-1b and HLA-E, residues Asn77, Thr80, Leu81, Leu116, and Ser147 that line the P9 pocket are conserved (Figs. 4B, 5). Furthermore, the substitution of Tyr7, Met45, Glu63, and Tyr159 that line the P9 pocket are conserved (Figs. 4B, 5). However, substitution at position 24 of Ile in Qa-1b for the polar Ser in HLA-E and at position 70 of the hydrophobic Met for the polar Thr results in a shallower cavity in Qa-1b. This variation is coincident with a shift of His63Cε1 of ∼1.1 Å.

of the main chain at P3, which may also impact on the positioning of the P4 residue. Indeed, variation between HLA-E structures is found in the P3 and P4 positions (10, 11, 52) (Fig. 6B). The HLA-EVMAPRALLL and HLA-EVMAPRTLLL structures adopt a similar conformation at P3/P4, which differs by ∼0.7 Å and ∼1.2 Å, at P3 and P4, respectively, compared with the conformation in the HLA-EVTAPRTLLL and HLA-EVMAPRTLFL structures (10, 11, 52). This variation is coincident with a shift of His63Cε1 of ∼1.1 Å.

The surface of Qa-1b is colored according to the sequence conservation with HLA-E shown in Fig. 4B. Conserved residues are colored green, and nonconserved residues are colored white. Residues of HLA-E that contact the CD94-NKG2A receptor are labeled. Residues contacting the NKG2A are labeled in black, and those that contact CD94 are labeled in pink. Trp65 is labeled in blue, as it contacts both CD94 and NKG2A.

The combined shifts of P7 and Trp133 position the carboxylate of Glu152 2.7 Å closer to the floor of the peptide-binding groove (Fig. 4D, 4E), which is
concomitant with a small shift of the guanidinium group of P5-Arg (Fig. 4D).

Structural and mutagenesis studies have shown that CD94-NKG2A recognition of HLA-E is dependent on a cluster of basic residues on the α1 helix, including residues Arg65, Arg73, and Arg79, a hydrophobic patch that includes the P8 side chain that abuts this region together with an acidic region on the α2 helix spanning residues 154–162 (19, 20, 53). Comparison of the corresponding regions in Qa-1b showed a similar charge distribution, with an electrostatic patch on the α1 domain and an electro-negative patch on the α2 domain (Fig. 8), both of which are also conserved across Qa-1b allotypes. Despite these broad similarities, however, there are numerous differences between Qa-1b and the corresponding residues in HLA-E that have been shown to contact CD94-NKG2A (19), in particular residues 154 and 155, which directly interact with human NKG2A, and residues 72 and 73, the latter of which interacts with P6-Thr, a residue shown to be important for the interaction with hCD94-NKG2A (Fig. 7) (54). Similarly, mutation of Arg72 of Qa-1b has also been shown to disrupt recognition by mCD94-NKG2A (55).

Discussion

Qa-1b has a number of distinct roles in the immune system serving both to regulate innate immunity via interactions with members of the CD94-NKG2 receptor family and stimulate adaptive immunity presenting peptides for recognition by the αβ TCR (29, 56). The data presented in this work identify the structural features of Qa-1b that allow for the selective binding of the Qdm peptide. The key features of Qdm involved in Qa-1b binding, notably P2-Met, P7-Leu, and P9-Leu, are highly conserved across mammalian MHC class I sequences, consistent with observations that similar peptides derived from other mammalian class I leader sequences also bind Qa-1b (51).

Peptide elution studies suggested that Qa-1b imposes tight specificity on the bound peptide (28). Furthermore, binding of Qdm to Qa-1b was greatly reduced by substitution of P2-Met and P9-Leu with Ala, and the double substitution at P2 and P9 completely abrogated peptide binding (49). These observations are consistent with the structure of Qa-1b-AMAPRTLLL, which revealed deep pockets that could accommodate the P2-Met and P9-Leu side chains of the Qdm determinant. However, the structural data presented also show that residues lining the shallow binding pockets that accommodate the P3 and P6 side chains make critical contacts with Qdm, consistent with the observation that a poly-Gly peptide with P2-Met and P9-Leu did not bind to Qa-1b (51).

Whereas the Qdm peptide is the dominant peptide eluted from Qa-1b purified from healthy cells, there is evidence to suggest that Qa-1b can bind a diverse array of peptides (56, 57). In particular, Qa-1b isolated from TAP-deficient cells was shown to have a markedly broader repertoire of peptides than originally reported (56). These studies showed little evidence of enrichment of any amino acid at P2, suggesting that a variety of amino acids can be accommodated at this position. Similarly, whereas P9 of both the Qdm sequence and its human counterparts (residue 11) is a Leu, the peptides isolated from Qa-1b obtained from TAP-deficient cells were enriched for additional aliphatic amino acids, including Ile, Ala, Met, and Phe at this position. Indeed, a variety of defined peptides that are distinct from MHC-I leader sequences has been shown to bind to Qa-1b. These include peptides from Hsp60 (GMKFDRGYD) (58), Salmonella (GMQFDRGYLY) (58), insulin (ALWMRFLPL) (59), and influenza (FLARSALIL) (57). The structural data in this study suggest that it is improbable that the Hsp60- and Salmonella-derived peptides could be accommodated in a conformation similar to Qdm. First, the introduction of long side chains Lys and Gln at the shallow P3 pocket and Arg at the shallow P6 pocket would require conformational changes to the peptide-binding groove. Furthermore, the loss of the P5 salt bridge to Glu156 and the loss of the stabilizing Leu in the deep P7 pocket by the substitution to Gly would impact on peptide stability and conformation. Likewise, modeling of the insulin-derived peptide shows significant conformational adjustment of the shallow P3 and P6 pockets to accommodate the bulky Trp and Phe side chains. The influenza-derived peptide appears closest to the Qdm peptide in conforming to the needs of Met/Leu at P2 and Leu at P7 and P9 with small side-chain residues at P3 and P6. However, the introduction of Ser at P5 would alter the salt-bridge interaction with Glu156, potentially impacting on the receptor recognition surface of the molecule. Although these peptides have all been shown to stimulate a T cell response, their capacity to interact with CD94-NKG2A is unclear. Modeling based on the Qa-1b-AMAPRTLLL complex suggests that with the possible exception of the influenza peptide, these peptides are unlikely to adopt a conformation similar to that of Qdm.

There are a number of crystal structures of different MHC molecules binding the one peptide (2, 60, 61). Typically, these involve highly related allotypes that exhibit limited sequence differences, which nevertheless often result in biologically significant changes in conformation of the peptide and/or the MHC-I molecule (2, 61–63). In contrast, despite the comparatively low level of sequence identity between Qa-1b and HLA-E, remarkably, they present their respective peptide cargoes in a very similar manner for recognition by CD94-NKG2 receptors. Whereas previous studies have shown that Qdm peptide can bind HLA-E and be recognized by hCD94-NKG2A (42), we show that the Qa-1b-AMAPRTLLL complex does not bind hCD94-NKG2A consistent with previous data showing that HLA-E and Qa-1b tetramers fail to bind murine splenocytes or human PBMC, respectively (64). Thus, we suggest that the inability of hCD94-NKG2A to recognize Qa-1b-AMAPRTLLL most likely results from differences between the Qa-1b and HLA-E H chain rather than changes in orientation and sequence of the peptide per se.

Finally, a notable feature of Qa-1b and indeed its human counterpart, HLA-E, is the relative depth of the C and E pockets that accommodate P6 and P7, respectively. Consequently, the peptide sits deeper in the groove compared with typical pMHC-Ia complexes. Given that the primary function of MHC-Ia molecules is to display a diverse group of peptides for T cell recognition, the elevated positioning of the peptide backbone in MHC-Ia molecules may result in a greater range of ligands for TCR recognition. In contrast, the deep position of the peptide backbone in both Qa-1b and HLA-E, which is also a feature of peptide binding to a number of other nonclassical MHC class I molecules, including HLA-G (9), might reflect their primary role is the presentation of a conserved group of peptides for recognition by essentially invariant receptors that regulate innate immune responses.

Disclosures

The authors have no financial conflicts of interest.

References


