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

Li Zeng,*1 Lucy C. Sullivan,†1 Julian P. Vivian,* Nicholas G. Walpole,*
Christopher M. Harpur,† Jamie Rossjohn,* Craig S. Clements,*2 and Andrew G. Brooks†,2

The primary function of the monomorphic MHC class Ib molecule Qa-1β 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-1β function is unclear. We have assessed the interaction between Qa-1β and CD94-NKG2A and shown that they interact with an affinity of 17 μM. Furthermore, we have determined the structure of Qa-1β 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-1β. Whereas the Qa-1β-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-1β 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-1β 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-1β and HLA-E and provide a structural basis for understanding peptide repertoire selection and the specificity of the interaction of Qa-1β with CD94-NKG2 receptors. The Journal of Immunology, 2012, 188: 302–310.
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-E\textsuperscript{VMAFRTL1L1L} 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-E\textsuperscript{VMAFRTL1L1L} 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-1\textsuperscript{b} molecule. Early studies suggested that Qa-1\textsuperscript{b} is widely expressed (23) and involved in both innate and adaptive immune stimulation. Qa-1\textsuperscript{b} was subsequently identified as a nonameric 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-1\textsuperscript{b} (28).

Like HLA-E, Qa-1\textsuperscript{b} 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-1\textsuperscript{b} 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-1\textsuperscript{b} are orthologs (32). As Qa-1\textsuperscript{b} acts in an analogous role to HLA-E, acting as a checkpoint for class I expression by presenting leader peptides from other MHC-I molecules (33), it has been well characterized (19, 20, 42, 43), there has been no study investigating the HLA-EVMAPRTLLL complex (20). The structural analysis of this complex has 97.4% of residues within the favored region of the Ramachandran plot. Coordinates and structure factors are deposited in the PDB under accession number 4000.

**Results**

### Structural overview of Qa-1\textsuperscript{b}

The interaction between soluble murine and human CD94-NKG2A with Qa-1\textsuperscript{b} or HLA-E was analyzed by surface plasmon resonance (SPR) using a Bio-Rad ProteOn XPR\textsuperscript{36} 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 Sensord (Bio-Rad) by amine coupling (~300 response units of 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-1\textsuperscript{b} 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-1\textsuperscript{b} 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\textsubscript{D} values were analyzed using ProteOn Manager software version 3.0.1 (Bio-Rad) and Prism (GraphPad).

### Crystalization, data collection, structure determination, and refinement

Crystals of Qa-1\textsuperscript{b}-AMAPRTLLL were grown at 4 mg/ml protein in 6% PEG 4000, 10 mM NaCl, and 0.1 M sodium acetate (pH 6.3), using the hanging drop vapor diffusion technique at 20°C. The crystals were flash cooled at 100 K using 15% glycerol as a cryoprotectant. A 1.9 Å resolution data set was collected at GM/CA-CAT (Advanced Photon Source, Chicago, IL) with a MarMosaic 300 CCD. The data were processed and scaled with HKL2000 (35). The crystal structure was solved by molecular replacement, as implemented in PHASER (36). The search model was HLA-E (Brookhaven Protein Data Bank [PDB] accession number: 2ESV) with all sequence differences mutated to alanine and the peptide removed. The progression of refinement was monitored by the Rfree value (5% of the data) with neither a σ nor a low-resolution cutoff being applied to the data. The structure was refined with REFMAC (37) and simulated annealing implemented in PHENIX (38) interspersed with rounds of model building in COOT (39). The final structure was validated with MolProbity (40, 41) and has 97.4% of residues within the favored region of the Ramachandran plot.

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-1\textsuperscript{b} 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-1\textsuperscript{b} was also expressed in E. coli and refolded with β\textsubscript{2}-microglobulin and the Qdm peptide, and the interaction between refolded Qa-1\textsuperscript{b} and mCD94-NKG2A was assessed by SPR. mCD94-NKG2A bound Qa-1\textsuperscript{b}-AMAPRTLLL with rapid binding kinetics that precluded the accurate determination of both on and off rates, similar to the interaction between hCD94-NKG2A and HLA-E (Fig. 1) (43). Equilibrium-binding analyses yielded a K\textsubscript{D} of ~17 μM. Given the capacity of both Qa-1\textsuperscript{b} 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-1\textsuperscript{b} and HLA-E could interact with either mCD94-NKG2A or hCD94-NKG2A was assessed by SPR. hCD94-NKG2A bound HLA-E\textsuperscript{VMAFRTL1L1L} 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-1\textsuperscript{b}-AMAPRTLLL or between mCD94-NKG2A and HLA-E\textsuperscript{VMAFRTL1L1L} (Fig. 1B, 1D).
The asymmetric unit comprised one hetero-
molecules H-2K b (44) and H-2D b (45) and with the murine
majority of the structural differences between Qa-1b and the other
1.1, 1.2, and 1.4 Å, respectively (over all main-chain atoms). The
dimer, and the structure was solved by molecular replacement
between 1 and 277 of the H chain and residues 1–99 of
and 24.9%, respectively. The final model contains all residues

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

binding cleft, highlighting that subtle changes in this region reflect
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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-1b. 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 N\textsuperscript{\alpha} 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 Tyr\textsuperscript{171} that are conserved in 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 hydroxyl groups of Tyr\textsuperscript{84} and Ser\textsuperscript{143} and to the side-chain amino group of Lys\textsuperscript{146}, as well as forming water-mediated hydrogen bonds to the side chains of Asn\textsuperscript{77} and Thr\textsuperscript{80} (Fig. 3, Table II).

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

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commodate the P2-Met, P3-Ala, P6-Thr, P7-Leu, and P9-Leu (Fig. 4B–F). The pockets accommodating P2, P7, and P9 are all relatively deep, whereas those in which P3 and P6 sit are significantly shallower (Fig. 5). Previous studies had established that P2-Met served as a dominant anchor residue for peptide binding to Qa-1b (49). This residue sits in the deep B pocket, making a series of van der Waals interactions within a hydrophobic cavity lined by Tyr2, Ile24, Met45, Met70, and Tyr99 (Fig. 4B). The residues Met70, Tyr99, and Tyr159 from the B pocket also combine with Trp16 to shape the shallow P3 pocket (Fig. 4C). Similarly, the P6-Thr also sits in a shallow hydrophobic pocket formed by the side chains of Phe28 and Trp71 and the aliphatic side chain of Asn73 (Fig. 4D). The P7-Leu is located in a deep pocket accommodated by van der Waals interactions with Glu152, Ile24, Trp133, and Glu152 (Fig. 4E). Finally, the C-terminal peptide residue, P9-Leu, is tethered by a series of main-chain hydrogen bonds as well as being anchored via side-chain interactions with Asn77, Thr80, Leu81, Tyr84, Leu95, Glu116, and Ser143 (Fig. 4F).

Substitution at the P4 and P8 positions of the Qdm peptide had little effect on binding to Qa-1b (49). From the structure, these 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-Ia 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 Glu52. By contrast, the hydrogen bond from Tyr39 of Qa-1b to the peptidyl amide of P3 is not maintained by the His39 substitution in HLA-E (10) (Fig. 4C). Conceivably, this may allow greater flexibility in the positioning

**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, nitrogens 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 peptidom was 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 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-EVTAPRTLLL and HLA-EVMAPRTLFL 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-

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FIGURE 6. Overview of the structural conservation of the Qa-1b and HLA peptide-binding groove. A, Structural superposition of the α1 and α2 domains of Qa-1b (blue) with the three HLA-E structures, as follows: PDB accession number 3BZE (10) (red), 3BZF (10) (yellow), and 1KTL (52) (gray), demonstrating the highly conserved structure of the Ag-binding cleft. B, Conformation of peptides bound to Qa-1b and HLA-E, as follows: Qdm (green), VMAPRTLFL (pink), VMAPRTLLL (yellow), and VTAPRTLLL (gray). The α1 and α2 domains were superposed, as in A.

FIGURE 7. Structure-based sequence conservation of Qa-1b with HLA-E. A, Surface representation of Qa-1b-Qdm. Qdm is shown in yellow. 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 blue, as it contacts both CD94 and NKG2A. B, Structure-based sequence alignment of the α1 and α2 domains of Qa-1b and HLA-E. Strictly conserved residues are highlighted in green boxes.

FIGURE 8. Comparison of the electrostatic potential on the surface of Qa-1b and HLA-E. Electronegative and electropositive patches shown to complement binding regions on CD94 and NKG2A, respectively, are labeled. Likewise, a patch of nonconserved residues shown to be important for the interaction with CD94 is indicated. Although the overall charge distribution between the two proteins is similar, it is likely that differences in electrostatic interactions determine differences in CD94-NKG2A recognition. The potentials are displayed as surfaces contoured at levels of −5 kT/e (red) and +5 kT/e (blue).
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, Arg⁷⁵, 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 (GMQFDRGYL) (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 Glu¹⁵² 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 suggests 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 Glu¹⁵², 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**


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