Structural Basis of Diverse Peptide Accommodation by the Rhesus Macaque MHC Class I Molecule Mamu-B*17: Insights into Immune Protection from Simian Immunodeficiency Virus

Yan Wu,*† Feng Gao,*‡ Jun Liu,*§ Jianxun Qi,*† Emma Gostick,‖ David A. Price,‖*# and George F. Gao*†,*$,**

The MHC class I molecule Mamu-B*17 has been associated with elite control of SIV infection in rhesus macaques, akin to the protective effects described for HLA-B*57 in HIV-infected individuals. In this study, we determined the crystal structures of Mamu-B*17 in complex with eight different peptides corresponding to immunodominant SIVmac239-derived CD8+ T cell epitopes: HW8 (HLEVQGYW), GW10 (GSHLEVQGYW), MW9 (MHPAQTSQW), QW9 (QTSQWDWPW), FW9 (FQWMGYELW), MF8 (MRHLVEPEF), IW9 (IRYPKTFGW), and IW11 (IRYPKTFGWLW). The structures reveal that not only P2, but also P1 and P3, can be used as N-terminal anchor residues by Mamu-B*17-restricted peptides. Moreover, the N-terminal anchor residues exhibit a broad chemical specificity, encompassing basic (H and R), bulky polar aliphatic (Q), and small (T) residues. In contrast, Mamu-B*17 exhibits a very narrow preference for aromatic residues (W and F) at the C terminus, similar to that displayed by HLA-B*57. Flexibility within the whole peptide-binding groove contributes to the accommodation of these diverse peptides, which adopt distinct conformations. Furthermore, the unusually large pocket D enables compensation from other peptide residues if P3 is occupied by an amino acid with a small side chain. In addition, residues located at likely TCR contact regions present highly flexible conformations, which may impact TCR repertoire profiles. These findings provide novel insights into the structural basis of diverse peptide accommodation by Mamu-B*17 and highlight unique atomic features that might contribute to the protective effect of this MHC I molecule in SIV-infected rhesus macaques.


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The atomic coordinates and structure factors for all complexes presented in this article have been submitted to the Protein Data Bank (http://www.pdb.org) under accession numbers 3RWC, 3RWD, 3RWE, 3RWG, 3RWH, 3RWI, and 3RWJ.

Address correspondence and reprint requests to Prof. George F. Gao, CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100010, China. E-mail address: gao@im.ac.cn

Abbreviations used in this article: EC, elite controller; β2m, human β2-microglobulin; β2m, rhesus macaque β2-microglobulin; MHC I, MHC class I; Pc, C-terminal; pMHC I, peptide–MHC class I; Pc, C-terminal; β2m, rhesus macaque β2-microglobulin.
(36), which suggests that this MHC I molecule could play important roles in the control of viral replication throughout the course of infection. However, the efficacy of immunodominant CD8+ T cell responses can be impaired by the selection of viral escape variants (38). Indeed, the emergence of escape mutations frequently correlates directly with the loss of viral suppression (38–40). Nevertheless, this phenomenon can impact viral fitness and reflects the potency of certain CD8+ T cell response (41). Many escape mutations within viral epitopes involve anchor residue substitutions that impede peptide binding to the corresponding MHC I molecule. In contrast, much of the observed sequence variation within Mamu-B*17–restricted epitopes does not seem to impair peptide–MHC I (pMHC I) binding substantially (42). Moreover, in the Mamu-B*17 setting, the C-terminal tryptophan anchor residues remain conserved, and amino acid substitutions only rarely affect the N-terminal anchors at positions 1, 2, or 3. Although variants of the HW8 epitope (Viff66–73) can display substitutions in the N-terminal anchor residue, they maintain the ability to bind Mamu-B*17, which likely reflects amino acid compatibility and pocket B flexibility. Similarly, the Ile-to-Thr replacement at the N-terminal amino acid residue of the IW9 epitope (Nef165–173) does not impact either Mamu-B*17 binding or CD8+ T cell recognition; rather, this mutation hinders Ag processing and/or presentation (42). Collectively, these features indicate that Mamu-B*17–restricted epitopes are favorable targets for effective CD8+ T cell immunity. Indeed, Mamu-B*17+ elite controllers (ECs) have been shown to resist rechallenge with various SIV strains containing multiple escape mutations, and CD8+ T cell clones derived from ECs were more effective at suppressing viral replication than those derived from progressors (43).

The characteristics of peptide presentation by MHC I play a pivotal role in the antigenicity of an epitope (44–49). Accordingly, we determined the structures of eight SIVmac239–derived epitopes in complex with Mamu-B*17 to define the atomic basis for effective peptide epitope presentation by this protective MHC I molecule. Structural analysis revealed a novel peptide-binding strategy for the accommodation of diverse antigenic peptides, which informs our understanding of the association between Mamu-B*17 and the control of SIV replication.

Materials and Methods

Peptide synthesis

The peptides used in this study (Table I) were synthesized at The Journal of Immunology 6383

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<th>Name</th>
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<th>Position</th>
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*Peptides are derived from SIVmac239.

![Downloaded from www.jimmunol.org on December 12, 2011](http://www.jimmunol.org)
Table II. X-ray diffraction data processing and refinement statistics

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aValues in parentheses refer to the highest resolution shell of data.

bRmerge = \frac{\sum I_{hk} - \langle I \rangle}{\sum I_{hk}} \quad I_{hk} = \langle I \rangle \quad \text{where} \quad I_{hk} \quad \text{is the intensity of unique reflection} \quad hkl \quad \text{and} \quad \langle I \rangle \quad \text{is the average over symmetry-related observations of unique reflection} \quad hkl.

cRcryst = \frac{\sum |F_{obs} - F_{calc}|}{\sum F_{obs}} \quad \text{where} \quad F_{obs} \quad \text{and} \quad F_{calc} \quad \text{are the observed and calculated structure factors, respectively.}

dRfree is calculated as for Rcryst but using 5% of reflections sequestered before refinement.
II. As expected, the overall domain arrangements and topologies of these Mamu-B*17 structures are similar to those of other mammalian MHC I molecules. The extracellular region of the Mamu-B*17 H chain forms a bedlike shape containing three domains (α1, α2, and α3). Specifically, the bedrail is composed of antiparallel α1 and α2 helices, which are supported by an eight-stranded β-sheet bedplate. The bound antigenic peptide lies along the bedrail. The α3 domain and β2m occupy the standard positions below the bedplate (Fig. 1). Both Mamu-B*17 and HLA-B*57 are enriched in EC cohorts and associated with the control of SIV and HIV replication, respectively. Amino acid sequence alignments (Fig. 2) reveal that Mamu-B*17 is quite similar to HLA-B*57, displaying 85.14% identity with HLA-B*5703. Moreover, the overall three-dimensional structure of Mamu-B*17 is similar to that of HLA-B*5703, with a root mean square difference of 0.709 Å.

Various conformations of the bound peptides
Superposition of all eight peptides revealed the structural basis for length heterogeneity within Mamu-B*17–restricted epitopes (Fig. 3A). The majority of bound peptides adopt similar conformations with the P2 and C-terminal (Pc) residues serving as anchors, consistent with other pMHC I structures (Fig. 3A). Exceptions to this rule are HW8 and GW10. For HW8, His1 occupies pocket B to serve as the N-terminal anchor residue. For GW10, the two small amino acids Gly1 and Ser2 at the N terminus occupy pocket A, which results in P3 serving as an anchor residue. In accordance with previous studies, the primary anchor specificity at the C terminus was found to be associated with aromatic residues (W and F), which is similar to the situation for HLA-B*57–restricted peptides. Conversely, the N-terminal anchor residues for Mamu-B*17 appear to have a broad chemical specificity (R, H, T, Q) due to the adjustment of pocket B (discussed below). In addition, the secondary anchor residues (Pc-2 or Pc-3) also display diverse conformations (Fig. 3A).

![FIGURE 1.](image1) Overview of the structure of Mamu-B*17 in complex with bound peptide. Structure of the Mamu-B*17-HW8 complex. The H chain (α1, α2, and α3 domains) and L chain (β2m) are shown in cartoon representation and colored in green and cyan, respectively. Peptide HW8 (yellow) is shown in stick representation.

![FIGURE 2.](image2) Structure-based sequence alignment of Mamu-B*17 and other MHC I molecules. Cylinders above the alignment indicate α-helices. Black arrows denote β-strands. Residues highlighted in red are completely conserved, and residues in blue boxes are highly conserved (>80%). Green numbers denote residues that form disulfide bonds. Residues that play a critical role in the primary anchor pockets are highlighted with asterisks (residues in pocket B labeled in green, and residues in pocket F labeled in blue). Residues that are important for TCR recognition are highlighted with yellow boxes. The sequence alignment was generated using Clustal X (73) and ESPript (74).
Two comparisons are noteworthy (58, 59). First, compared with HW8, GW10 has two more N-terminal residues (Gly1 and Ser2). However, the conformations of the central regions of the two peptides are essentially identical. Moreover, the solvent-accessible surface area for the central regions of HW8 (P3–P7) and GW10 (P5–P9) is similar (HW8 230 Å², GW10 261.1 Å²) (Fig. 3B). In contrast, IW9 and IW11 present distinct conformations in the central region, although they share the first nine amino acids from their N termini. Specifically, IW11 contains two extra amino acids at the C terminus (Leu10 and Trp11), which induce a switch in the usage of the Pc anchor residue from Trp9 in IW9 to Trp11 in IW11 (Fig. 3C). This Pc anchor switch causes the central region (P4–P10) of IW11 to protrude out of the groove and into the solvent, with the main chain of IW11 shifting up to 7.6 Å higher than the equivalent regions of IW9. Furthermore, the solvent-accessible surface area of the central region of IW9 (P4–P8, 161.1 Å²) shows

FIGURE 3. Overlay of eight different peptides bound to the Mamu-B*17 molecule. A, Eight different epitopes bound to Mamu-B*17 show different conformations. The magenta circles indicate conserved C-terminal anchor residues and diverse N-terminal and secondary anchor residues. B, Superposition of the HW8 (green) and GW10 (wheat) peptides. The main-chain conformations of these two peptides are shown in cartoon-and-stick representation. The central exposed areas of HW8 and GW10 are identical, as highlighted below in orange surface representation (HW8: upper panel; GW10: lower panel). C, Superposition of IW9 (pale cyan) and IW11 (yellow) peptides. The main-chain conformations of these two peptides are shown in cartoon-and-stick representation. The C-terminal anchor residue Trp9 in IW9 shifts to serve as a secondary anchor in IW11, which causes the central region (P4–P10) of IW11 to form a bulged conformation. For Mamu-B*17-IW9, the IW9 peptide exposes Pro4, Lys5, Thr6, and Gly8 for potential TCR docking (orange surface representation, upper panel). For Mamu-B*17-IW11, the exposed area of IW11 is much larger, spanning Pro4, Lys5, Thr6, Phe7, and Leu10 (orange surface representation, lower panel).

FIGURE 4. Comparison of the binding capability in pocket B between the real Mamu-B*17-GW10 structure and an alternative molecular model. A, The real Mamu-B*17-GW10 structure. The side chain of His3 (pale cyan) is stabilized by forming a hydrogen bond and a salt bridge with Tyrα9 and Gluα45 (cyan), respectively. B, Model of Mamu-B*17-GW10. Ser2 (yellow) is considered as an anchor residue buried in pocket B. The side chain of Ser2 is too short to form strong hydrogen bonds with Tyrα9 and Gluα45 (cyan).
a significant difference compared with IW11 (P4–P10, 410.7 Å²), which suggests that they might elicit qualitatively different TCR repertoires. Strikingly, a similar result has been reported for HLA-B*5703 (60). Thus, our structures show two distinct strategies used by MHC I molecules to present N- or C-terminal truncated peptides, which may partially reflect the intrinsic characteristics of the peptides themselves. An alternative molecular model of GW10 in complex with Mamu-B*17 was produced using COOT and CCP4, in which the peptide uses Ser² as the P2 anchor, and Gly¹ hides in pocket A instead of protruding out of the peptide binding groove (Fig. 4). Compared to the real Mamu-B*17-GW10 structure, however, we found that the interaction (van der Waal force and hydrogen bonds) between Ser² and pocket B in the alternative model was much weaker than the interaction between His³ and pocket B in the real Mamu-B*17-GW10 structure. Thus, GW10 adopts a proper conformation with the lowest Gibb’s free energy, which is similar to the situation for HW8.

Two types of binding mode in pocket D and different nonameric peptide conformations

The four nonameric peptides (IW9, FW9, QW9, and MW9) and one decameric peptide (GW10), which protrudes the additional P1 residue out of the groove, were compared in further analyses. As noted above, the second amino acid of GW10 is the small residue Ser, which is buried in pocket A. Thus, in this case, P3–P10 of GW10 is equivalent to P2–P9 of the nonameric peptides. Analysis of these five structures revealed that the conformation of nonamers presented by Mamu-B*17 can be divided into two types according to the binding mode in pocket D (Fig. 5). Type I is common in mammalian MHC I molecules. In this mode, the P3 residue (referring to P2 as the anchor residue) occupies the D pocket, stacking against the side chain of Tyr⁰159. Both Mamu-B*17-IW9 and Mamu-B*17-FW9, which contain bulky aromatic residues at P3 (Tyr³ in IW9 and Trp³ in FW9) that fit pocket D well, display this conformation. In type II, the P3 residues are relatively small, which contributes to the spaciousness of pocket D and allows the accommodation of side chains contributed by other peptide residues. Mamu-B*17-QW9, Mamu-B*17-MW9, and Mamu-B*17-GW10 all fall into this category. Trp⁵ in QW9, Gln⁴ in MW9, and Val⁵ in GW10 occupy pocket D, respectively, due to the small side chains of the corresponding P3 residues (Ser² in QW9, Pro¹ in MW9, and Leu¹ in GW10), which results in distinct main chain conformations relative to type I. Furthermore, compared with pocket D in Mamu-A*01 (accessible surface area 235.7 Å²), which is optimal for binding the P3 proline side chain (50), pocket D in Mamu-B*17 is unusually large (average accessible surface area 321.4 Å²), thereby facilitating the formation of these distinct binding modes.

Unconventional flexibility of the binding groove in Mamu-B*17 leads to different bound peptide conformations

Previous studies have reported that water molecules (60–62), platform adjustment (60), or the presence of only one flexible pocket contribute to the accommodation variability of bound peptides. However, none of these binding modes involve flexibility of the whole groove. Further analysis of the Mamu-B*17 structures indicated that the side chains of residues on both the α1/α2 helices and the β-sheet can adopt different conformations, which confer important flexibility to the peptide-binding groove for the accommodation of different peptide conformations (Fig. 6A).

Gluc⁰⁴⁵, Argo⁶⁶, Gluc⁶⁹, and Hiso⁷⁴ located on the α1 helix are all involved in interactions with bound peptides. Furthermore, the side chains of these four residues present various conformations to accommodate different peptides. For example, in the Mamu-B*17-IW9 and Mamu-B*17-GW10 structures, the side chains of Gluc⁶⁹ extend in different directions as a consequence of the different P6 residues (corresponding to P7 in GW10) in these two peptides. Specifically, for IW9, the side chain of Thr⁶ hydrogen bonds to the OE1 atom of Gluc⁶⁹, which forces the side chain of Gluc⁶⁹ to orientate toward the peptide. In contrast, for GW10, the side chain of Gln⁷ shifts 2.37 Å to form hydrogen bonds with the OE1/OE2 atoms of Gluc⁶⁹, causing the side chain of Gluc⁶⁹ to protrude up out of the groove (Fig. 6B).

In contrast to the central location of flexible residues in the α1 helix, the flexible residues in the α2 helix that are involved in peptide binding are distributed from the N terminus to the C terminus. The structures of Mamu-B*17-QW9 and Mamu-B*17-MW9 reveal conformational shifts in the side chains of Argo¹⁵⁵ and Asno¹⁵⁰ (Fig. 6C). For Mamu-B*17-QW9, two salt bridges are formed between the basic Arg¹⁵⁵ guanidinium head group and the OD1/OD2 oxygen atoms of the QW9 acidic Asp⁷, respectively. For Mamu-B*17-MW9, the side chain of Argo¹⁵⁵ is much closer to the α2-helix due to hydrogen bonds with Asno¹⁵⁰. The side chain of Asno¹⁵⁰ is orientated toward the peptide and contributes to the stabilization of the side chain of peptide residue Ser² by forming a hydrogen bond between the ND2 atom and the oxygen atom of Ser².

Superposing whole residues comprising the peptide binding groove in the Mamu-B*17-IW9 and Mamu-B*17-IW11 structures revealed that Tyr⁵¹⁵ in the α2-helix of the H chain also contributes to different peptide accommodation modes (Fig. 6D). Indeed, the side chain extends in totally different directions as a result of interactions with different peptide residues. Specifically, in case of the IW11 peptide, the side chain of Trp⁵ is oriented toward the bottom of the peptide-binding groove, which forms a hydrogen bond to Lyso⁹⁷. The side chain of Tyr⁵¹⁵ pokes toward the N terminus of the peptide, and the hydroxyl group hydrogen bonds to the side chains of Lyso⁹⁷ and Hisa¹³⁴, respectively. In contrast, the side chain of Phe⁶ in IW9 is directed toward the α2-helix and occupies pocket E, which forces the phenolic group of Tyr⁵¹⁵ to swivel away and leave enough space to accommodate the aromatic side chain.
In the structures of Mamu-B*17 complexed with different peptides, H chain position 97 is located at the bottom of the peptide-binding groove as for other classical MHC I molecules. The conformational plasticity of Lys$^97$ contributes to the accommodation of different peptide residues that insert into the central region of the groove accordingly. For example, in the Mamu-B*17-FW9 structure, the Lys$^97$ side chain helps to stabilize the side chain of Glu$^7$ by forming a salt bridge. In the Mamu-B*17-MF8 structure, however, the side chain of Lys$^97$ is poked away from the side chain of Leu$^5$ in the MF8 peptide due to a steric clash.

**Unique primary anchor residues in pockets B and F of Mamu-B*17**

In most mammalian MHC I molecules, the peptide-binding motif generally specifies two or more positions (anchor positions) at which all suitable peptides have either one or a very limited set of permissible residues (63, 64). Strikingly, pocket B of Mamu-B*17 can bind a wide range of anchor residues. In accordance with previous data (36), the small residue T, the bulky residues R and H, and the bulky polar aliphatic residue Q, can all be accommodated in pocket B. On one hand, pocket B of Mamu-B*17 is much deeper compared with that of Mamu-A*01 and Mamu-A*02 (Fig. 7A). Sequence analysis reveals that position 63 and position 67 in Mamu-A*01 and Mamu-A*02 are glutamine and methionine, respectively (Fig. 2), the side chains of which are much longer than their counterparts in Mamu-B*17, thereby resulting in a relatively shallow pocket B. In contrast, the small alanine side chains in both positions 63 and 67 contribute to the spaciousness of pocket B in Mamu-B*17, enabling the accommodation of bulky anchor residues (Fig. 7A). On the other hand, the unique charged Glu$^{45}$, which is located at the bottom of pocket B, also plays an

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**FIGURE 6. Flexibility in the Mamu-B*17 peptide-binding groove.** A, Conformational rearrangements of residues in the peptide-binding groove of Mamu-B*17. The peptide-binding groove is displayed in white cartoon model format. Residues labeled in red are described further in B–E. B, Glu$^{69}$ located on the α1-helix exhibits different side-chain conformations in the Mamu-B*17-FW9 (pink) and Mamu-B*17-GW10 (green) structures. Glu$^7$ of peptide GW10 and Thr$^9$ of peptide IW9 are shown in both stick and sphere format, with hydrogen bonds and salt bridges indicated by dotted lines. C, The side chains of Asn$^{150}$ and Arg$^{155}$ adjust to accommodate different residues in the Mamu-B*17-QW9 (orange) and Mamu-B*17-MW9 (limon) structures. D, Tyr$^{152}$ located on the α2-helix displays two conformations. For Mamu-B*17-IW11 (cyan), Trp$^9$ in the IW11 peptide lies along the peptide-binding groove. The aromatic ring of Trp$^9$ is parallel to the side chain of Tyr$^{152}$. For Mamu-B*17-FW9 (pink), the phenyl ring of Phe$^7$ in the FW9 peptide points toward the α2-helix, and the Tyr$^{152}$ side chain swivels away to leave enough space. E, At the bottom of the groove, the side chain of Lys$^97$ displays flexibility with respect to peptide accommodation. In the Mamu-B*17-FW9 (salmon) structure, Glu$^7$ in the FW9 peptide forms a salt bridge with Lys$^97$. In the Mamu-B*17-MF8 (blue) structure, Lys$^97$ is poked away from the side chain of Leu$^5$ in the MF8 peptide due to a steric clash.
important role in the binding of various peptide anchor residues (Fig. 7B). For IW9, the side chain of Glu<sub>45</sub> engages in two of the four hydrogen bonds that are formed by the guanidinium moiety of peptide Arg<sub>2</sub>, the other two being formed with Tyr<sub>9</sub> and Ser<sub>24</sub>. For FW9, the side chain of Glu<sub>45</sub> rotates to connect to the short side chain of peptide Thr<sub>2</sub> via a water molecule, which is stabilized by Ala<sub>63</sub> located on the α<sub>1</sub>-helix. The side chains lengths of peptide Gln<sub>2</sub> in FW9 and peptide His<sub>3</sub> in GW10 are similar and adopt a similar conformational orientation with respect to Glu<sub>45</sub> and Tyr<sub>9</sub>, although the interaction between peptide Gin<sub>2</sub> and Glu<sub>45</sub> is a little bit weaker than that between peptide His<sub>3</sub> and Glu<sub>45</sub>. Furthermore, the charged residues Arg<sub>66</sub> and Glu<sub>163</sub> form a salt bridge above pocket B, which forces anchor residues to insert deeply into this pocket.

The large volume of Mamu-B<sup>17</sup> pocket F is composed of hydrophobic amino acids (Ile<sub>81</sub>, Tyr<sub>9</sub>, and Tyr<sub>118</sub>), which makes it suitable for binding large aromatic C-terminal side chains (W and F). Notably, Ala<sub>81</sub> at the bottom of pocket F plays an important role in the accommodation of large aromatic anchors, although it does not generate many contacts with the anchor residues. Compared with Leu<sub>81</sub> in Mamu-A<sup>01</sup>, the small side chain of Ala<sub>81</sub> in Mamu-B<sup>17</sup> provides pocket F with sufficient volume to accommodate large aromatic anchors. Thus, pocket F of Mamu-B<sup>17</sup> can accommodate large aromatic anchors, whereas pocket F of Mamu-A<sup>01</sup> can only bind to relatively small side chain residues. The C-terminal binding motif of Mamu-B<sup>17</sup>- restricted peptides is similar to that for HLA-B<sup>57</sup>-restricted peptides. Ser<sub>116</sub> and Ile<sub>23</sub> in Mamu-B<sup>17</sup> also contribute to the

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**FIGURE 7.** Pockets B and F of Mamu-B<sup>17</sup>. A, Comparison of the binding grooves among Mamu-B<sup>17</sup>, Mamu-A<sup>01</sup> (1ZVS), and Mamu-A<sup>02</sup> (3JTS), viewed from above. Pocket B and pocket F are indicated as surface representations in 90% gray. The α1/α2-helices and peptides are shown in cartoon format. Anchor residues are shown in stick format. Position 63 and 67 (warm pink sticks) in Mamu-B<sup>17</sup> are alanines, the side chains of which are much shorter than the corresponding Glu<sub>63</sub> and Met<sub>67</sub> in Mamu-A<sup>01</sup> and Mamu-A<sup>02</sup>; the Mamu-B<sup>17</sup> pocket B is therefore deeper. Ala<sub>81</sub>, Ile<sub>81</sub>, and Ser<sub>116</sub> (green sticks) contribute to a much larger pocket F in Mamu-B<sup>17</sup> compared with Mamu-A<sup>01</sup>, which has a relative shallow pocket F composed of Leu<sub>81</sub>, Leu<sub>95</sub>, and Tyr<sub>118</sub>. This enables Mamu-B<sup>17</sup> pocket F to accommodate aromatic anchor residues. B, Binding strategies in pocket B. Four different anchor residues have the ability to bind pocket B; these are shown in pale cyan stick and sphere representations with hydrogen bonds and salt bridges indicated by dotted lines. For IW9 (magenta), the side chain of Arg<sub>2</sub> is stabilized by forming hydrogen bonds and salt bridges with Tyr<sub>9</sub>, Ser<sub>24</sub>, and Glu<sub>45</sub>, respectively. For QW9 (orange), the side chain of Thr<sub>2</sub> is too short to insert into the bottom of pocket B, which needs a water molecule to mediate the interactions among Thr<sub>2</sub>, Glu<sub>45</sub>, and Ala<sub>63</sub>. For FW9 (salmon) and GW10 (cyan), the side chains of Gln<sub>2</sub> and His<sub>3</sub> hydrogen bond to Tyr<sub>9</sub> and Glu<sub>45</sub>.
reveals that Glu\textsubscript{a} crucial residues for TCR interactions (67). Sequence analysis files (Fig. 8). Position 69 and position 155 have been reported as important role in mediating pMHC I interactions with the TCR helices, including residues 65–76 and residues 149–156, play an important function.

Flexible TCR contact region

Previous studies have indicated that key residues on the \(\alpha 1\) and \(\alpha 2\) helices, including residues 65–76 and residues 149–156, play an important role in mediating pMHC I interactions with the TCR (65–67). Notably, these residues in Mamu-B*17 present highly flexible conformations, which may influence TCR repertoire profiles (Fig. 8). Position 69 and position 155 have been reported as crucial residues for TCR interactions (67). Sequence analysis reveals that Glu\textsubscript{69} and Arg\textsubscript{155} in Mamu-B*17 are unique (Fig. 2), as the corresponding residues in other primate MHC I molecules are noncharged (S and Q). These unique features may contribute to a more flexible juxtaposition at the TCR–pMHC I interface, with potential implications for TCR repertoire engagement.

Discussion

The Mamu-B*17 allele is prevalent among rhesus macaques and consistently associated with efficacious control of SIV replication. In this article, we describe the structures of eight different immunodominant SIV\textsubscript{mac} 239-derived CTL-epitope peptides complexed with Mamu-B*17, thereby providing the first insights into the atomic basis of Ag presentation by this protective MHC I molecule.

The peptide-binding specificity of Mamu-B*17 has been characterized previously in functional studies (36). Pocket B and pocket F are clearly the dominant peptide anchor sites, as observed for other MHC I complexes. N-terminal peptide anchor residues display a broad chemical specificity, encompassing basic residues (H and R), a bulky polar aliphatic residue (Q), and a small residue (T). This diverse profile is determined by two main structural features of the Mamu-B*17 molecule. First, pocket B of Mamu-B*17 is much deeper and larger than that of either Mamu-A*01 or Mamu-A*02 (50, 68), which provides more space for bulky residues. In contrast, pocket B of Mamu-A*01 can only accommodate small residues (S and T) (50). Second, the conformational adjustment of side chains and water molecule compensation confer substantial plasticity within pocket B. Single substitution analog assays have shown that negatively charged residues (D and E) are not tolerated at position 2 (36), which may be partially explained by the Glu\textsubscript{45} residue positioned at the bottom of pocket B. Pocket F in the Mamu-B*17 molecule is particularly distinctive in that it confers a preference for bulky aromatic residues, especially tryptophan, as the C terminus of the bound peptide. Many HLA-B*57 suballeles show similar C-terminal anchor residue preference due to the presence of identical key residues in this pocket (60). Previous studies have failed to detect the emergence of natural mutations that change the C terminus tryptophan anchor residue (69). Given that tryptophan is a rare amino acid, encoded only by one codon, these observations indicate that such variation would likely impact viral fitness and provide a rationale for the protective effect of Mamu-B*17–restricted epitope presentation.

The conformations of the eight peptide epitopes complexed to Mamu-B*17 described in this study are distinct, which suggests the potential to elicit a broad range of protective CD8\textsuperscript{+} T cell responses. In addition, many studies show that water molecules, platform adjustment, or the presence of only one flexible pocket contribute to the accommodation of different peptides (60–62). In contrast, Mamu-B*17 has a whole flexible peptide-binding groove to achieve this. Furthermore, Mamu-B*17 appears to maintain the ability to bind variant epitopes, which may elicit cross-reactive TCRs or specific de novo responses (43). It is tempting to speculate that the unique flexibility within the Mamu-B*17 binding groove may enable the accommodation of natural epitope variants, thereby providing a novel explanation for the containment of viral replication by this MHC I molecule.

TCR cross-reactivity (18, 70) or variant-specific de novo responses (71, 72) might be important for the maintenance of SIV control in ECs. Among the structures determined in this study, two sets of related peptides present distinct outcomes. The GW10 and HW8 peptides display identical main-chain and side-chain conformations, although GW10 contains two more amino acids (Gly\textsuperscript{1} and Ser\textsuperscript{3}) at the N terminus. Thus, HW8 and GW10 might elicit similar TCR repertoires. In contrast, IW9 and IW11 display distinct main-chain conformations across the central region. Specifically, IW11 extends two more amino acids (Leu\textsuperscript{10} and Trp\textsuperscript{11}) at the C terminus, inducing an anchor residue shift in pocket F. Consequently, the central region of IW11 bulges out of the groove, which likely plays a dominant role in TCR recognition. The identical main-chain conformations of the P1 to P4 and the Pc-1 to Pc-2 residues in IW9 and IW11 lock both the N terminus and C terminus, respectively. Two related epitopes, KF8 and KF11, show similarly distinct features when bound to HLA-B*5703 (60). The structures of Mamu-B*17 complexed with different peptides therefore provide insights into the relationship between different epitopes. However, extrapolations to TCR repertoire engagement remain untested and require further exploration.

In summary, the current study provides novel insights into the structural basis of diverse peptide accommodation by Mamu-B*17 and highlights unique atomic features that might contribute to the protective effect of this MHC I molecule in SIV infection.

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