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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 (QTSQWDWP), FW9 (FQWMGYELW), MF8 (MHRVLEPF), I9W (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 under accession numbers 3RWC, 3RWD, 3RWE, 3RWG, 3RWJ, and 3RWI.

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, Building 3, No. 1 Beichen West Road, Chaoyang District, Beijing 100101, 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; P2, C-terminal; pMHC I, peptide–MHC class I; P2, rhesus macaque β2-microglobulin which provides a useful model to inform HIV pathogenesis and study the efficacy of vaccine candidates (1, 2). In the past decade, most work on SIV-specific CD8+ T cell responses in the rhesus macaque model has centered on the MHC class I (MHC I) molecule Mamu-A*01 (3–20). More recently, however, the scope of immunological studies in this model has expanded to include additional MHC I molecules that display distinct biological properties. Mamu-B*17, which is present in >12% of captive Indian rhesus macaques (21), is associated with the control of SIV replication in vivo (22–26), although it should be noted that inheritance of this allele alone does not predict a favorable outcome and that the immunological basis for this association remains unclear. In humans, it is well-established that HLA-B*27 and HLA-B*57, the gene products of which present HIV-derived epitopes that elicit highly effective CD8+ T cell responses (27–31), are associated with reduced virus loads and delayed disease progression in HIV-infected individuals (27, 32–35). By analogy, the study of Mamu-B*17–positive rhesus macaques provides an opportunity to understand how specific MHC I molecules might impact AIDS virus replication in a controlled setting (31).

The peptide-binding specificity of Mamu-B*17 has been identified, together with a range of presented SIV-derived epitopes that exhibit diverse lengths and antigenicities (36). Strikingly, no immunodominant Gag-derived epitopes have been described (36). In contrast, many targeted epitopes emanate from the smaller regulatory/accessory proteins Nef and Vif (Table I) (37). Given the critical roles of these proteins with respect to infectivity and pathogenicity, such epitopes may be appealing targets for vaccine development.

CD8+ T cell responses restricted by Mamu-B*17 can be detected during both the acute and chronic phases of SIV infection.
initiated using commercial kits (Hampton Research). All crystallizations were performed using the hanging drop vapor diffusion technique. Plates were incubated at 291 K and examined after 72 h and 1 wk. Crystals of Mamu-B*17-HW8, Mamu-B*17-FW9, Mamu-B*17-MW9, and Mamu-B*17-GW10 were observed both in Index 44 (0.1 M HEPES [pH 7.5] and 25% w/v polyethylene glycol 3350) and Index 72 (0.2 M sodium chloride, 0.1 M HEPES [pH 7.5], and 25% w/v polyethylene glycol 3350). Crystals of Mamu-B*17-MF8, Mamu-B*17-IW9, and Mamu-B*17-IW11 were observed in Index 80 (0.2 M ammonium acetate, 0.1 M HEPES [pH 7.5], and 25% w/v polyethylene glycol 3350). Crystal of Mamu-B*17-QW9 were observed in Index 68 (0.2 M ammonium sulfate, 0.1 M HEPES [pH 7.5], and 25% w/v polyethylene glycol 3350). All crystals were soaked in reservoir solution supplemented with 20% glycerol as a cryoprotectant, then flash-cooled and maintained at 100 K in a cryostream. Data collection was performed in-house on a Rigaku R-AXIS IV++ image plate with a Rigaku MicroMax007 rotating-anode x-ray generator (Rigaku) operated at 40 kV and 20 mA (Cu Kα; λ = 1.5418 Å). Data were processed and scaled using HKL2000 (51).

### Results

**The overall structures of Mamu-B*17 with eight different SIVmac239-derived peptides**

The structures of Mamu-B*17 complexed with eight different immunogenic SIV-derived peptides were determined in this study.
<table>
<thead>
<tr>
<th>HW8</th>
<th>GW10</th>
<th>IW9</th>
<th>IW11</th>
<th>FW9</th>
<th>QW9</th>
<th>MW9</th>
<th>MF8</th>
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<tr>
<td><strong>Data collecting</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Space group</td>
<td>P2</td>
<td>P2</td>
<td>P1</td>
<td>P1</td>
<td>P2</td>
<td>H3</td>
<td>P2</td>
</tr>
<tr>
<td>Unit cell dimensions (a, b, c)</td>
<td>67.96, 44.60, 80.63</td>
<td>82.96, 44.60, 81.75</td>
<td>45.22, 69.03, 81.98</td>
<td>68.81, 45.29, 129.03</td>
<td>95.69, 95.69, 80.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit cell dimensions (α, β, γ)</td>
<td>90.00, 96.94, 90.00</td>
<td>90.00, 95.99, 90.00</td>
<td>90.00, 96.11, 90.00</td>
<td>90.00, 96.33, 90.00</td>
<td>90.00, 96.33, 90.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>54.96–2.26 (2.38–2.26)</td>
<td>50.00–2.01 (2.08–2.01)</td>
<td>50.00–2.60 (2.69–2.60)</td>
<td>50.00–2.60 (2.69–2.60)</td>
<td>50.00–2.35 (2.43–2.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of reflections</td>
<td>88043 (12328)</td>
<td>32167 (7659)</td>
<td>32635 (3169)</td>
<td>28767 (2814)</td>
<td>20992 (2000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of molecules in the asymmetric unit</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redundancy (%)</td>
<td>3.9 (3.8)</td>
<td>4.2 (4.2)</td>
<td>3.8 (3.8)</td>
<td>3.4 (3.4)</td>
<td>7.2 (6.6)</td>
<td>5.6 (5.5)</td>
<td>5.5 (5.6)</td>
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<tr>
<td>Completeness (%)</td>
<td>99.7 (99.0)</td>
<td>95.7 (92.7)</td>
<td>97.0 (95.1)</td>
<td>96.8 (95.2)</td>
<td>98.4 (96.2)</td>
<td>99.9 (100)</td>
<td>96.9 (94.7)</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>22.48–2.70</td>
<td>24.39–2.00</td>
<td>27.01–2.50</td>
<td>27.27–2.60</td>
<td>28.55–2.40</td>
<td>24.63–2.60</td>
<td>50.00–2.10</td>
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<td>Refinement</td>
<td>Rcryst (%)</td>
<td>21</td>
<td>25.6</td>
<td>0.004</td>
<td>17.3</td>
<td>0.01</td>
<td>20.9</td>
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<tr>
<td>Rfree (%)</td>
<td>17.3</td>
<td>20.9</td>
<td>0.005</td>
<td>19.6</td>
<td>27.7</td>
<td>0.004</td>
<td>24.9</td>
</tr>
<tr>
<td>Ramachandran plot quality</td>
<td>12.4 (51.2)</td>
<td>27.1</td>
<td>0.004</td>
<td>24.6</td>
<td>0.005</td>
<td>23.6</td>
<td>0.01</td>
</tr>
<tr>
<td>No. of residues in favored region (∼98.0% expected)</td>
<td>368 (97.4%)</td>
<td>731 (97.9%)</td>
<td>728 (96.3%)</td>
<td>734 (96.6%)</td>
<td>370 (97.9%)</td>
<td>364 (96.0%)</td>
<td>370 (97.6%)</td>
</tr>
<tr>
<td>No. of residues in allowed region (∼2.0% expected)</td>
<td>10 (2.6%)</td>
<td>7 (1.8%)</td>
<td>25 (3.3%)</td>
<td>26 (3.4%)</td>
<td>8 (2.1%)</td>
<td>15 (4.0%)</td>
<td>9 (2.4%)</td>
</tr>
<tr>
<td>No. of residues in outlier region (%)</td>
<td>0 (0%)</td>
<td>1 (0.3%)</td>
<td>3 (0.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

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

**Notes:**

1. \( Rmerge = \frac{\sum_{i=1}^{n} I_{hk} - \langle I \rangle}{\sum_{i=1}^{n} I_{hk}} \), where \( I \) is the intensity of unique reflection \( hkl \) and \( \langle I \rangle \) is the average over symmetry-related observations of unique reflection \( hkl \).
2. \( Rcryst = \frac{\sum_{i=1}^{n} F_{obs} - F_{calc} \sum_{i=1}^{n} F_{calc}}{\sum_{i=1}^{n} F_{obs}} \), where \( F_{obs} \) and \( F_{calc} \) are the observed and calculated structure factors, respectively.
3. \( Rfree \) is calculated as for \( Rcryst \) but using 5% of reflections sequestered before refinement.
(Table I). X-ray data collection statistics are summarized in Table 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 (\(\alpha_1, \alpha_2, \) and \(\alpha_3\) domains). Specifically, the bedrail is composed of antiparallel \(\alpha_1\) and \(\alpha_2\) helices, which are supported by an eight-stranded \(\beta\)-sheet bedplate. The bound antigenic peptide lies along the bedrail. The \(\alpha_3\) domain and \(\beta_2m\) 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.](http://www.jimmunol.org/)

Overview of the structure of Mamu-B*17 in complex with bound peptide. Structure of the Mamu-B*17-HW8 complex. The H chain (\(\alpha_1, \alpha_2, \) and \(\alpha_3\) domains) and L chain (\(\beta_2m\)) are shown in cartoon representation and colored in green and cyan, respectively. Peptide HW8 (yellow) is shown in stick representation.

![FIGURE 2.](http://www.jimmunol.org/)

Structure-based sequence alignment of Mamu-B*17 and other MHC I molecules. Cylinders above the alignment indicate \(\alpha\)-helices. Black arrows denote \(\beta\)-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

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**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 Pro5, 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 Pro5, Lys5, Thr6, Phe7, and Leu10 (orange surface representation, lower panel).

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**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 Gibbs’s free energy, which is similar to the situation for HW8.

Two types of binding mode in pocket D and different nonamer 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 Tyro159. 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, Gln5 in MW9, and Val6 in GW10 occupy pocket D, respectively, due to the small side chains of the corresponding P3 residues (Ser2 in QW9, Pro3 in MW9, and Leu3 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).

Glu45, Arg66, Glu69, and His74 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 Glu69 extend in distinct 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 Glu69, which forces the side chain of Glu69 to orient 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 Glu69, causing the side chain of Glu69 to protrude 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 Arg155 and Asn150 (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 Arg155 is much closer to the α2-helix due to hydrogen bonds with Asn150. The side chain of Asn150 is orientated toward the peptide and contributes to the stabilization of the side chain of peptide residue Ser7 by forming a hydrogen bond between the ND2 atom and the oxygen atom of Ser7.

Superposing whole residues comprising the peptide binding groove in the Mamu-B*17-IW9 and Mamu-B*17-IW11 structures revealed that Tyra152 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 orientated toward the bottom of the peptide-binding groove, which forms a hydrogen bond to Lyso97. The side chain of Tyr152 pokes toward the N terminus of the peptide, and the hydroxyl group hydrogen bonds to the side chains of Lyso97 and His81, respectively. In contrast, the side chain of Phe8 in IW9 is directed toward the α2-helix and occupies pocket E, which forces the phenolic group of Tyr152 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\textsuperscript{a97} 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\textsuperscript{a97} side chain helps to stabilize the side chain of Glu\textsuperscript{7} by forming a salt bridge. In the Mamu-B*17-MF8 structure, however, the side chain of Lys\textsuperscript{a97} is poked away from the side chain of Leu\textsuperscript{5} in the MF8 peptide due to a steric clash.

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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\textsuperscript{45}, which is located at the bottom of pocket B, also plays an

**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\textsuperscript{69} located on the \textalpha{1}-helix exhibits different side-chain conformations in the Mamu-B*17-IW9 (pink) and Mamu-B*17-GW10 (green) structures. Glu\textsuperscript{7} of peptide GW10 and Thr\textsuperscript{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\textsuperscript{150} and Arg\textsuperscript{155} adjust to accommodate different residues in the Mamu-B*17-QW9 (orange) and Mamu-B*17-MW9 (limon) structures. D. Tyr\textsuperscript{152} located on the \textalpha{2}-helix displays two conformations. For Mamu-B*17-IW11 (cyan), Trp\textsuperscript{8} in the IW11 peptide lies along the peptide-binding groove. The aromatic ring of Trp\textsuperscript{8} is parallel to the side chain of Tyr\textsuperscript{152}. For Mamu-B*17-IW9 (pink), the phenyl ring of Phe\textsuperscript{7} in the IW9 peptide points toward the \textalpha{2}-helix, and the Tyr\textsuperscript{152} side chain swivels away to leave enough space. E. At the bottom of the groove, the side chain of Lys\textsuperscript{97} displays flexibility with respect to peptide accommodation. In the Mamu-B*17-FW9 (salmon) structure, Glu\textsuperscript{7} in the FW9 peptide forms a salt bridge with Lys\textsuperscript{97}. In the Mamu-B*17-MF8 (blue) structure, Lys\textsuperscript{97} is poked away from the side chain of Leu\textsuperscript{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 Gluα45 engages in two of the four hydrogen bonds that are formed by the guanidinium moiety of peptide Argα2, the other two being formed with Tyrα9 and Serα24. For QW9, the side chain of Gluα45 rotates to connect to the short side chain of peptide Thrα2 via a water molecule, which is stabilized by Alaα63 located on the α1/α2-helix. The side chains lengths of peptide Glnα2 in FW9 and peptide Hisα3 in GW10 are similar and adopt a similar conformational orientation with respect to Gluα45 and Tyrα9, although the interaction between peptide Ginα2 and Gluα45 is a little bit weaker than that between peptide Hisα3 and Gluα45. Furthermore, the charged residues Argα66 and Gluα163 form a salt bridge above pocket B, which forces anchor residues to insert deeply into this pocket.

The large volume of Mamu-B*17 pocket F is composed of hydrophobic amino acids (Ileα81, Tyrα123, and Tyrα118), which makes it suitable for binding large aromatic C-terminal side chains (W and F). Notably, Alaα81 at the bottom of pocket F needs a water molecule to mediate the interactions among Thrα2, Gluα45, and Alaα63. For FW9 (salmon) and GW10 (cyan), the side chains of Ginα2 and Hisα3 hydrogen bond to Tyrα9 and Gluα45.

FIGURE 7. Pockets B and F of Mamu-B*17. A, Comparison of the binding grooves among Mamu-B*17, Mamu-A*01 (1ZVS), and Mamu-A*02 (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*17 are alanines, the side chains of which are much shorter than the corresponding Gluα63 and Metα67 in Mamu-A*01 and Mamu-A*02; the Mamu-B*17 pocket B is therefore deeper. Alaα81, Ileα95, and Serα116 (green sticks) contribute to a much larger pocket F in Mamu-B*17 compared with Mamu-A*01, which has a relative shallow pocket F composed of Leuα81, Leuα95, and Tyrα116. This enables Mamu-B*17 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 Argα2 is stabilized by forming hydrogen bonds and salt bridges with Tyrα9, Serα24, and Gluα45, respectively. For QW9 (orange), the side chain of Thrα2 is too short to insert into the bottom of pocket B, which needs a water molecule to mediate the interactions among Thrα2, Gluα45, and Alaα63. For FW9 (salmon) and GW10 (cyan), the side chains of Ginα2 and Hisα3 hydrogen bond to Tyrα9 and Gluα45.
shape of pocket F; these residues are conserved in HLA-B*5701. Although tyrosine occupies position 116 in HLA-B*5703 (Fig. 2), the side chain is orientated toward the central region of the groove, allowing similar F-anchor preference.

Flexible TCR contact region

Previous studies have indicated that key residues on the α1 and α2 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<sup>69</sup> and Arg<sup>155</sup> 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 SIVmac239-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<sup>45</sup> 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<sup>+</sup> 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<sup>1</sup> and Ser<sup>3</sup>) 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<sup>10</sup> and Trp<sup>11</sup>) 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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Disclosures

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