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Conserved MHC Class I Peptide Binding Motif Between Humans and Rhesus Macaques

John L. Dzuris, John Sidney, Ettore Appella, Robert W. Chesnut, David I. Watkins, and Alessandro Sette

Since the onset of the HIV pandemic, the use of nonhuman primate models of infection has increasingly become important. An excellent model to study HIV infection and immunological responses, in particular cell-mediated immune responses, is SIV infection of rhesus macaques. CTL epitopes have been mapped using SIV-infected rhesus macaques, but, to date, a peptide binding motif has been described for only one rhesus class I MHC molecule, Mamu-A*01. Herein, we have established peptide-live cell binding assays for four rhesus MHC class I molecules: Mamu-A*11, -B*03, -B*04, and -B*17. Using such assays, peptide binding motifs have been established for all four of these rhesus MHC class I molecules. With respect to the nature and spacing of crucial anchor positions, the motifs defined for Mamu-B*04 and -B*17 present unique features not previously observed for other primate species. The motifs identified for Mamu-A*11 and -B*03 are very similar to the peptide binding motifs previously described for human HLA-B*44 and -B*27, respectively. Accordingly, naturally processed peptides derived from HLA-B*44 and HLA-B*27 specifically bind Mamu-A*11 and Mamu-B*03, respectively, indicating that conserved MHC class I binding capabilities exist between rhesus macaques and humans. The definition of four rhesus MHC class I-specific motifs expands our ability to accurately detect and quantitate immune responses to MHC class I-restricted epitopes in rhesus macaques and to rationally design peptide epitope-based model vaccine constructs destined for use in nonhuman primates. The Journal of Immunology, 2000, 164: 283–291.

In contrast to this significant body of knowledge, little is known about the exact nature of the motifs recognized from other species including nonhuman primates. Recent studies indicate that human supertypes extend to chimpanzee (19) and that several, as yet unidentified, chimpanzee class I molecules are associated with a binding specificity resembling that of HLA-A2 or HLA-B7. By contrast, when the motif of the rhesus macaque class I molecule, Mamu-A*01, was defined (20), it was found to be characterized by the somewhat uncommon requirement for three anchor residues located at positions 2, 3, and the C terminus of the peptide, including an original specificity for proline at position 3. The feature of the three anchor residues located at positions 2, 3, and the C terminus has been noted in the case of HLA-A*01 (3), but apart from this isolated incidence, it is rather uncommon. This apparent difference between peptide binding of humans and macaques MHC class I is consistent with the extensive differences seen between human and rhesus macaque class I molecules (21).

The importance of defining nonhuman primate-associated motifs comes from the fact that nonhuman primate models of infectious diseases are important components of studies aimed at elucidating disease pathogenesis as well as useful elements for testing strategies aimed at development of specific vaccines and immunotherapies. Because of relatively abundant availability of animals, and of the close similarities of the immune systems and disease susceptibilities between macaques and humans, rhesus macaques represent one of the best models for the study of the immunobiology of a number of important diseases, including Lyme disease (22), hepatitis virus (23, 24), rotavirus (25), malaria (26, 27), and SIV (28, 29).

In particular, SIV infection of rhesus macaques serves as an excellent model to study the HIV infection and the immunologic responses to HIV in humans. Similar to HIV infection of humans, infection with SIV causes an AIDS-like disease in the majority of infected macaques (30). SIV and HIV are also similar in that both have tropism for CD4+ T cells (31, 32) and that structurally
Materials and Methods

Cells

Stable transfectants expressing rhesus macaque MHC class I molecules Mamu-A*11, Mamu-B*03, Mamu-B*04, and Mamu-B*17 were generated by transferring each cloned cDNA into the HLA class I-deficient human B cell line 721.221 as previously described. Cells were grown in RPMI 1640 (Life Technologies, Rockville, MD) supplemented with 15% FBS (Gemini Bioresources, Calabasa, CA), L-glutamine, penicillin (100 IU/ml), streptomycin (100 µg/ml), and G418 (250 µg/ml; Life Technologies).  

Peptides and 125I labeling

Peptides were obtained as lyophilized crude products from Chiron Technologies (San Diego, CA) or synthesized at Epimmune using standard t-Boc or F-moc solid-phase synthesis methods (36) and purified by standard HPLC methods. Peptides were stored in stock solutions at either 10 mg/ml or 100 µg/ml in 100% DMSO (Sigma, St. Louis, MO) and diluted in RPMI 1640 for use in the cellular assays. HPLC-purified peptides were radiolabeled with 125I according to the chloramine-T method (37).

Live cell binding assays

Peptide binding to Mamu class I molecules was measured as previously described (4, 20). Briefly, Mamu class I-transfected 721.221 cells (106 cells/ml) were preincubated overnight at room temperature in RPMI 1640 supplemented with 15% FBS, L-glutamine, penicillin (100 IU/ml), and streptomycin (100 µg/ml). Cells were then washed twice in RPMI 1640 and resuspended to a final concentration of 1.25 × 106 cells/ml in RPMI 1640 supplemented with 3 µg/ml β2-microglobulin (Scripps Clinic and Research Foundation, La Jolla, CA). Aliquots of 2 × 105 cells/well were incubated at 37°C for 4 h in 96-well U-bottom microtiter plates with 100 µl (10 µl) of specific radiolabeled peptide (1–10 nM) and in the presence of the protease inhibitor mixture (final concentrations of 250 µg/ml PMSF (Sigma), 1.07 mg/ml EDTA (Sigma), 62.5 µg/ml pepstatin A (Sigma), 60 µg/ml TLCK (Calbiochem, La Jolla, CA), and 325 µg/ml phenanthroline (Sigma)). Peptides representing CTL epitopes, or analogues thereof, were used as radiolabeled probes. Specifically, peptide GDYKLIY was used for binding to Mamu-A*11, peptide RHRILDYL for binding to Mamu-B*03, peptide QQYMNTPW for Mamu-B*04 binding, and peptide IRYPKTFGW for Mamu-B*17 binding (38). Following a 4-h incubation at 20°C, unbound peptide was removed by three washes in serum-free medium, followed by one passage through a FBS gradient. The amount of a bound labeled peptide was determined by counting pelleted cells on a gamma scintillation counter. For competitive inhibition assays, a dose range (0.001–100 nM) of unlabeled competitor peptide was coincubated with the radiolabeled probe and the cells. The concentration of peptide yielding 50% inhibition of the binding of the radiolabeled probe peptide (IC50) was then calculated.

Results

Establishment of binding assays for four different Mamu class I alleles

Previous studies using SIV-infected rhesus macaques had identified SIV Nef- and Env-derived CTL epitopes restricted by the four macaque class I alleles, Mamu-A*11, -B*03, -B*04, and -B*17 (38). These epitopes were used to establish binding assays for the respective Mamu molecules. Specifically, the Mamu-A*11-restricted CTL epitope Env 497–504 (GDYKLIY), the Mamu-B*03-restricted epitope Nef 136–146 (ARRHRLDMYL), the Mamu-B*04-restricted epitope Nef 62–70 (QQYMNTPW), and an analogue of the Mamu-B*17-restricted epitope Nef 165–173 (1RFKPTFGW; F167→Y) were 125I-radiolabeled and tested for their capacity to bind 721.221 cells transfected with the relevant rhesus macaque class I alleles. Fig. 1 shows the results of a representative experiment. Approximately 7% of the Mamu-A*11-restricted epitope, Env 497, bound Mamu-A*11-expressing cells. The specificity of interaction was demonstrated by the fact that little or no binding to cells expressing either Mamu-B*03, -B*04, or -B*17 was detected. Similarly, radiolabeled Nef 136, Nef 62, and Nef 165 (F167→Y) peptides bound cells expressing Mamu-B*03, -B*04, and -B*17, respectively. The percentage of bound radiolabeled peptide ranged from 5 to 11.5%. In contrast, little or no binding of peptides to cells expressing the irrelevant MHC restriction was noted.

Assay specificity was further established by demonstrating competitive inhibition by the corresponding unlabeled version of the same peptide (Fig. 2). Typical inhibition curves are shown for each of the radiolabeled peptides binding to its respective MHC class I restriction element. Unlabeled peptide concentrations of 10–50

FIGURE 1. Binding of 125I-labeled peptides to rhesus macaque MHC class I-transfected 721.221 cells. One to 10 nM radiolabeled peptides (100,000 cpm) were incubated with 721.221 cells transfected with Mamu-A*11, -B*03, -B*04, and -B*17 as described in Materials and Methods.

Pocket analysis

On the basis of x-ray crystallographic studies of the structure of HLA class I molecules (14, 39, 40), residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket, and to determine the specificity for the residue in the second position of peptide ligands. Similarly, the polymorphic residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket for the C-terminal residue of peptide ligands. For pocket analyses, the residues comprising the B and F pockets of HLA alleles were compiled from the database published by Parham et al. (41). The sequences for Mamu-A*01 (42) and Mamu-B*03 (21) were also compiled from the published literature. The B and F pocket residues for Mamu-A*11, Mamu-B*04, and -B*17 were determined from DNA sequencing.

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nM were sufficient to inhibit 50% of the binding of the radiolabeled peptide, in good agreement with IC_{50} values reported for other molecules using live cell binding assays (4, 20). In conclusion, the results presented herein illustrate the establishment of sensitive binding assays, specific for four different macaque class I molecules.

Size requirements of macaque class I/peptide interactions

The epitope length required for optimal binding to these macaque class I molecules was analyzed next. Panels of truncated or extended peptides were synthesized for each of the different epitopes and tested for their ability to inhibit binding of the radiolabeled indicator peptide to cells transfected with relevant macaque class I molecules (Table I). In the case of the Mamu-A*11 molecule, the previously defined optimal 8-mer epitope, Env 497–504 (GDYKLVEI), and the LGDYKLVEI 9-mer epitope bound to Mamu-B*03 cells expressing Mamu-B*03, -B*04, and -B*17, respectively, as described in Materials and Methods. Mismatched competitor peptides (open symbols) ARRHRILDMLY, GDYKLVEI, IRFPKTFGW, and QQYMTNPW served as controls for the binding to Mamu-A*11, -B*03, -B*04, and -B*17, respectively.

In conclusion, a good correlation exists between the results of CTL assays and peptide binding assays, thus emphasizing the biological relevance of the assays established and demonstrating that

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

**FIGURE 2.** Dose-dependent inhibition of the binding to rhesus macaque class I-transfected 721.221 cells by excess unlabeled peptide. Peptide/Mamu combinations used were (filled symbols) GDYKLVEI to 721.221 cells expressing Mamu-A*11, peptide ARRHRILDMLY to 721.221 cells expressing Mamu-B*03, peptide QQYMTNPW to 721.221 cells expressing Mamu-B*04, and peptide IRFPKTFGW to 721.221 cells expressing Mamu-B*17. Peptides GDYKLVEI, RRHRILDMLY, QQYMTNPW, and IRFPKTFGW were radiolabeled and used for the binding to Mamu-A*11, -B*03, -B*04, and -B*17, respectively, as described in Materials and Methods. Mismatches competitor peptides (open symbols) ARRHRILDMLY, GDYKLVEI, IRFPKTFGW, and QQYMTNPW served as controls for the binding to Mamu-A*11, -B*03, -B*04, and -B*17, respectively.

![Table 1](http://www.jimmunol.org/)

**Table 1. Size requirement of rhesus macaque class I/peptide interactions**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Peptide Sequence</th>
<th>Length (aa)</th>
<th>IC_{50} (nM)^a</th>
</tr>
</thead>
<tbody>
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<td>Mamu-A*11</td>
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<td>56 *^b</td>
</tr>
<tr>
<td>Mamu-B*03</td>
<td>GGKLYLVEI</td>
<td>8</td>
<td>44</td>
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<tr>
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<td>—</td>
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<tr>
<td>Mamu-B*03</td>
<td>SATRRHRILDMLY</td>
<td>12</td>
<td>—</td>
</tr>
<tr>
<td>Mamu-B*04</td>
<td>QGQYMTNPW</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Mamu-B*17</td>
<td>PGIRFPKTFGW</td>
<td>11</td>
<td>—</td>
</tr>
</tbody>
</table>

^a IC_{50} values represent the concentration of test peptide required to outcompete 50% of the radiolabeled peptide, GDYKLVEI, RRHRILDMLY, QQYMTNPW, and IRFPKTFGW (Mamu-A*11, -B*03, -B*04, and -B*17, respectively). Values represent the average of two to three assays performed in duplicate. ^b Dashes indicate IC_{50} values >1000 nM.
FIGURE 3. Definition of peptide binding motifs of various rhesus macaque MHC class I molecules. A, Relative binding of Mamu-A*11 single amino acid substituted analogues of Env 497–504. Binding is normalized to the binding of the unsubstituted epitope (31 nM). B, Relative binding of Mamu-B*03 single amino acid substituted analogues of Nef 136–146. Binding is normalized to the binding of the unsubstituted epitope (32 nM). C, Relative binding of Mamu-B*04 single amino acid substituted analogues of Nef 62–70. Binding is normalized to the binding of the unsubstituted epitope (17 nM). D, Relative binding of Mamu-B*17 single amino acid substituted analogues of Nef 165–173. Binding is normalized to the binding of the unsubstituted epitope (27 nM). The dashed lines denote levels at which 3-fold and 10-fold reductions in binding are achieved as compared with the unsubstituted peptides.
Definition of a Mamu-A*11-specific peptide binding motif

To define specific motifs, single substitution analogues of the relevant CTL epitopes were synthesized and tested in the appropriate binding assays. For each epitope, five to seven different substitutions were introduced at each position. The effect of conservative, semiconservative, and nonconservative substitutions was investigated. We arbitrarily defined main anchor positions as those associated with at least a 10-fold reduction in binding capacity for the majority of analogues tested. A position was defined as a secondary anchor position if at least a 3-fold reduction in binding was recorded for >50% of the substitutions tested.

The results of the analysis of single substitution analogues of the Mamu-A*11 CTL epitope Env 497–504 (GDYKLVEI) are shown in Fig. 3A. Analogs with non- and semiconservative substitutions at position 2 (P2) and P8 were associated with significantly reduced binding capacities. At P2, the L, K, or N substitutions for D resulted in 20- to 115-fold reductions in binding. In contrast, a D substitution affected binding while a K, L, F, or N substitution resulted in 15- to 20-fold reductions in binding capacity. All substitutions for the C-terminal L were also associated with 10- to 15-fold decreases in binding capacity. This indicated P2 and the C terminus as dominant anchor positions. Greater than 3-fold reductions for a majority of the analogues were also observed at P1 and P5, indicating that they may represent secondary anchor sites. In conclusion, these data define a Mamu-B*03 motif based on a positive charged arginine residue at P2 and an aliphatic C terminus, with possible secondary anchor roles for P1 and P5 (Table II).

Definition of a Mamu-B*04-specific peptide binding motif

The Mamu-B*04 binding capacity of a panel of Nef 62–70 analogues was determined next (Fig. 3C). Six to seven single amino acid substitutions were evaluated at each position. Most notably, all substitutions for the G residue at P2 resulted in an almost complete loss of binding capacity. Even the conservative A substitution resulted in a >1500 binding reduction, indicating a strict requirement for G at that position. A majority of the substitutions tested at P4 were associated with >3-fold loss of activity (A, L, F, N, and D). In particular, an N substitution resulted in a >10-fold decrease in binding, and a D substitution decreased binding by >100-fold. Significant effects were also noted at P5. Substitutions such as A, K, and N resulted in 10- to 100-fold reductions in binding capacity, while a D substitution affected binding >100-fold. Finally, >3-fold decreases in binding capacity were also associated with a majority of substitutions tested at P6. In conclusion, these results suggest a crucial role for P2 and an important role also for P4, P5, and P6 in Mamu-B*04 binding (Table II). A similar crucial role for a glycine residue in P2 has previously been reported only in the case of the murine MHC class I molecule Dd (7). While no narrow affirmative requirement for the C terminus is evident, there does seem to be an accommodation for an uncharged residue. However, the Mamu-B*04 motif described herein is original in its lack of...
strict dependence on a C-terminal anchor and the presence of three contiguous secondary anchors in P4, P5, and P6.

**Definition of a Mamu-B*17-specific peptide binding motif**

Finally, a panel of Nef 165–173 analogues was tested for Mamu-B*17 binding. This analysis revealed a crucial role for the W residue at P9 (Fig. 3D). Three of the five substitutions tested at the P9 C terminus resulted in 90-fold reductions in binding. A conservative F substitution was well tolerated, while the hydrophobic L substitution reduced binding capacity by 8-fold. No other clear main anchor position was revealed by this analysis. However, reductions in binding capacity of at least 3-fold were associated with 50% or greater of the substitutions made at P2, indicating its possible role as a secondary anchor. In summary, these results indicate that the presence of an aromatic residue at the P9 C terminus is the main determinant of Mamu-B*17 binding capacity (Table II).

**Pocket analysis of rhesus macaque class I alleles**

Next, the P2 and C termini motif specificities, defined as described above and summarized in Table II, were correlated with the predicted structure of the B and F pockets of various rhesus macaque class I molecules on the one hand and of human HLA class I molecules of known motif specificities on the other hand.

In the case of the B pocket (Table III), it was noted that class I molecules with preference for small residues, such as Mamu-A*01, HLA-A*0101, and HLA-B*5801, are associated with the consensus motif of M or T at residue 45, N at residue 66, and M at residue 67 (16). In contrast, we found that other alleles, associated with different known P2 specificities, such as Mamu-A*11, HLA-B*4001, HLA-B*4402, Mamu-B*03, HLA-B*2705, and HLA-B*3901, are not associated with the presence of this motif. Similarly, alleles associated with a P2 preference for negatively charged residues are associated with the presence of K at residue 45, I at residue 66, S at residue 67, and N at residue 70. We found that Mamu-A*11, which has similar P2 specificity, also shares this B pocket consensus motif (Table III). This motif is not found in any of the other Mamu molecules that we have analyzed to date.

Finally, a B pocket consensus motif characterized by E at position 45 and small residues in position 67 (such as A or C) and a positive charged residue in position 70 is apparently associated with a P2 specificity for positively charged residues, such as the one displayed by HLA-B*27 and HLA-B*39 (43–45). We found that Mamu-B*03, which has a similar P2 preference, specifically displays a similar motif characterized by E, A, and H in P45, P67, and P70, respectively (Table III).

In the case of the F pocket, alleles with preference for peptides with positively charged C termini are associated with an F pocket motif of D at positions 77 and 116, T at position 80, and L at position 81 (16). In particular, we hypothesized that the simultaneous presence of the two negative charges, D at positions 77 and 80, in combination with a positive charge at position 81, is a critical determinant for binding to Mamu-B*03 (Table III).
116, is crucial for high-affinity engagement of the positively charged C termini (16). Such a motif is not found in either human HLA alleles (16) or rhesus macaque class I alleles with a preference for hydrophobic or aromatic residues at the C termini of their ligands (Table IV).

In conclusion, the analysis presented herein suggests that common features exist in the B and F pockets of human and macaque alleles and that these common features correlate with similarities in the peptide binding motifs associated with such molecules.

**Overlap in peptide binding repertoire between rhesus macaque (Mamu) and human (HLA) class I molecules**

Whether a significant overlap in peptide binding repertoire exists between HLA-B*4402 and Mamu-A*11 on one hand and HLA-B*2705 and Mamu-B*03 on the other hand was tested next. The human HLA molecule B*4402 is associated with a motif characterized by E at P2 and Y or F at the C terminus (46). This motif overlaps significantly with the motif defined in the current study for Mamu-A*11. Similarly, the reported motif of HLA B*2705, characterized by R at the P2 anchor and an aromatic/aliphatic C-terminal anchor (43, 44), overlaps significantly with the one herein defined for Mamu-B*03. As mentioned above, the analysis of polymorphic pockets of Mamu-A*11 and Mamu-B*03 also suggested that a significant overlap might exist in the peptide binding repertoire of these alleles with the human HLA-B*4402 and HLA-B*2705 class I molecules, respectively.

To test this hypothesis, a panel of peptides corresponding to previously described HLA-B*4402 naturally occurring sequences of bacterial or viral origin (46–50) were synthesized and tested for their binding capacity to Mamu-A*11 (and Mamu-B*03 as a specificity control). A total of five HLA-B*44 peptides were tested, each a 9-mer peptide and each with the negatively charged E residue at P2. Three peptides had aromatic residues at the C terminus, and two peptides had L residues at the C terminus. Four of these five peptides bound Mamu-A*11 with IC50 in the 100- to 500-nM range. In contrast, none of these peptides bound appreciably to Mamu-B*03 (Table V).

Next, a panel of peptides corresponding to HLA-B*27 endogenously bound sequences (43) were synthesized and tested for binding to Mamu-B*03 (and Mamu-A*11 as a specificity control). A total of six HLA-B*27 peptides were tested (one 10-mer and five 9-mer peptides) each with a positively charged residue (arginine) at P2. Four peptides had aromatic residues at the C terminus, and two peptides had L residues at the C terminus. Two of the six peptides bound to Mamu-B*03 with IC50 below 100 nM, and two peptides bound with IC50 in the 100- to 500-nM range (Table V). None of these peptides bound appreciably to Mamu-A*11.

In conclusion, the results presented in this section demonstrate a significant overlap in peptide binding specificity between specific rhesus macaque class I molecules and certain human HLA class I molecules of similar motif specificity.

**Discussion**

In the series of experiments presented here, we describe cell binding assays that allow the quantitation of peptide binding to rhesus macaque MHC class I molecules. These assays were used to define peptide binding motifs specific for four different rhesus macaque class I alleles (Mamu-A*11, -B*03, -B*04, and -B*17). Some features of these motifs appear to be unique to class I molecules of rhesus macaque origin. However, the motifs associated with rhesus macaque Mamu-A*11 and -B*03 molecules are very similar to those associated with the human class I HLA-B44 and -B27 molecules, respectively (44, 46), establishing a previously unappreciated functional overlap between the binding repertoire of rhesus macaque and human class I molecules.

Our observations demonstrate that live cell binding assays can be used as a general method to measure peptide binding to rhesus class I MHC molecules. Additionally, the data provides a first glimpse at the relation between MHC binding affinity and immunogenicity in rhesus macaques. Previous data suggests that a binding affinity of 500 nM or less (and preferably 50 nM or less) is associated with immunogenicity in humans (51). The four SIV CTL epitopes tested here bind to their restricting macaque class I molecule with affinities <50 nM, suggesting that this correlation can also be extended to rhesus macaque class I molecules. As in the case with both human and murine class I, epitopes of 8–11 aa bind optimally to rhesus macaque class I molecules (6).

The present study defines four new rhesus macaque peptide binding motifs. Thus, together with the previously described Mamu-A*01 motif (20), a total of five macaque class I motifs have now been described. As discussed below, clarification of these motifs should greatly facilitate quantitation of immune responses and design of specific vaccine immunotherapies in the rhesus macaque.

The crucial features of the five macaque motifs thus far described can be analyzed in the context of the large body of information available for motifs of both murine and human origin. Several original features are apparent. First, most of the macaque motifs thus far defined do not strictly follow the P2/C-terminal main anchor paradigm that applies to most human class I motifs. For example, the Mamu-A*01 peptide motif did not possess strong P2 and C-terminal anchors, and its dominant anchor was found at P3 (20). Precedents for P3 anchors have been reported in the context of both the murine (H-2Dd) and human (HLA-A*01 and -B*08) systems (16, 52–53).

Similarly, we show herein that Mamu-B*04 displays a strict requirement for G at P2 and lacks a defined requirement for the C terminus. A prominent influence on binding capacity is associated with the central portion of the peptide, with secondary anchor residues in P4, P5, and P6. This motif spacing is unique to rhesus class I molecules studied to date, although murine H-2Dd motif also requires a G at P2 (7). Conversely, the Mamu-B*17 motif does require an aromatic residue at the C terminus, but the P2 plays only a relatively minor and secondary role.

These original features of the macaque MHC class I peptide binding motifs are not unexpected given the relatively large sequence diversity and phylogenetic difference between human and rhesus macaque MHC class I molecules (21). However, it was also noted that the Mamu-A*11 and -B*03 peptide binding motifs were strikingly similar to the HLA-B44 and -B27 motifs, respectively. These motif similarities were correlated with structural similarities in both B and F pocket structures of the relevant HLA-MHC class I molecules. The functional consequence of such structural similarities was demonstrated by the capacity of HLA-B44 ligands to specifically bind Mamu-A*11 and by the capacity of known endogenously bound HLA-B27 ligands to specifically bind to Mamu-B*03. As expected, this overlap in repertoire was only partial as previously seen in the case of HLA alleles belonging to the A3 or B7 supertypes (16). We do acknowledge the fact that the data regarding the HLA-B*4402 and -B*2705 ligand binding is generated by a different assay. However, the fact that the peptides used in the live cell binding assays were peptides isolated as naturally processed binding epitopes does to our mind suggest that this binding is biologically relevant by definition.

It should be noted that these four rhesus MHC class I peptide binding motifs can and will be further refined as more data points...
will be generated. Additionally, secondary effects might be revealed by the use of ligands with less optimal residues occupying the primary MHC class I pockets.

It is interesting to interpret these findings in the context of the recently described human HLA class I supertypes. A series of studies has demonstrated that the majority of human HLA class I can be classified in one of nine major supertypes (16-18). Recent data also demonstrates that HLA supertypes extend to the chimpanzee MHC class I molecules (19). Based on the results presented herein, it can be concluded that HLA supertypes similarly extend to rhesus macaques. The functional overlap in repertoires of many primate class I molecules could be reflective of a common ancestry of the class I genes encoding these molecules. Alternatively, convergent evolution could explain these structural and functional similarities.

The results presented herein are also important because they enable future studies aimed at the identification of epitopes that would be recognized in the context of macaque MHC class I alleles.

Identification of macaque MHC class I-restricted peptide binding motifs will facilitate development of a variety of different rhesus macaque disease models, including Lyme disease (22), hepatitis virus (23, 24), rotavirus (25), and malaria (26, 27). Such advances could include the capacity to detect accurately and map specific immune responses, as a tool toward understanding disease course and ethiopathology (57), as well as enabling the design and testing of specific vaccine and immunotherapy strategies.

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References


