Distinct conformations of a peptide bound to HLA-DR1 or DRB5*0101 suggested by molecular modelling

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Abstract

The conformation of peptides when bound to different HLA class II molecules is of interest in the study of specificity and function of responding T cells. Here, we report the investigation of the HLA-DR binding profiles of an immunodominant and HLA-promiscuous mycobacterial peptide, p38G. Its binding affinities were found to be high for DR1, moderate for DR2, DR7 and DR8, low for DR4, DR5, DR6 and DR9, and below detection for DR3. The minimum peptide length required for binding was, in the majority of cases, nine residues and 11 in two instances (DR2 and DR4). Peptide binding to DR2 was attributed to the DRB5*0101 and not to the DRB1*1501 gene product. Substitution analysis of the amino acid residues involved in binding to DR1 and DRB5*0101 identified F-354 as the common primary contact residue (P1), while allele-specific differences were found in positions P4, P6 and in the C-terminal anchor residue (valine at P9 for DR1 or lysine at P10 for DRB5*0101). Computer-assisted evaluation of these empirical data produced a molecular model, suggesting that the peptide binds to DR1 in an elongated conformation, similar to that of other peptide MHC class II complexes. In contrast, the DRB5*0101 bound peptide is likely to be kinked, which so far was considered characteristic only for peptides within MHC class I complexes. The different conformations imposed on the same peptide by distinct HLA alleles may represent an important mechanism for the control of T cell responses.

Introduction

T cell recognition of proteins involves binding of distinct peptide regions to the polymorphic, allele-specific domains of MHC molecules (1). The diversity of immunodominant sequences recognized in the context of the widely polymorphic HLA genotypes in man represents an inherent obstacle for the development of synthetic peptides as potential diagnostic tools or vaccine subunits. Therefore, a special interest is attached to promiscuous, immunodominant peptides, which have been identified in several microbial antigens (2–10), endogenous proteins (11) and potential autoantigens (13). The mechanism of their recognition involves the binding of different key residues within the same epitope core sequence to different MHC class II molecules and/or TCR (12–14, 18). A better understanding of the structural features of immunogenic peptides in relation to multiple DR alleles is necessary for the rational design of permissively recognized variant peptides of biological interest. Furthermore, the affinity of MHC peptide binding, which will influence the density of MHC peptide complexes at the cell surface, and the conformation of MHC peptide complexes may influence interaction with the TCR and the development of different cytokine secretion patterns of T cells (16, 18).

For the detailed analysis of key residues, we selected the immunodominant epitope p38G (amino acids 350–369) from the 38 kDa antigen of Mycobacterium tuberculosis. This peptide stimulates proliferation of peripheral blood mononuclear cells (PBMC) in the majority of healthy tuberculin-positive individuals (19), thus implying that it is recognized in the context of several different HLA alleles. Although the same epitope core was found to be recognized when tested with T
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Table 1. Binding properties of p38G in relation to different HLA-DR alleles

<table>
<thead>
<tr>
<th>HLA</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Binding core&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HLA-DR contact residues&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>1.8 ± 0.2</td>
<td>FQPLPPAVV</td>
<td>F-354, L-357</td>
</tr>
<tr>
<td>DR2</td>
<td>17 ± 1.9</td>
<td>HFQPLPPAVVK</td>
<td>F-354, A-360, K363</td>
</tr>
<tr>
<td>DR3</td>
<td>&gt;1000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DR4</td>
<td>200 ± 25</td>
<td>HFQPLPPAVVK</td>
<td>F354</td>
</tr>
<tr>
<td>DR5</td>
<td>59 ± 16</td>
<td>FQPLPPAVV</td>
<td>F-354, L-357, V-362</td>
</tr>
<tr>
<td>DR6</td>
<td>75 ± 21</td>
<td>FQPLPPAVV</td>
<td>F-354, L-357</td>
</tr>
<tr>
<td>DR7</td>
<td>21 ± 10</td>
<td>FQPLPPAVV</td>
<td>F-354, V-302</td>
</tr>
<tr>
<td>DR8</td>
<td>12 ± 3.0</td>
<td>FQPLPPAVV</td>
<td>F-354, L-357, A-360</td>
</tr>
<tr>
<td>DR9</td>
<td>210 ± 40</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

<sup>a</sup>p38G peptide concentration which inhibits by 50% binding of 1.6 µM bio<sub>3</sub>CLIP to DR3 and DR9 or binding of 1.8 µM bio<sub>3</sub>H3A307 to other DR alleles.

<sup>b</sup>Minimal length of p38G which shows at least 30% of binding capacity of the 20-mer peptide.

<sup>c</sup>The p38G residues whose substitution by alanine or leucine led to at least five times lower or higher DR binding affinity: IC<sub>50</sub>p38G/IC<sub>50</sub> variant >5.

NT, not tested.

Methods

Synthetic peptides

Peptides of >85% purity were produced using solid-phase/ Fmoc chemistry in-house or purchased from Chiron (San Diego, CA). Sequence integrity was verified by mass spectrometry and homogeneity by reverse-phase HPLC. No significant differences in binding affinities were found between different batches of the same peptide sequence. N-terminally biotinylated peptides were produced by incubation on a shaker of the fully side-chain protected peptide-resin in different batches of the same peptide sequence. N-terminally biotinylated peptides were produced by incubation on a shaker of the fully side-chain protected peptide-resin in a solution containing 1.5 mol equivalents of both Fmoc-9-fluorenylmethoxycarbonyl-L-lysine and 9-fluorenylmethoxycarbonyl-L-lysine in dimethyl sulfoxide (50 ml/mmol) for 2 h. The p38G peptide was synthesized as a 20-mer (DOVHFOQPLPPAWKVLSDALI) or biotinylated 20-mer (bio<sub>20</sub>p38G) and as truncated or substituted variants (Table 1). Biotinylated reference peptides for binding competition assays were represented by the HA/306-18 influenza haemagglutinin peptide (APKYVKONTLKLAT) with an added N-terminal alanine and the human invariant chain 104–119 (CLIP) peptide (VSKMRM-ATPLMOALP). The reference for selective binding to HLA-DRB<sub>1</sub><sup>1</sup>1501 using purified HLA-DR2, containing both B1 and B5 β chains, was served by the HLA-A3 (153–166) peptide (AEQLRAYLDGTGVE).

Cells

HLA-DR homozygous B cell lines HOM-2, MGAR, VAVY, BOLET, SWEIG, AMALA, MOU, MADURA and DKB, obtained from The European Collection of Animal Cell Cultures, were grown in RPMI 1640 medium, supplemented with 10% FCS, 1.0 mM glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. The HLA class II-negative plasmacytoma cell line J588L (20) transfected with DRA<sup>1</sup>101 and DRB<sup>5</sup>101 was grown in the same medium supplemented with HMX selective media (5 µg/ml hypoxanthine, 15 µg/ml mycophenolic acid, 200 µg/ml xanthine and 0.5 mg/ml G418).

Purification of HLA-DR molecules

HLA-DR molecules were purified using methods described elsewhere (21,22). Briefly, Epstein-Barr virus-transformed lymphoblastoid B cell lines (LBC) at 10<sup>8</sup> cells/ml were lysed in PBS containing 1% NP-40, 0.5 mM phenylmethylsulphonyl fluoride and 0.05% Na<sub>3</sub>N<sub>3</sub> for 2 h at 4°C, and then centrifuged at 10,000 g for 1 h. Supernatants were passed at 0.5 ml/min through 5 ml Protein A Sepharose-CL4B columns, covalently coupled to the L243 mAb. The column was then washed with 50 ml PBS, 10 mM n-octyl β-D-glucopyranoside and eluted with pH 11.0 buffer (0.15 M NaCl, 0.05 M NaHCO<sub>3</sub>, 10 mM n-octyl β-D-glucopyranoside), with 1.0 ml fractions collected directly into 0.25 ml of 1 M Na<sub>2</sub>HPO<sub>4</sub> (pH 6.8). The HLA-DR protein-containing fractions were pooled, concentrated using Centricon-30 ultra-filtration membranes (Amicon, Beverly, MA) and protein concentrations were determined using the Microtitre BCA assay (Pierce, Rockford, IL).

HLA-DR-peptide binding assays

(i) Purified HLA-DR binding assay (22). The reaction mixture in 0.5 ml Eppendorf tubes contained the HLA-DR molecules (0.5 µM), a biotinylated reference peptide (1.6 µM bio<sub>3</sub>CLIP or 1.8 µM bio<sub>3</sub>H3A306–18) and competitor peptide (0.1–1000 µM) in a total volume of 30 µl of 'binding buffer' (0.1 M sodium citrate, pH 6.0 ± 0.2% NP-40). After incubation at room temperature for 48 h, the MHC peptide complexes were diluted with 100 µl of 'blocking buffer' (PBS ± 5% non-fat dry milk + 0.1% Tween-20), divided into triplicate sets and transferred to a 96-well microtitre plate, pre-coated with 1 µg/well of monomorphic anti-HLA-DR mAb (L243). After 2 h incubation with shaking at 4°C, the plates were washed six times with PBS + 0.1% Tween-20 (PBST) and the quantity of bound biotinylated peptide was determined colorimetrically using streptavidin peroxidase and tetramethyl benzidine (Sigma, Poole, UK).

(ii) Cellular binding assay (23). Washed LBC in serum-free medium were incubated in 96-well microtitre plates (10<sup>5</sup> cells/well) with the reference peptide bio<sub>3</sub>H3A306–18 (17 µM) and different concentrations of competitor peptides (1–1000 µM). After overnight incubation, the cells were lysed using PBS + 1% NP-40, the lysates were transferred to a 96-well microtitre plate pre-coated with 1 µg/well of L243 and subsequently tested for bio<sub>3</sub>H3A306–18 binding by the procedure described above for purified HLA-DR molecules.

In both assays, absorbances in the presence of the refer-
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Fig. 1. Recognition of p38G in the context of B5*0101. (A) Binding of p38G to DRB5*0101, but not to DRB1*1501. Different concentrations of DRB1*1501-specific peptide, HLA-A3/153-66, and DRB5*0101-specific peptide, HA/306-18, were added to compete with the binding of 8 μM biotinylated p38G to HLA-DR2. Absorbance in the absence of inhibitor was 0.3-0.4 and background was <0.02. (B) Proliferation of p38G-specific T cell line in the context of HLA-DRB5*0101. T cells were incubated with different concentrations of p38G in the presence of J558L cells transfected with DRB5*0101 molecule. Mean c.p.m. of [3H]thymidine uptake ± SD from three experiments; background: 4218 ± 1365 c.p.m. in the absence of peptide has been subtracted.

Generation and assay of T cell lines

A T cell line was derived from a DR15, DQ5-typed, healthy, purified protein derivative-positive donor using previously established methods (24). Briefly, 1 x 10^7 PBMC in 10 ml RPMI 1640 medium were stimulated with 50 μg/ml p38G; T cell blasts isolated after 10 days were incubated with peptide-preloaded, irradiated (3000 rad) autologous PBMC and rIL-2 (20 U/ml) for 7 days, and re-stimulated weekly in 24-well plates. T cell lines were rested for 7 days before each assay. Proliferation of the T cell line was tested by incubation of 2 x 10^4 T cells/well with 6 x 10^4/well of γ-irradiated (5000 rad) HLA-DRB5*0101-transfected BCL (see above) and different concentrations of p38G for 72 h. After pulsing with 1 μCi/well [3H]thymidine for 16 h, cells were harvested with an automatic cell harvester (Skatron, Lier, Norway) and the incorporation of radioactivity was determined by liquid scintillation counting.

Computer-assisted molecular modelling

The model of the DRA:DRB5*0101 molecule was built from that of the HLA-DR1 molecule, (25,26), using the COMPOSER suite of programs, (28,29) contained within SYBYL (Tripos Associates, St Louis, MO). In brief, the sequence of the DRB5*0101 chains was built into the co-ordinates of the MHC class II β chain of the HLA-DR1:influenza HA/306-18 structure using COMPOSER and subjected to energy minimization. To model the peptide in either DR1 or DRB5*0101, candidate residues for the P1 position were used to align the test sequence within the binding cleft. Alignments that gave unacceptable contacts were eliminated and the remaining alignments were ranked by inspection of the peptide side chain interactions with the MHC molecule, particularly those with the P1, P6 and P9 pockets, and by calculating interaction energies for those pockets (30). The optimal model was refined using a gradient torsional optimization algorithm. Initially the main chain of the molecule and the Cβ carbon atoms were fixed. Following convergence of the side refinement, all atoms in the molecule were released and the optimization was performed on the entire model. In the case of the peptide bound to DRB5*0101, a satisfactory choice of ligands for the P1 and P9 pockets resulted in the introduction of a kink in the peptide; residues 1-3 and 8-10 were initially held constant while the conformation of residues 4-7 was optimized; subsequently the conformation of the whole peptide was optimized as described above.

Results

Binding affinity and minimum length of p38G in multiple DR alleles

Purified DR proteins or LBC, homozygous for nine different HLA alleles were used to determine quantitative differences in the binding affinities of p38G. The results, in Table 1,
showed that p38G inhibited binding of the reference peptides to eight different DR alleles, with the single exception of DR3. The binding affinities were found to be high for DR1 (IC$_{50} < 10$ μM), moderate for DR2, DR5, DR6, DR7 and DR8 (IC$_{50} = 10$–100 μM), and low for DR4 and DR9 (IC$_{50} > 100$ μM).

The minimum peptide length required for DR binding was ascertained using truncated peptides, varying in length between eight and 15 amino acids. The results (Table 1) showed that most alleles, i.e., DR1, DR5, DR6, DR7 and DR8, required at least nine residues (354–362), whereas a minimum of 11 residues (353–363) was required for binding to DR2 and DR4.

Substitutions of single residues within the p38G epitope core with alanine or leucine (pepscan) were used to identify the contact residues for the seven positively binding HLA-DR alleles. The results (summarized in Table 1) showed that the F-354 to A substitution abrogated peptide binding to all seven alleles, hence allowing us to identify it as the N-terminal primary anchor residue (P1). In contrast, inhibition or enhancement of peptide binding caused by substitutions of L-357 (P4), A-360 (P7), V-362 (P9) and K-363 (P10) varied considerably between the tested alleles.

DR2 recognition of p38G is targeted to the DRB5$^*$0101 and not to the DRB1$^*$1501 molecule

HLA-DR2 binding of p38G, described in the preceding paragraphs, has been characterized by lower affinity, longer peptide length and the role of positively charged K-363, when compared with DR1. We next used specific peptide probes (47) to find out which of the two expressed constituent loci, DRB5$^*$0101 or DRB5$^*$0101, was responsible for the p38G binding. The results showed that the binding of $^{\text{lep}}$p38G to purified DR2 protein was completely inhibited by the DRB5$^*$0101-specific HA/306–18 peptide (IC$_{50} = 3.7 ± 1.0$ μM) but not by the DRB1$^*$1501-specific HLA-A3/153–166 peptide (Fig. 1A).

It was of further interest to test if T cells can effectively recognize p38G in the context of HLA-DRB5$^*$0101. For this purpose, a T cell line derived from a HLA-DR2-typed donor (DR15, DR51, DQ5, DQ6) were stimulated with peptide in purpose, a T cell line derived from a HLA-DR2-typed donor (DR15, DR51, DQ5, DQ6) were stimulated with peptide in the presence of antigen presenting cells which were co-

Allele-specific effects resulted from substitutions of several core residues. Thus, L-357 at position P4 (relative to F-354 as P1) was found to be more important in binding to DR1 than to DRB5$^*$0101, as shown by the selective, 1000-fold decrease in binding to DR1, resulting from substitutions with charged residues such as lysine or aspartic acid. Substitutions of P-359 (position P6) with either large (L, Y or Q) or charged (K or D) residues decreased 6- to 500-fold peptide binding selectively to DR1, suggesting the requirement of a small, uncharged residue. The effects of substitutions of A-360 (P7) had a different pattern for the two alleles: only the negatively charged aspartic acid produced a pronounced decrease in binding to DR1, whereas all substitutions except threonine led to a 6- to 8-fold decrease in binding to DRB5$^*$0101. Substitutions with the charged aspartic acid or lysine at position V-362 (P9) also profoundly decreased binding to DR1. In contrast, lysine substitution at P9 significantly enhanced binding to DRB5$^*$0101.
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resulted from aspartic acid or glutamine substitutions of the C-terminal K-363 (P10). This abrogated binding to DRB5*0101 but had no effect on binding to DR1 (Fig. 2). Hence, a distinct role of the C-terminus toward a DR pocket is represented by K-363 (P10) for DRB5*0101 but by V-362 (P9) for the DR1 allele. The steric aspects were further analyzed using a shorter, P-358 deletion peptide analogue (P358/var) with a proximal P9 placement for K-363. The results (Fig. 3) showed that P358/var compared with the native p38G peptide bound with significantly lower affinity ($P < 0.01$) to DR1 ($IC_{50} = 12.3 \pm 1.5$ and $2.3 \pm 0.3$ nM, respectively) but with higher affinity ($P < 0.005$) to DRB5*0101 ($IC_{50} = 1.9 \pm 0.3$ and $11.8 \pm 0.8$ nM respectively). This result confirmed the optimal length of the binding pocket for a peptide nonamer for both alleles, but with a distinct preference of the C-terminal valine for DR1 and lysine for the DRB5*0101 allele.

In order to further ascertain the role of C-terminal positively charged residue in peptide binding to DRB5*0101 we have tested a set of five peptides with lysine (K) shifting from relative position 7 to 11 within a polyalanine matrix (Fig. 4). Peptide binding to DR1 was found to be 5-fold decreased only if lysine was at P9. In contrast, binding to DRB5*0101 was 10-fold increased by placing lysine at P9. Furthermore, lysine placed at either P7 or P11 led to 3- to 5-fold decrease in binding to DRB5*0101. The peptides with lysine at P8 and P10 bound to DRB5*0101 with affinity similar to parental 38G peptide. These data have confirmed a role for the positively charged residue as a C-terminal anchor in peptide binding to DRB5*0101.

**Molecular model of the p38G complexed with HLA-DR1 and HLA-DRB5*0101**

Based on the binding data presented above, we have constructed molecular models of the p38G complexes with both HLA-DR1 (Fig. 5A) and HLA-DRB5*0101 (Fig. 5B) molecules. The conformation of the peptide bound to DR1 is essentially the same as those of the influenza haemagglutinin 306–18 and CLIP peptides bound to DR1 (26,31). The conserved interactions that the DR molecule makes with the peptide backbone constrain the conformation of the peptide so it has been assumed that most peptides will, in fact, be bound in a similar manner. The striking feature of the DRB5*0101 model is that, in order to accommodate F-354 as the N-terminal (P1) and K-362 as a C-terminal anchor residues, the conformation of the peptide must differ from that seen in DR1. We suggest that this results from two factors: DRB5*0101 has a strong preference for a positively charged C-terminal anchor residue and the presence of proline residues at P5 and P6 prevents the formation of bidentate hydrogen bonds by Asn-a60, weakening the interaction between the MHC molecule and the central part of the peptide. The kink introduced into the peptide also disrupts the normal hydrogen bonds formed between Gln-a7 and the backbone of the P4 residue, in this case L-357. The p38G with deleted P358 (P358/*var) can be accommodated in DRB5*0101 without a kink, and can form hydrogen bonds to Gln-a7; this is the likely explanation for the increased binding affinity of P358/*var.

**Discussion**

This is the first study on the MHC binding properties of p38G, which has previously been characterized as a genetically permissive (promiscuous) epitope for both human and murine T cells (9,19). Using purified HLA-DR molecules, we have demonstrated significant differences in the binding affinities of p38G to a wide range of DR alleles. Such variations may explain why a proportion of sensitized human T cells fail to proliferate in vitro in response to p38G. The results from the peptide DR binding assay attribute the 'gap' in the repertoire to DR3, which was the single allele (out of nine tested) which failed to give any demonstrable binding of p38G. It should be noted that in mice of B10 background, T cells of only one haplotype (H-2A{sup f}) out of eight tested had failed to respond (9). Results from both species corroborate the conclusion that p38G represents a selectively, rather than universally, promiscuous epitope. Furthermore, the results of DR binding confirm those from T cell assays (9) as to the position of the minimal binding and stimulatory 9-mer epitope core.
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(FQPLPPAVV), with the exceptions of DR2 and DR4, which require an additional C-terminal lysine (discussed below).

In our binding assays, relatively high concentrations of the biotinylated indicator peptide (1.6–1.8 μM) giving ~50% maximal binding were used. Similar concentrations (0.5–15 nM) of biotinylated peptide were used by others (23,32–34). The IC50 values obtained in assays based on biotinylated indicator peptide tend to be in the micromolar range, rather than the nanomolar range of assays based on radiolabelled peptides and gel filtration (7,21,35,36). Despite the lower sensitivity of our binding assay, adequate resolution between the tested peptides was obtained.

Detailed analysis of the p38G binding profile identified a major difference in the binding of the C-terminal part of the peptide to the DR1 (DRB1*0101) and DRB5*0101 molecules. The essential role of the positively charged K-363 seems to be a unique feature for DRB5. However, K-363 at P10 is one residue further than the corresponding V-362 contact residue for DR1. Hence, either the peptide binding cleft of DRB5*0101 is longer or the peptide is forced to bulge outwards in its centre, so as to fit in its normally-sized binding cleft. Our results support the second explanation, since shifting the position of K-363 from P10 to P9 significantly increased the peptide's binding affinity. Consequently, our data indicate that the conformations of p38G bound to DR1 and DRB5*0101 are distinct. Peptide binding together with molecular modelling
suggest that p38G is bound to DR1 in an extended conformation, similar to that seen in the HA306-18-DR1 complex (26), whilst p38G bound to DRB5*0101 has a bulge in its central region and is anchored at its C-terminal end by K-363 (P10), binding into the P9 pocket.

Our results support the view that different MHC alleles may impose different conformations on the same bound peptide. It is known that complexing with MHC class I molecules causes longer peptides to bulge in their centres, so that their termini fit into the binding groove (38). This feature has been thought to be characteristic of class I molecules, as they specifically interact with the peptide's terminal groups, allowing the central part of the peptide to adopt a less constrained conformation (39). On the other hand, MHC class II molecules have been thought to bind peptides with their central parts fixed by multiple interactions with the MHC molecule, while their termini are relatively free to move (25,26). However, the results of our studies with p38G suggest that peptides may bulge, even when bound to class II molecules. Although the enforced conformation of the bound peptide appears to be at the expense of its inherent DRB5*0101 binding affinity, we have demonstrated that the moderate binding affinity attained was sufficient for the effective stimulation of a T cell line of corresponding specificity.

Substitution analysis of p38G binding to DR1 indicates a binding motif consisting of an aromatic or large hydrophobic residue at P1, an uncharged residue at P4, a small and uncharged residue at P6, and a hydrophobic residue at P9. This finding is in line with previous reports of the DR1 binding motif (7,26,27). The structural motifs of peptide binding to DR2 are of particular interest in view of the known association of this DR specificity with various diseases (40,41), including tuberculosis and leprosy (42,45), but the respective roles of the B1 and B5 loci is not known. The peptide binding motif of DRBS*0101 consists of an aromatic residue at P1, an aliphatic (I, L, V or M) or polar (Q) residue at P4 and possibly a positively charged residue at P8 or P9 (46,47). The results of our detailed peptide binding analysis broadly agreed with these motifs and expanded the structural knowledge. In the light of our own data, we suggest that the DRBS*0101 motif consists of an aromatic or large hydrophobic residue at P1, a small residue at P6 and a positively charged residue at P9. In addition, negatively charged residues are not permissible at P7.

The main feature of the DRBS*0101 binding motif appears to be a strong need for a positively charged contact residue at P9, but the flexible peptide conformation allows contact with P8 or P10 as alternatives. This view is supported by the reported finding of positively charged residues at C-termini of the endogenous peptides eluted from DRBS*0101 (47). The data obtained using peptides with lysine shifting from relative position 7 to 11 within a polyalanine matrix showed that peptide with lysine at P9 binds with highest affinity to DRBS*0101, whereas peptides with lysine at P8 or P10 and peptides with lysine at P7 or P11 bind with moderate or low affinity respectively. These results confirmed that the positively charged residue at P9 is optimal for binding to DRBS*0101. This view is supported also by the reported finding of positively charged residues at C-termini of the endogenous peptides eluted from DRBS*0101 (47). The binding pocket of the DRBS*0101 molecule, which accommodates the P9 residue, has three negatively charged residues (β30, β37 and β57) which may explain the preference for positively charged residues. Furthermore, it has been proposed that the positively charged peptide residues are necessary for the stability of DRBS*0101 molecule (C. Zamoyska et al., submitted for publication). Finally, the possible relationship of MHC peptide conformations to the resulting T cell functions will need to be explored in the light of the recent association of MHC–peptide binding affinity with the cytokine secretion patterns of stimulated T cells (16–18).

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Abbreviations

LBC Epstein Barr virus-transformed lymphoblastoid B cell line
PBMC peripheral blood mononuclear cell

References


