Selective contact during TCR recognition

Ming-Hsien Lin Feng1,2, Yu-Chi Shen1,2, Ding-Li Chou2,3, Ming-Zong Lai1,3 and Yen-Chywan Liaw2

1Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical School, Taipei, Taiwan, ROC
2Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC
3Graduate Institute of Immunology, National Taiwan University, Taipei, Taiwan, ROC

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Abstract

Recent structural analysis of the peptide–MHC complex reveals that an antigenic peptide binds to MHC in only one conformation and that side chains anchoring in the binding pocket would not contact TCR. The identification of all the MHC-anchoring residues on an antigenic peptide is a prerequisite to understand how a given peptide interacts with the TCR. In a combination of binding analysis and model simulation, model peptide λ repressor cl 16–26 was shown to bind to I-Ek through four anchor residues (Leu18, Ile21, Glu23 and Lys26), a pattern found in many I-Ek-binding peptides. TCR reactivity analysis clearly indicates a great variation in the interaction with cl 16–26 by T cells generated from different strains of I-Ek-bearing mice. Most of the T cells generated from A/J mice reacted with the central region of cl 16–26, while there is a great diversity on the recognition of cl 16–26 by T cells from C3H and B10.BR mice. Despite the diverse interactions with antigenic peptide by these T cells, most TCR–I-Ek contacts are limited to the central region of the I-Ek β-chain. T cells recognizing only the N-terminal part of cl 16–26 were found to contact I-Ek at nearly the same residues as T cells interacting with the C-terminal of cl 16–26. TCR–I-Ek recognition was apparently independent of TCR–cl 16–26 contact. The discordant TCR–peptide and TCR–MHC interactions may represent a unique feature of TCR recognition.

Introduction

The TCR recognizes peptide binding to a MHC molecule (1–3). The understanding of the three-way interaction between TCR, peptide and MHC molecule has been greatly facilitated by recent rapid progress in the delineation of the MHC structure. Crystallographic studies revealed a peptide-binding groove formed by two α-helices lying parallel on a β-sheet on both class I and class II MHC molecules (4–7). Sequencing of the class I-binding peptides identified a motif shared by peptides that bind to the same allele (8). The anchor residues of the peptide, characteristic of each binding motif, are shown to dock in the allele-specific pockets inside the binding groove of class I MHC molecules (9–14). Allele-specific anchors were also identified in a number of class II MHC (15–18). The binding of peptide to class II is similarly mediated by positioning these residues into the specific pockets inside the MHC (19). Since antigenic peptides bind to MHC in only one conformation (9–14,19,20), a MHC anchor residue can be excluded from TCR contact and vice versa. Identification of all the anchor residues on a peptide is thus a prerequisite to elucidate its TCR recognition. In most commonly employed methods, peptides with site-specific mutations are tested on binding analysis. However, not all mutations in the anchor residue will affect the specific MHC binding (21,22), thus a few anchor residues may not be revealed through such analysis. In the present study, we have used both binding analysis and model simulation to identify all the anchor residues of I-Ek-binding peptide λ repressor cl 16–26. Two of the I-Ek-anchoring residues of cl 16–26 were first identified through competitive binding assay of cl 16–26 variants. The information was used to simulate an interaction between cl 16–26 and I-Ek based on the high homology between I-Ek and HLA-DR1. In terms of the selective interaction with the TCR-contacting sites on cl 16–26, great variation was found between different T cells. Interestingly, the diverse TCR–antigen interaction was accompanied with a restricted TCR–I-Ek contact. The relatively conserved TCR–I-Ek interaction may represent a unique feature of TCR recognition.

Methods

Animals and cell lines
A/J, B10.BR and C3H mice were obtained from National Cheng-Kung University School of Medicine. CTLL 2 and HT-2

Correspondence to. M.-Z. Lai, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan, ROC
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were obtained from the ATCC (Rockville, MD). BW5147(αβ⁺) (23) was a gift from Dr W. B. Born (University of Colorado Health Sciences Center, Denver, CO). T cell hybridomas were produced by fusion of BW5147(αβ⁺) with lymph node (popliteal, para-aortic and inguinal) cells obtained from mice immunized with the respective antigenic peptide and stimulated in vitro with the same antigen (24–28). The hybridoma was selected and subcloned by limited dilution as previously described (24–28). Chinese hamster ovary (CHO) cells expressed I-Ék with various mutations at TCR-contacting sites were gifts of Dr M. M. Davis (Stanford, Palo Alto, CA). The mutations were at α57S→N, α61Q→R, α65A→V, α69A→V, α72A→V, α79A→K, β59E→K, β64Q→R, β69E→K, β73A→V, β77V→Q, β81H→Y and β84E→K (29).

Peptides

Peptides were synthesized and purified as previously described (24,26). The sequences of peptides used in this study were: pigeon cytochrome c (PCC) 81–104, IFAGIKKAF-RADLIAYLKQATA; moth cytochrome c (MCC) (29,102) 88–103, ANERADIYLRQR; λ repressor cl 12–26, LEDARLRLKAIYEKKK; λ repressor cl [15E]12–26, LEDARLRLKAIYEKKK; λ repressor cl [19Y]12–26, LEDARLRLKAIYEKKK; λ repressor cl [21Y]12–26, LEDARLRLKAIYEKKK; λ repressor cl [23A]12–26, LEDARLRLKAIYEKKK; λ repressor cl [25D]12–26, LEDARLRLKAIYEKKK; λ repressor cl [26H]12–26, LEDARLRLKAIYEKKK; λ repressor cl [19H]12–26, LEDARLRLKAIYEKKK; λ repressor cl [19S]12–26, LEDARLRLKAIYEKKK; λ repressor cl [17E]12–26, LEDARLRLKAIYEKKK; λ repressor cl [17Y]12–26, LEDARLRLKAIYEKKK; λ repressor cl [16E]12–26, LEDARLRLKAIYEKKK; λ repressor cl [16Y]12–26, LEDARLRLKAIYEKKK; λ repressor cl [16L]12–26, LEDARLRLKAIYEKKK.

Lymphocyte proliferation and IL-2 assay

All cultures and assays were performed in RPMI with 10% FCS (Gibco, Grand Island, NY), 10 mM glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml), and 2x10⁻⁵ M 2-mercaptoethanol. For the T cell hybridoma assay, 1x10⁵ T cells, 5x10⁴ TA3 cells and dilutions of the appropriate antigens were plated in duplicate in microtiter plates to a final volume of 0.15 ml. Cultures were incubated for 18–24 h, and 50 µl of supernatant was harvested and the IL-2 content determined by assay on the IL-2-dependent cell line HT-2 (24–26).

The affinity of each mutated cl 12–26 peptide for I-Ék was determined by the previously described competition assay (27), i.e. to measure its ability to competitively inhibit the binding of PCC 81–104 to I-Ék. Glutaraldehyde-fixed TA3 was pulsed with inhibitor peptides for 2 h and then co-incubated with PCC peptide for an additional 4 h. Unbound peptide was washed away and I-Ék-associated PCC 81–104 was determined by the activation of 2B4.

FACS analysis of the binding of the biotinylated peptide

[99R,103R] MCC 88–103 was biotinylated at the N-terminal amino group by reacting with sulfosuccinimidyl-6-(biotin-amido) hexanoate (Pierce, Rockford, IL) and purified as described (30). The direct binding of antigenic peptide to live APC was performed according to Busch et al. (30). Binding mixtures (200 µl) contained 10¹⁰ of splenic B lymphocytes, 1–10 µg of biotinylated peptide, with or without cl 12–26 variants, and were incubated at 37°C for 3 h. After washing four times with PBS at 4°C, the cells were incubated with phycoerythrin-labeled streptavidin (Caltag, South San Francisco, CA). Stained cells were analyzed on a FACScan. The specificity of binding was confirmed by inhibition with I-Ék-specific antibody (data not shown).

Molecular model building

The coordinates of HLA-DR1 (7,19) were obtained from Dr J. H. Brown (Harvard University, Cambridge, MA) and were used as a template for model building of I-Ék by employing the graphics program QUANTA on a Silicon Graphics Crimson workstation. Under the QUANTA environment, a multiple-sequence alignment algorithm (31) was used to align the I-Ék sequence to the sequence of DR1. Since the sequences were perfectly aligned without any gaps, there was no need for gap annealing. The coordinates of whole residues for the identical ones and of the main chain for others were copied from DR1. The coordinates of the side chain were built and relaxed by using the 'regularization modeling' technique in QUANTA. The complete structure was obtained by 200 cycles of the 'region regularization' tool using the 'adopted basis set NR' protocol in both minimization stages in order to remove close contacts and reduce strains. Based on overall homology of I-Ék with DR1, cl 16–26 was modeled according to the backbone of HA 306–318 on DR1 (19) cl 16–26 was then manually moved along the binding groove of I-Ék by one residue at a time. For each position, the structure was further minimized by heating up to 2000 K for 1.2 ps (1200 steps), equilibration at 300 K for 0.5 ps (500 steps), and being subjected to 200 cycles of Powell conjugate gradient minimization and 300 cycles of conjugate gradient minimization to optimize hydrogen bonds, ion pair and hydrophobic interactions. The final positioning of cl 16–26 was attained because of an optimum accommodation of Leu18 and Lys26 and maximum interaction with the I-Ék molecule.

Results

Leu18 and Lys26 are critical for I-Ék binding

Previous studies with truncated cl 12–26 analogs revealed residues critical for I-Ék binding. The removal of Lys26 reduces the binding of cl 12–26 to I-Ék protein by >98% (32), indicating a critical role for Lys26. In contrast, the removal of up to four N-terminal residues had no effect on the binding of cl 12–26 to the I-Ék molecule (32) and on T cell recognition (data not shown). cl 16–26 (sequence RRLKAIYEKKK) thus contains the full motif necessary for binding to the I-Ék protein. The potential I-Ék anchor residues of cl 12–26 were further delineated by using mutant peptides with substitution at residues 16–26. Only one substitution, usually with the residue chemically distinct, was made at each position for cl 16–26. This is because we aimed to identify one or two anchor residues of cl 16–26 for the proper orientation of the peptide.
Fig. 1. I-Ek competition analysis for cl 12-26 peptides with substitution at positions 16-26. (A and B) Each peptide was assayed for its ability to inhibit the binding of PCC 81-104 to I-Ek on TA3. Glutaraldehyde-fixed TA3 was preincubated with serial dilutions of each peptide (concentration indicated) for 2 h, followed by 2 h incubation with 10 nM PCC 81–104. Free peptide was washed away and the amount of the bound PCC peptide assayed by the activation of 2B4. The dashed line indicates T cell activation in the absence of competitors. (C) The ability of mutant cl peptides to inhibit the binding of biotinylated [103R] MCC 88–103 peptide. TA3 was incubated with 10 ng of biotinylated MCC peptide together with the indicated peptide for 2 h, and was stained with phycoerythrin-labeled streptavidin and analyzed on a FACSscan. Open curve, binding in the absence of inhibitor; solid curve, binding in the presence of inhibitor peptide. The concentration of inhibitor peptide added is shown at the right-hand side of each diagram.

During modeling, no mutated peptide at Ala20 was used because the small side chain of Ala is unlikely as a major anchor residue. The I-Ek-binding affinity of these mutant peptides was assessed by their ability to inhibit the binding of PCC 81–104 to I-Ek in a competition assay (27). As shown in Fig. 1(A and B), peptides with substitutions at Leu18 and Lys26 were the least effective in competing for I-Ek-specific binding. Replacement at other positions of cl 16–26 had only a minimum effect on their I-Ek-binding ability (data not shown for [17E], [19E], [24E] and [25E] cl 12–26 because they are similar to that of the wild-type peptide). We also examined how each mutant peptide competed with the binding of biotinylated MCC 88–103 to I-Ek on TA3. The substitution that affects the ability to block the binding of MCC peptide was
also found to be at residues 18 and 26 (Fig. 1C, data not shown for [35E] cl 12–26), further supporting the idea that Leu18 and Lys26 are the anchor residues. Both anchors are found in almost all I-E<sup>k</sup>-binding peptides (Fig. 2).

**Accommodation of cl 12–26 into the I-E<sup>k</sup> molecule**

The crystal structure of the human class II molecule DR1 has recently been determined (7,19). The I-E<sup>k</sup> protein shares striking sequence homology with the DR1 protein, in which 75 out of 84 residues on the a1 domain and 69 of 84 residues on the b1 domain are either identical or similar. The homology is even higher after excluding the difference in polymorphic residues (e.g. 10 of the different residues in b chain are in the polymorphic positions (7)). Moreover, all the residues on DR1 (a51, a53, a9, a62, a69, a76, b81, b82, b71, b61, b57) that form hydrogen bonds with the HA peptide main chain (19) are identically present on the I-E<sup>k</sup> protein. Because of such high homology between them, the coordinates of the whole residues for the identical ones and of the main chain for the dissimilar ones were copied from DR1. After introducing side chains of dissimilar residues, energy minimization generated a structure which retains most of the features of DR1. There are two prominent pockets (pockets 1 and 9 in ref. 19) on both ends of the binding groove of the I-E<sup>k</sup> molecule (Fig. 3). Due to the above-mentioned homology with DR1, cl 16–26 was also modeled according to the backbone of HA 306–318 bound to HLA-DR1 (19). The final positioning of cl 16–26 was attained by the optimum accommodation of Leu18 and Lys26 and maximum interaction with the I-E<sup>k</sup> protein. The orientation of the cl 16–23 backbone was parallel to that of HA 306–313. Due to the anchor of Lys26 into the C-terminal pocket, the orientation of cl 24–26 was different from that of HA 314–318 at this part of the peptide (diagram not shown).

The proposed alignment of cl 16–26 directs residues Ile21 and Glu23, in addition to Leu18 and Lys26, toward the binding groove of the I-E<sup>k</sup> molecule (Fig. 2). The simulated interaction at pockets 1 and 9 are highly homologous to that of HA 306–318/DR1. The amino group of Lys26 was placed in the proximity of Glu9 from the β-chain on the bottom of the binding groove (diagram not shown). The positioning of Leu318 in DR1 and Lys26 in I-E<sup>k</sup> is in good agreement, in that DR1 differs from I-E<sup>k</sup> in two out of the six amino acids forming the pocket 9 (β9 W→E and α72 I→Y). The replacement of Ile85 and Phe86 of the E<sup>k</sup> β-chain by the smaller Val85 and Gly86 in DR1 may explain the fact that pocket 1 of I-E<sup>k</sup> adapts Leu18 while the same pocket of DR1 accommodates the bulky Phe/Tyr (17,19,33). The modeling places the side chains of Ile21 and Glu23 of cl 16–26 in the central depression of I-E<sup>k</sup>. This depression was also demonstrated in the recent NMR study of the E<sup>k</sup> molecule (20), and the similar structures were refined in the HA 306–318/DR1 structure as pockets 4 and 6 (19). Moreover, a motif with these four anchors can be identified in most of the I-E<sup>k</sup>-binding peptides (Fig. 2). The identified pattern contains the previously suggested I-E<sup>k</sup>-binding motif (Leu18, Glu23 and Lys26) (18,21,34). Leu18, Ile21, Glu23 and Lys26 were thus defined as I-E<sup>k</sup>-anchor residues for cl 16–26 (Fig. 4).

**Restricted cl 16–26 contact by T cells generated from A/J mice**

Excluding these four anchor residues, other residues on cl 16–26 should be accessible to TCR (Fig. 4). Twenty-four cl 12–26-specific T cell hybridomas independently generated from A/J, B10.BR and C3H mice (24–26,28) were used in T cell reactivity analysis (Tables 1 and 2). The complete dose–response curves of cl 12–26 and its mutants were obtained on each T cell. The ability of the mutant peptide to simulate T cells was indicated by the concentration required to reach half-maximum response. Because most of the potential TCR contact sites of cl 16–26 are either Arg or Lys, substitution with Glu should have a similar effect on TCR recognition. In addition, cl 16–26 mutants in which Arg/Lys were replaced by another dissimilar amino acid Tyr were also examined in selected T cells. The substitution of each Arg/Lys with Glu or Tyr affected the recognition of cl 16–26 mostly to the same
extent (Table 3). A discrepancy was found with the C123 T cell for the recognition of [\(^{16}\text{E}\)] and [\(^{16}\text{Y}\)] cl 12-26. Further examination using [\(^{16}\text{L}\)] cl 12-26 identified a similar reactivity as [\(^{16}\text{E}\)] cl 12-26 on C123 (Table 3). Fine antigen specificity for many T cells was determined by the recognition of cl 12-26 with Glu substitution.

A great discrepancy on the effect of substitution of each potential TCR-contacting site was revealed. A preferential interaction with Lys24 over Lys25 was seen in six out of the seven T cell hybridomas generated from A/J (Table 1). The reactivities to [\(^{24}\text{E}\)] cl 12-26 are always an order lower than those to [\(^{25}\text{E}\)] cl 12-26, supporting the idea that Lys24 is more critical for TCR contact than Lys25 in these T cells. On the modeled cl 16-26 (Fig. 4), both side chains of Lys24 and Lys25 are clearly accessible to TCR. Tyr22 is a TCR-contacting residue that has been previously characterized, a T cell such as 8I cannot recognize cl 12-26 by a replacement of Tyr22 with Phe (24,25). The substitution of Tyr22 with His or Asp resulted in a peptide incapable of stimulating most of the T cells. On the N-terminal part of cl 16-26, Lys19 is another critical TCR-contacting residues. All A/J T cells were almost non-responsive to [\(^{19}\text{E}\)] cl 12-26 (Tables 1 and 2).

![Fig. 3. Top view of cl 16-26 and the pockets of I-E\(^{k}\) in the modeled antigen-MHC complex. The color code for atoms of I-E\(^{k}\) and cl 16-26 is, white, carbon; red, oxygen; blue, nitrogen. Lys26 of cl 16-26 is adapted of the right end pocket (pocket 9 as in ref 19), Glu23 and Ile21 are accommodated in the central pockets (pockets 4 and 6), Leu18 is at the left end pocket (pocket 1). The van der Waals surface of I-E\(^{k}\) is in yellow. The Ig-like domains (a2 and b2) of I-E\(^{k}\) have been removed for easier illustration. The modeling of I-E\(^{k}\) and the simulated binding of cl 16-26 are described in Methods.](https://academic.oup.com/intimm/article-abstract/8/1/45/859491)

### Table 1. Reactivities of T cells from A/J mice to cl 12–26 mutants

<table>
<thead>
<tr>
<th>T cell</th>
<th>cl 12–26 mutant</th>
<th>[(^{16}\text{E})]</th>
<th>[(^{17}\text{E})]</th>
<th>[(^{19}\text{E})]</th>
<th>[(^{20}\text{H})]</th>
<th>[(^{20}\text{D})]</th>
<th>[(^{24}\text{E})]</th>
<th>[(^{25}\text{E})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3I</td>
<td></td>
<td>10</td>
<td>&gt;300(^b)</td>
<td>&gt;300(^b)</td>
<td>&gt;1000(^b)</td>
<td>93</td>
<td>&gt;300(^b)</td>
<td>30</td>
</tr>
<tr>
<td>7II</td>
<td></td>
<td>0.5</td>
<td>250</td>
<td>110</td>
<td>&gt;1000(^b)</td>
<td>&gt;1000(^b)</td>
<td>&gt;500(^b)</td>
<td>4.5</td>
</tr>
<tr>
<td>8I</td>
<td></td>
<td>0.5</td>
<td>23.5</td>
<td>550</td>
<td>&gt;1000(^b)</td>
<td>&gt;1000(^b)</td>
<td>&gt;500(^b)</td>
<td>40</td>
</tr>
<tr>
<td>10I</td>
<td></td>
<td>1</td>
<td>100</td>
<td>&gt;500(^b)</td>
<td>10</td>
<td>&gt;1000(^b)</td>
<td>&gt;1000(^b)</td>
<td>40</td>
</tr>
<tr>
<td>22I</td>
<td></td>
<td>2.1</td>
<td>40</td>
<td>&gt;300(^b)</td>
<td>100</td>
<td>70</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>26IV</td>
<td></td>
<td>3.2</td>
<td>&gt;500(^b)</td>
<td>&gt;1000(^b)</td>
<td>&gt;1000(^b)</td>
<td>&gt;500(^b)</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>51V</td>
<td></td>
<td>&gt;500(^b)</td>
<td>&gt;500(^b)</td>
<td>&gt;500(^b)</td>
<td>1.2</td>
<td>5.6</td>
<td>0.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

\(^a\)Each mutated peptide was assayed on the T cell indicated and the complete dose-response curve was obtained. The concentration required for each mutant to reach the half-maximum response was divided by the concentration of wild-type peptide (cl 12–26) required to activate the half-maximum T cell response. This number thus represents the inverse of efficacy of the mutant to stimulate T cells. 3I, 7II, 8I, 10I, 22I, 26IV and 51V are cl 12–26-specific T cell hybridomas generated from A/J mice (21–23).

\(^b\)The T cell response generated by the mutated peptide is not comparable with those stimulated by cl 12–26 in magnitude. At the indicated fold of concentration, mutant peptide failed to reach half-maximum T cell response.
stringent requirement for Arg17 in contrast to Arg16 for A/J T cell recognition. The mutation of Arg17 dramatically reduced the T cell response, while mutation of Arg16 did not affect recognition in these T cells (Tables 1 and 2). Most of the T cell clones derived from A/J mice displayed a rigid requirement for Arg17, Lys19, Tyr22 and Lys24 on cl 12-26 (Tables 1 and 3). This spans a region of at least 8 amino acids with four TCR-contacting residues. The exception is 51V, the T cell which may see only the N-terminal part of cl 16-26 on I-E<sup>k</sup>.

Diversity on the interaction with cl 16-26 by T cells derived from B10.BR and C3H mice

In contrast to the highly restricted antigen recognition by A/J T cells, T cells generated from B10.BR and C3H mice displayed a diverse reactivity pattern (Table 2). For B17 and

![Fig. 4. Side view of the cl 16-26 peptide in I-E<sup>k</sup>](https://academic.oup.com/intimm/article-abstract/8/1/45/859491/6465941) The orientation of cl 16-26 in the binding complex with I-E<sup>k</sup> as generated in Fig. 3. The four anchor residues Leu18, Ile21, Glu23 and Lys26, and some of the potential TCR-contacting residues are indicated.

B18 from B10.BR mice, the reactivity with cl 16-26 was similar to that of A/J T cells. B9 and B26 interacted with Arg17, Lys19, Tyr22, Lys24 and Lys25, while B16 displayed a
stringent requirement for all the potential TCR contact sites on cl 16-26 (Tables 2 and 3). B2 and B15 recognized mainly the central region of cl 16-26, and a fine specificity difference can be further identified between them. For T cells from C3H, C6 shared the same reactive pattern as most of the T cells from A/J. The interaction of C90 with cl 16-26 is analogous to B2 (Table 3), whereas the reactivity pattern of C26 is closer to that of B15 (Table 2). An additional way of interaction with cl 16-26 was seen with C123 (Arg16, Arg17, Lys19 and Tyr22) and C128 (Lys19, Tyr22, Lys24 and Lys25) (Tables 2 and 3). Except for B17, B18 and C6, the preferential recognition of Lys24 over Lys25 was not seen in T cells from B10.BR and C3H mice.

**TCR-I-E<sup>α</sup> interaction in the context of cl 16-26 recognition**

CHO cells transfected with the I-E<sup>α</sup> gene containing different mutations at potential TCR-contacting sites (29) were used to present cl 16-26 to these I-E<sup>α</sup>-restricted T cells. The dose-response curve of cl 12-26 on each I-E<sup>α</sup> mutant was constructed, and the ratio between the concentration required to reach half-maximum response on wild-type I-E<sup>α</sup> and that in I-E<sup>α</sup> mutants and in the context of cl 16-26 recognition. Selected residues of I-E<sup>α</sup> were determined (Table 4). Despite the fact that the criteria is slightly different from that used by Ehrlich et al. (29), the results obtained with 3I were almost identical (Table 4). A few TCR contact sites on I-E<sup>α</sup> (e.g. α57S and β89E) are located at the outer edge of the α-helices (Fig. 5), suggesting that I-E<sup>α</sup>-TCR contact is extensive. Similar to T cells specific for MCC B8-103 (29), variation of the specific TCR-I-E<sup>α</sup> contact was found between different T cells (Tables 4 and 5). For example, mutations at α65A or α6BA greatly reduced the efficacy to present cl 12-26 to B2, B9, B26, C6, C108 and C123 T cells, but not to other T cells (Fig. 6). However, despite such fine variation, almost all cl 12-26-specific T cells displayed identical sensitivity to mutations at central α-helical regions of the I-E<sup>β</sup> chain (Table 4 and Fig. 6). Mutation at β69E or β77T abolished the recognition of cl 16-26 by 17 of 23 T cells analyzed. In addition, the 51V T cell recognized the N-terminal segment of cl 16-26, yet did not specifically discriminate mutations at the left end of the I-E<sup>α</sup> molecule (e.g. α57S, β84E or β81H) (Table 4 and Figs 5 and 6). Similarly, B24 preferentially interacts with the central and C-terminal part of cl 16-26 (Lys19, Tyr22, Lys24 and Lys25), but B24 did not contact the right part of the I-E<sup>α</sup> protein (α65A, α72A or α79E) (Fig. 6). Furthermore, many of these TCR-contacting sites (α61Q and β73A) on the I-E<sup>α</sup> molecule are distal from the TCR-contacting residues of cl 16-26 (Fig. 5). This indicates that TCR-I-E<sup>α</sup> contact is apparently not in coordination with TCR-cl 16-26 recognition.

**Table 4. Reactivities of T cells to cl 12-26 presented by CHO cells transfected with I-E<sup>α</sup> mutants**

<table>
<thead>
<tr>
<th>Mutations</th>
<th>3I</th>
<th>7II</th>
<th>8I</th>
<th>10I</th>
<th>26IV</th>
<th>51V</th>
<th>B17</th>
<th>B18</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>α57S→N</td>
<td>11</td>
<td>11</td>
<td>0.8</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
<td>3.6</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>α61Q→R</td>
<td>100</td>
<td>0.9</td>
<td>1</td>
<td>0.7</td>
<td>&gt;1000</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>α65A→V</td>
<td>1</td>
<td>1</td>
<td>0.7</td>
<td>0.8</td>
<td>1</td>
<td>1.2</td>
<td>2.3</td>
<td>0.28</td>
<td>0.2</td>
</tr>
<tr>
<td>α68A→V</td>
<td>1</td>
<td>1.2</td>
<td>0.2</td>
<td>0.8</td>
<td>1</td>
<td>1.2</td>
<td>2.3</td>
<td>0.28</td>
<td>0.2</td>
</tr>
<tr>
<td>α72A→V</td>
<td>1.2</td>
<td>1.2</td>
<td>28</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>1.1</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td>α79E→K</td>
<td>1.3</td>
<td>1</td>
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*Each CHO cell with mutated I-E<sup>α</sup> (27) was used to present cl 12-26 to the T cell indicated and the complete dose-response curve was obtained. The number is the ratio of the concentration required to reach the half-maximum response on each I-E<sup>α</sup> mutant to the concentration required to activate half-maximum T cell response.*

---

**Fig. 5. Location of selected TCR-contacting sites on modeled I-E<sup>α</sup>***

*In the context of cl 16-26 recognition. Selected residues of I-E<sup>α</sup> identified in Table 4 as critical TCR-contacting residues are marked in the modeled I-E<sup>α</sup> molecule. α65A is included for its close approximation to Tyr22 (see text). Only the side chains of TCR contact sites and of protein a are shown. A indicates the α-chain and B indicates the β-chain of I-E<sup>α</sup>.*
Peptide-dominated TCR interaction

Discussion

cl 16-26–I-E\k binding

Based on the unrefined coordinates of DR1, an interaction between cl 16-26 and I-E\k protein is proposed. Leu18, Ile21, Glu23 and Lys23 of cl 16-26 were oriented to adapt into pockets 1, 4, 6 and 9 of the I-E\k protein respectively. Even though the modeled interaction lacks the fine molecular contact revealed by the recent crystal structure of HA 306-318/DR1 (19), a few structural features are apparent. Lys26 of cl 16-26 forms a salt bridge with Glu9 of the I-E\k \beta-chain. This specific salt bridge has been suggested to dictate the interaction between the MCC peptide and the I-E\k molecule (18). Notably, Glu9 of the \beta-chain is not present in the I-A\d protein, demonstrating why Lys26 is not an I-A\d anchor despite the binding of cl 12-26 to I-A\d (24,32). A hydrophilic anchor residue found in many I-E\k-binding peptides (Fig. 2) is likely to fit into pocket 6 in the central groove. The limited size of pocket 6 (diagram not shown) may even favor a smaller residue in this position. This is demonstrated by the finding that replacing the corresponding anchor Thr with Ala in HA peptide increases DR1 binding (35). Similarly, I-E\k binding was not reduced when Glu23 of cl 16-26 was replaced with Ala (Fig. 1B). Pocket 4 in the central groove may accommodate additional residues of a size no larger than Ile, as in the case of cl 16-26 (Fig. 2). Moreover, it may easily be seen why the bulky residues (Tyr22 for the cl peptide) flanked by these two anchor residues would have to point away from the groove. This is in good agreement with the fact that Tyr22 of the cl peptide is the major TCR-contacting site (24,26,32). If the central regions of other I-E\k-binding peptides are also positioned in such a manner, I-E\k-bound peptide would not have the middle bulge demonstrated in class I bound peptides (36). Therefore, based on the known HA 306-318 structure, the constructed cl 16-26-I-E\k model agrees very well with the existing information. A similar analysis can be used to probe peptide–MHC interactions in other DR and I-E molecules, which are also highly homologous to DR1. In addition, most of the potential TCR-contacting sites can be identified after locating the anchor sites (indicated by an open triangle in Fig. 2).

It has been shown that the same class I molecule uses a different combination of pockets to bind different peptides (11). Similarly, not all the four anchor residues identified in the present analysis are necessarily used by all I-E\k-binding peptides. This may explain the fine difference in the specific anchor residue in different I-E\k-binding peptides. For example, through the use of global amino acid replacement on MCC 93-103, Reay et al. (21) identified Ile95, Gin100, Lys103 but not Leu98 (corresponding to Ile21 of cl 16-26) as the I-E\k anchors. Instead, Leu98 of MCC 93-103 is suggested to be a neutral residue for both MHC binding and TCR recognition (21). Pocket 4 in DR1 has been described as a "secondary anchor" (37), which is consistent with the possibility that it may engage only a subset of peptides. An additional discrepancy is seen with Hb68-76 ([TAFNEGLK]), in which 73E is suggested to be a TCR contact site (38). The resolution of these differences likely requires an exact determination of I-E\k crystal structure.

Fig. 6. Efficiency of mutant I-E\k to present cl 12-26 to T cells. The number used for each position is the ratio of the cl 12-26 concentration required to activate half-maximum response on each I-E\k mutant to the concentration required to reach half-maximum response on wild-type I-E\k (Table 4). For those I-E\k mutants with enhanced T cell response, '<' is used when a ratio falls between 0.1 and 0.5.
TCR contact with cl 16-26

The exact localization of the anchor residues on cl 16-26 enables us to delineate the interaction of different TCR with the same peptide-I-E<sup>k</sup> complex. Because most of the potential TCR contact sites are positively charged residues, even single substitution with glutamic acid allows a differentiation between each residue. Additional analysis with Tyr-substituted cl 12-26 on selected T cells yields mostly similar reactivity (Table 3). Given that a peptide binding to an MHC protein adopts only a single conformation, the enormous number of reactivity patterns seen with 22 different TCR is interesting. The diversity is generated by the fact that different segments of cl 16-26 bound to I-E<sup>k</sup> molecule can be independently recognized by different T cells. For example, C123 interacted with the N-terminal part of cl 16-26, C128 preferentially recognized the C-terminal portion of cl 16-26, C27 contacted only the central segment of cl 16-26, while B16 interacted with almost all the potential TCR contact sites of cl 16-26 (Tables 2 and 3). Moreover, there is an apparent distinction in the diversity between T cells from AJJ mice and from C3H and B10.BR mice. Most T cells derived from AJJ preferentially reacted with Arg17, Lys19, Tyr22 and Lys24, a pattern seen in only three T cells from other mice (B17, B18 and C6). In contrast, the interactions with cl 16-26 are highly heterogeneous for T cells from B10 BR and C3H mice. We have recently observed that there is no TCR repertoire flexibility in AJJ mice, but a limited flexibility can be observed in B10 BR and C3H mice (28). Whether this may be correlated with the different recognition diversity awaits further delineation.

Independent antigen and MHC recognition by TCR

Since any peptide is deeply buried in the binding groove of MHC (6, 11, 13, 19), TCR-MHC contact is inevitable in the TCR recognition of antigenic peptide. That the residue on the outer edge of α-helices (e.g. 69E) is in contact with TCR (Fig. 5) supports extensive TCR-I-E<sup>k</sup> contact. The observed TCR-I-E<sup>k</sup> contacts cannot be correlated with a specific TCR usage. The same regions of I-E<sup>k</sup> were critical for most MCC 88-103-specific T cells (29) and most cl 16-26-specific T cells (Fig. 6). However, V<sub>p</sub>3 and V<sub>p</sub>11 are used by most MCC 88-103-specific T cells, whereas V<sub>p</sub>1 and V<sub>p</sub>2 are used by selected cl 12-26-specific T cells (3I, 7I, 2I, 2I6, 5I, C6 and C25) in this study (24-26,28). There is also no similarity in the CDR3 regions between these two group of T cells. Hence the recognition of the central α-helical region of the I-E<sup>k</sup> β-chain is not attributed to a specific TCR sequence. The present result serves as another example that TCR-MHC contacts cannot be inferred from the usage of the specific TCR element (29).

A surprising observation is that most cl 16-26-specific T cells interact with I-E<sup>k</sup> at almost the same sites despite some fine variation (Table 4 and Fig. 6). The 51V T cell, which recognized the N-terminal part of cl 16-26, did not preferentially interact with the left part of I-E<sup>k</sup>. Similarly, B24 did not contact the right end of I-E<sup>k</sup> despite the critical interaction with the C-terminus of cl 16-26. Instead, both 51V and B24 interacted with the central part of the α-helices of the I-E<sup>k</sup> β-chain, in a manner not different from most of the cl 12-26-specific T cells. Moreover, residue α65A of the I-E<sup>k</sup> protein that is approximately close to Tyr22 was not necessarily recognized by TCR that are in contact with Tyr22 (Figs 5 and 6). Additionally, Glu69 of the I-E<sup>k</sup> β-chain is very close to Lys25 in the modeled cl 16-26-I-E<sup>k</sup> complex (Fig. 5), yet T cells from AJJ that recognize the more distally located βGlu69 did not interact with Lys25. The TCR-MHC interaction and the TCR-antigen contact are apparently not coordinated.

The observation that most of the TCR recognizing the distinct segment of cl 16-26 are in contact with the same region of the I-E<sup>k</sup> molecule is intriguing. The discordant interactions between TCR-I-E<sup>k</sup> and TCR-peptide represent a unique feature of TCR recognition. Interestingly, such a mode of interaction can be accounted for by current knowledge of TCR recognition. The predicted homology between TCR and Ig has recently been confirmed by the crystal structure of the TCR β-chain (39). In the widely accepted model of TCR-antigen-MHC interaction, the first and second complementarity-determining regions (CDR) interact with MHC α-helices, and the third CDR interacts with the antigenic peptide (40-42). Even though the CDR1,2-MHC and CDR3-peptide interactions may not be clearly separable from each other (43), a preferential contact with MHC by CDR1,2 is expected to be less variable than the CDR3-antigen interaction. In addition, since the role of the MHC molecule is mainly to present peptide (3), a less flexible TCR contact may be inevitable in the context of a structural support for the optimum interaction of peptide with TCR (26, 40, 44). Interestingly, variation can be still observed in a few T cells in their interactions with I-E<sup>k</sup> (Fig. 6). As suggested by Ehrlich et al (29), such flexibility in MHC contact reflects the dominant effect of antigen and represents the case that peptides dictate TCR recognition (29, 40, 44). This is consistent with the fact that T cells (B2, B9, B26 and C123) contacting I-E<sup>k</sup> in a pattern distinct from other T cells also recognized cl 16-26 distinctively (Table 2). It may be suggested that the presentation by the I-E<sup>k</sup> molecule already enables the recognition of the distinct segment of cl 16-26 by a diverse population of T cells in a more homologous I-E<sup>k</sup>-TCR contact. However, the TCR-MHC contact is not necessarily so rigid that a higher fluidity in MHC contact can be observed under a dominant TCR-antigenic peptide interaction. Further structural studies may help understand how the flexibility and rigidity of MHC contact is determined during TCR interaction.

Acknowledgements

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Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CHO</td>
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<tr>
<td>cl</td>
<td>λ repressor cl protein</td>
</tr>
<tr>
<td>MCC</td>
<td>moth cytochrome c</td>
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<tr>
<td>PCC</td>
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