The Crystal Structure of a T Cell Receptor in Complex with Peptide and MHC Class II

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The crystal structure of a complex involving the D10 T cell receptor (TCR), a 16-residue foreign peptide antigen, and the I-Aα self major histocompatibility complex (MHC) class II molecule is reported at 3.2 angstrom resolution. The D10 TCR is oriented in an orthogonal mode relative to its peptide-MHC (pMHC) ligand, necessitated by the amino-terminal extension of peptide residues projecting from the MHC class II antigen-binding groove as part of a mini β sheet. Consequently, the disposition of D10 complementarity-determining region (CDR) loops is altered relative to that of most pMHCI-specific TCRs; the latter TCRs assume a diagonal orientation, although with substantial variability. Peptide recognition, which involves P–1 to P8 residues, is dominated by the Vα domain, which also binds to the class II MHC β1 helix. That docking is limited to one segment of MHC-bound peptide offers an explanation for epitope recognition and altered peptide ligand effects, suggesting a structural basis for allotypicity, and illustrates how bacterial superantigens can span the TCR-pMHCII surface. The adaptive immune response is dependent on the specific recognition function of αβ T lymphocytes (1). Each T cell detects a protein fragment (that is, peptide) of a self protein or cell-associated pathogen derived from either viral, bacterial, fungal, parasitic, or tumor cell origin bound to an MHC molecule. The physical binding of the peptide-MHC (pMHC) complex to the TCR then initiates a series of signal transduction events. Once triggered, T lymphocytes release cytotoxic molecules or inflammatory cytokines (or both) that destroy the infected or otherwise altered cells through various effector mechanisms. For a given αβ T lymphocyte, immune recognition is mediated by a clonotypic αβ heterodimeric structure (T) noncovalently associated with the monomorphic CD3 signaling components.

Sequence analysis of TCR αβ heterodimers first suggested that they would share with antibodies a common structure (2, 3). However, direct evidence supporting this notion has been provided only in the last several years, initially from crystal structures of αβ TCR components (4) and subsequently through analysis of intact αβ TCR heterodimers alone (5) or in complex with pMHC (6, 7). As anticipated, aside from the Vα domain, the three-dimensional (3D) structure of the TCR resembles an antibody Fab fragment such that each of the α and β chains consists of canonical immunoglobulin (Ig)-like variable and constant domains, with the hypervariable complementarity-determining regions (CDRs) from the two variable domains (Vα and Vβ) forming the ligand binding site for pMHC within the immunoreceptor module.

Class I and class II MHC molecules have evolved to facilitate T cell detection of pathogens residing in distinct intracellular compartments (8–10). Although the domain organization of the class I and class II MHC extracellular segments is different, these molecules possess a very similar overall antigen-presenting groove consisting of α1 plus α2 domains and α1 plus β1 domains for class I and class II MHC, respectively (11–13). For both molecules, the α helices of these two domains form the sides of the antigen-binding groove with the floor created by an eight-stranded β sheet arising from both domains. However, unique structural features of the two MHC classes dictate the binding of peptides differing in length and composition (14). The bipartite nature of the immune recognition molecules expressed on antigen-presenting cells is reflected at the level of αβ T lymphocytes by the evolution of two subsets bearing specialized MHC binding structures, termed CD4 and CD8 (15, 16). CD8 cells are cytolytic precursor and effector cells, whereas CD4 cells comprise the helper T cell subset that initiates inflammatory responses. CD4 and CD8 molecules have been termed coreceptors because CD4 binds to the membrane-proximal β2 domain of class II MHC whereas CD8 (αα and αβ) isoforms bind to the corresponding α3 domain of class I MHC (17, 18).

At present, four distinct class I–restricted TCRs have been crystallized in complex with their specific pMHCI ligands (6, 7). Rather extensive interactions with the pMHC α helices have suggested a common “diagonal” docking mode, regardless of TCR specificity or species origin, in which the TCR Vα domain overlays the class I MHC α2 helix and the Vβ domain overlays the MHC α1 helix. As a result, the CDR1 and CDR3 loops of the TCR Vα and Vβ domains make the major contacts with the peptide, whereas the two CDR2 loops interact primarily with the MHC. Given the distinct nature of class II versus class I MHC expression, peptide binding, and the differential interactions with CD4 and CD8 T cell subsets, it was of interest to structurally define the TCR-pMHCII interaction. We now report the x-ray crystal structure of a TCR-pMHCI ternary complex. The complex contains the V module of the D10 TCR [single chain (sc) D10] derived from AKR/J (H-2k) mouse T cell clone D10.4 and a fragment of conalbumin (CA) bound to the self-I-Aα molecule (19, 20). A marked difference in TCR docking topology relative to TCR-pMHCI complexes is noted.

Overview of the Complex Structure

The crystal structure of the scD10-CA/I-Aα complex was determined with molecular replacement and alternative cycles of model building and refinement (Table 1). In the asymmetric unit there are two complexes related to each other by a 115° rotation. The complex A and D10-B pack together to form layers perpendicular to the longest Y axis, whereas the I-Aβ molecules B connect the layers, thus leaving spaces filled with large amounts of solvent. The structures of the two complexes are very similar. The root-mean-square deviation (r.m.s.d) value of Ca superposition is only 0.8 Å for the whole complex. Consequently, only complex A is discussed.

Figure 1 is a ribbon drawing of the scD10-CA/I-Aα complex. Figure 2 is an omit map around the CA peptide. In Fig. 1, the view is down the peptide-binding groove of the MHC molecule, hence the longer dimension of the MHC molecule with α and β chains on either
side. The immediately striking observation is that the scD10 molecule sits on top of the MHC with its longer dimension crossing the bound peptide in an orthogonal manner, rather than the “diagonal” mode commonly recognized in structures of TCR-pMHC I complexes (6, 7). The Vα domain of scD10 contacts the β1 helical region of I-Aª, whereas the Vβ domain reaches the α1 helical region. Contacts between the D10 TCR and the CA/ I-Aª pMHC ligand are listed in the supplementary table (21). In contrast to the class I pMHC-TCR ternary structures, the much longer peptide stretches out both sides of the TCR-MHC complex. In particular, the COOH-terminal residues have no interaction with either TCR or MHC. The orthogonal orientation for the TCR-pMHCII interaction noted herein excludes the possibility that direct TCR contact with COOH-terminal peptide flanking residues is the basis for any observed functional dependence on this peptide segment in T cell recognition (22).

Although there have been several structures of class I–restricted αβ TCRs or derivative fragments (4–7), our scD10 represents the crystal structure of a class II–restricted αβ TCR V module in complex with its cognate pMHC partner. The structure of the Vα- Vβ heterodimer is very similar to the recently published nuclear magnetic resonance (NMR) structure of an unligated scD10 (23). The rmsd’s for all of the backbone atoms of residues in β strands between structures in the NMR ensemble and the crystal structure are 1.3 and 1.4 Å for Vα and Vβ domains, respectively. Notably, there does not appear to be any significant 3D structural difference between TCRs that recognize peptides bound to class I versus class II MHC molecules. The human class I HLA-A2/Tax-specific B7 TCR is by far the most structurally similar to our murine class II–specific scD10. Virtually the entire V module of these two TCRs can be superimposed. The rmsd values of the superposition for the entire Vα domain’s 110 Cα atoms (excluding the first residue, which is not seen in the density map of our scD10 structure) and 107 Cα atoms of the Vβ domain (excluding part of the CDR3) are only 0.98 and 0.72 Å, respectively. Moreover, if

Table 1. Crystalllographic analysis. Crystals of the ternary complex were grown by the conventional hanging-drop vapor diffusion method at room temperature. The Escherichia coli–expressed scD10 (57) and glycosylated CA/I-Aª from CHO Lec3.2.8.1 cells (58) were mixed at a 1:1 molar ratio to a final concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5). The protein solution was further mixed with a crystallization buffer of 8% PEG 8000, 0.1 M tris (pH 8.5), 0.01 M KCl and then sealed against a reservoir concentration of 23 mg/ml in 0.1 M tris-HCl buffer (pH 8.5).

![Fig. 1. Structure of the scD10-CA/I-Aª complex. Ribbon diagram showing the overall orientation of the scD10 V module to CA/I-Aª (pMHC). Domains are color-coded and labeled as follows: TCR Vα (green), Vβ (blue), MHC α-chain (light green), and MHC β chain (orange). The secondary structures, β strands, and α helices of all component domains are defined by the program DSSP (63). The peptide is drawn in red tracing of the Cα atoms with NH₂- and COOH- terminals labeled. The three glycans (GlcNAc) (gray) are drawn in ball-and-stick format. This figure was generated with the program MOLSCRIPT (64).](Image 404x171 to 568x437)
the two Vα domains are superimposed, then the orientations of the two Vβ domains differ only by a 3.7° rotation, indicating that Vα-Vβ dimerization is very similar for these two TCRs as well.

**The Orthogonal Binding Mode**

The orthogonal docking mode was not correctly predicted by either extensive mutagenesis studies (19) or modeling with a scD10 NMR structure in conjunction with a CA/I-Ak crystal structure as a starting point (23). To establish a quantitative and comparative measurement of binding orientation among TCRs and their pMHC ligands, we have defined an angle between two vectors. One vector passes through the mass centers of the Vα and Vβ domains of the TCR, while the other is drawn from the NH2-terminal Ca atom at the P1 position (the first residue bound in the P1 pocket of the MHC) to the COOH-terminal Ca atom (the P9 position, the last buried residue) of the bound peptide. The angle for scD10-CA/I-Ak complex is 80°, very close to a right angle. Table 2 lists the orientations of the two Vα domains of the TCR, while the other is drawn from the NH2-terminal Ca atom at the P1 position (the first residue bound in the P1 pocket of the MHC) to the COOH-terminal Ca atom (the P9 position, the last buried residue) of the bound peptide. The angle for scD10-CA/I-Ak complex is 80°, very close to a right angle. Table 2 lists the orientation angle calculated for all known TCR-pMHC complex structures. For class I complexes, the peptide vector is defined between the anchoring residues at the two termini. The angles for TCR-pMHC complexes span a broad range, from diagonal (45°) to close to orthogonal (70°). The difference between orthogonal and diagonal docking is illustrated by comparing the scD10-CA/I-Ak structure to the 2C-dEV8/H-2Kb (pMHCII) complex (Fig. 3, A and B).

Garboczi et al. (6) argue that in pMHCII structures, there are two high “peaks” near the NH2-termini of the α-helical regions forming the side wall of the peptide binding groove. These two “peaks” limit the TCR-pMHC class I binding to a diagonal mode such that the TCR can fit at a low enough point on the MHC surface to contact the entire complexed antigenic peptide. Teng et al. (7) have compared three TCR-pMHC class I complex structures and identified a common docking mode of the TCR relative to the MHC with substantial variation of twist, tilt, and shift, however (Table 2 legend). Furthermore, we have noticed that the inherent left-handed twist of the eight-stranded β sheet that forms the platform of the binding groove is the structural basis for the breaks in the two helical regions, resulting in the formation of high “peaks.” In this context, an MHC class II molecule is similar to an MHC class I molecule because all MHC molecules have the same platform. However, there are distinct features to the mode of peptide binding between the two classes. In the class I system, the 8- to 10-residue peptide has its termini anchored into two binding pockets whose unique chemical environments determine the polarity of the bound peptide. In addition, the bulky side chains of the conserved Trp167α and Tyr84α from the MHC molecule occlude the peptide-binding groove at both ends. In class II MHC, these blocking side chains are replaced by smaller ones or are reoriented (or both); the open ends eliminate the peptide length restriction. Moreover, the peptide (15 to 20 amino acids long) binds to the class II MHC molecule with hydrogen bonds not only at the termini, but throughout the entire peptide through main-chain atoms (14). Figure 4A shows the hydrogen-bonding pattern between the CA peptide and the I-Ak molecule that is conserved in other pMHC class II structures. Compared with the class I system, the P–3 to P–1 segment is an extension. This extension plays a unique role in the orthogonal docking mode. The peptide binding groove is much wider in the middle relative to its tapered ends so that the MHC class II molecule needs to use side chains of multiple conserved residues from α1 and β1 helical regions to reach the peptide mainchain atoms. The residues include asparagine and glutamine, which form bidentate hydrogen bonds to the peptide backbone. This hydrogen bonding pattern determines the peptide binding polarity in the class II MHC system (11–14). An important characterization of class II MHC molecules is that the α1 helix is two turns shorter in the NH2-terminus than the corresponding class I MHC molecule α1 helix (Fig. 4A). In particular, from Arg52α to Glu58α, the α1 helix is replaced by an extended strand that reaches close enough to the NH2-terminal extension segment of the bound peptide to form a mini parallel β sheet through the use of main-chain atoms. The pair of main-chain—main-chain hydrogen bonds between Arg63α to the MHC class II molecule and the P–2 and P1 residues at the NH2-terminal part of the peptide are conserved among all known pMHCII structures. The beginning of the α1 helix, Glu57α, is at the high “peak,” so from Glu54α to Arg52α toward the NH2 terminus the chain runs down, away from the TCR binding surface. However, the left-handed twist of the mini β sheet then forces the NH2-terminus of the peptide to point in the opposite direction, curving up toward the TCR binding surface. Together,

**Table 2. The orientation angle of a TCR onto a pMHC ligand.**

<table>
<thead>
<tr>
<th>TCR-peptide/pMHC complex</th>
<th>Orientation angle° (&quot;)</th>
<th>MHC class</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10-CA/I-Ak</td>
<td>80</td>
<td>II</td>
</tr>
<tr>
<td>2C-dEV8/H-2Kb</td>
<td>45</td>
<td>I</td>
</tr>
<tr>
<td>N15-VSV8/H-2Kb</td>
<td>54</td>
<td>I</td>
</tr>
<tr>
<td>A6-Tax/HLA-A2</td>
<td>56</td>
<td>I</td>
</tr>
<tr>
<td>B7-Tax/HLA-A2</td>
<td>70</td>
<td>I</td>
</tr>
</tbody>
</table>

*The orientation angle of a TCR on MHC is defined as the angle between two vectors determined for the orientation of the TCR and pMHC, respectively. The vector representing the TCR direction is drawn from the mass center of Vα to the mass center of Vβ. The vector representing the pMHC complex direction is drawn from the NH2-terminal Cα atom to the COOH-terminal Cα atom of the peptide in the case of MHC class I. In the case of MHC class II, the vector is drawn from the P1 residue to the P9 residue of the peptide. Note that in Teng et al. (7), twist and tilt were used for semi-quantitative comparison among different TCR-MHC complexes. Essentially, the twist and tilt angles are two projections of the orientation angle more accurately defined here. Whereas the twist is a top view from the TCR toward the MHC, the tilt is a side view, perpendicular to the bound peptide.
the extended NH$_2$-terminus of the bound peptide and the MHC molecule now form a broader high “peak,” or a small protruding “ridge” (Fig. 4B). For comparison, Fig. 4C is the same view of pMHC$_I$ taken from the 2C-dEV8/H-2K$^b$ structure. Our scD10-CA/I-

A$^\alpha$ structure shows that a diagonal TCR docking would result in a collision between the V$_{\alpha}$ domain of TCR and the pMHC$_I$ on the “left” side as viewed in Fig. 4B. Moreover, the tilt angle of a TCR relative to an MHC molecule (see Table 2 legend for the definition of tilt angle) exacerbates this potential clash by maintaining the V$_{\alpha}$ domain in close proximity to MHC. We propose that while the TCR-pMHC class I docking may have more variation in terms of the orientation angle as demonstrated in Table 2, the topology of TCR binding to pMHC class II may be more closely restricted to an orthogonal mode due to the “ridge” described above. It is interesting that the protrusion of the peptide’s NH$_2$-terminus has been suggested as a site for disruption by DM in the process of exchanging CLIP for an antigenic peptide in the MHC class II molecule ($^{24}$).

The Interface

The interaction between D10 and CA/I-A$^\alpha$ buries 1718 Å$^2$ of surface area, 861 Å$^2$ from the pMHC and 857 Å$^2$ from the TCR as determined by a 1.7 Å probe ($^{25}$). Twenty-three percent of the pMHC buried surface involves the peptide. In general, the size of the buried surface is comparable to that previously reported for three class I ternary structures (1700 to 1880 Å$^2$). However, the Sc value (the shape correlation statistic, a measurement of the degree of geometric match between two juxtaposed surfaces, where interfaces with Sc = 1 fit perfectly, whereas interfaces with Sc = 0 effectively define topologically uncorrelated surfaces) ($^{26}$) of the interface between the scD10 V$_{\alpha}$V$_{\beta}$ module and the CA/I-A$^\alpha$ ligand is 0.70, higher than for class I TCR-pMHC interfaces whose Sc values range from 0.45 to 0.64 ($^{27}$, $^{28}$). Moreover, the number of atomic contacts ($^{29}$) in our class II complex structure is about twice as many as those for the class I complexes. For I-A$^\alpha$, 68 atomic contacts exist with D10. By contrast, there are just 27 H-2K$^b$ contacts with the 2C TCR, and 27 and 34 HLA-A2 contacts for the A6 and B7 TCRs, respectively. These results suggest a much better shape complementarity of the scD10-CA/I-A$^\alpha$ interface, and agree well with the higher affinity of D10 for its pMHC ligand relative to that of 2C, for example (1 to 2 μM versus 100 μM) ($^{6}$, $^{7}$, $^{30}$). Of particular relevance is the finding that the additional interface atomic contacts can largely be ascribed to contacts between the TCR and the I-A$^\alpha$, rather than between the TCR and the CA peptide ($^{21}$). Assuming that these results are representative for other class II MHC-specific TCRs, the dominance of this TCR-MHC class II interaction may explain why expression of a single pMHC class II complex in the thymus can select many different TCRs ($^{31}$). Our data also show that despite having roughly the same buried surface area, the complementarity of TCR-pMHC recogni-
tion surfaces can vary substantially from a low extreme to one even better than that of an antigen-Fab complex, as is the case for the scD10-CA/I-A^b complex. In complexes like 2C-dEV8/H-2K^b, a few large cavities (6, 7) contribute to poor shape complementarity. The presence or absence of such cavities may vary for different TCR-pMHC complexes, thereby influencing the shape of complementarity.

Of the total buried surface area, V_α accounts for 519 Å^2, whereas V_β accounts for 338 Å^2 of the TCR buried surfaces. This result is consistent with the notion that V_α dominates in the interaction, which is generally true for the class I system as well. Our calculations show that the buried surface areas of V_α and V_β are 480 and 430 Å^2 for the 2C-dEV8/H-2K^b complex, 576 and 319 Å^2 for A6-Tax/HLA-A2, and 555 and 260 Å^2 for B7-Tax/HLA-A2, respectively. Perhaps more importantly, amongst different TCR-pMHC complexes, the variation in buried surfaces is significantly smaller for V_α than for V_β. Given that the rotation angle of known TCRs relative to their MHC ligands varies by as much as 35° (Table 2), these data suggest that the pivot point is closer to V_α, so that the V_α domain location on the pMHC will not change as much as the V_β domain, which can alter dramatically (compare Fig. 3, A and B). Differences in the disposition of CDR loops reflect this pivot point (Fig. 3, C and D). Variability in TCR docking also arises from differences in the tilt angle as described by Teng et al. (7) and noted in the Table 2 legend. The extreme is the A6-Tax/HLA-A2 structure, where the large tilt essentially precludes CDR1β and CDR2β from making contact with the MHC molecule (6, 7). Given that V_α is critical for TCR selection in thymic development as well as mature T cell activation (32), this V_α dominance in immune recognition is not unexpected.

Comparison between the scD10 TCR interaction with CA/I-A^b analyzed here and the 2C TCR interaction with dEV8/K^b (6, 7) shows how a single TCR V_β8.2 domain can bind in distinct orientations to class I and class II pMHC ligands (Fig. 3, C and D). In the 2C-dEV8/K^b complex, the germ line V_β8.2 segment recognizes the K^b α1 helical MHC residues Gln57, Val76, and Arg79 through CDR2, and the K^b α2 helical residues Lys39, Gln49, and Ala69 through CDR1 (6, 7). In the scD10-CA/I-A^b complex, the identical germ line V_β8.2 segment recognizes the I-A^b α1 helical residues Lys43, Gln55, and Leu60 through CDR2 and Gln55 through CDR1. Given that these two docking interactions are to highly conserved MHC class I and to highly conserved class II residues, respectively, it is tempting to speculate that the V_β domain plays a major role in MHC recognition by both classes of TCRs and perhaps in pre-TCRs as well (33).

**Research Articles**

**Antigenic Peptide Recognition**

Although the peptide in our ternary structure is 16 residues long, designated as from P–3 to P13, the TCR interaction is restricted to the P–1 to P8 segment. The supplementary table (21) lists all the contacts to the peptide. It is noteworthy that of 27 atomic contacts with the peptide, 23 involve V_α and only 4 involve V_β. This dominance of the V_α domain in peptide recognition was not appreciated previously, although early molecular modeling efforts correctly suggested that an orthogonal TCR docking mode was possible (3). The spiral conformation of bound peptide (Fig. 4) (14) dictates that of the deeply buried peptide residues, only those at positions P2, P5, and P8 are accessible to the TCR molecule. The Trp at the P7 position is an excep-

![Fig. 4. The high point “ridge” in pMHCII ligands is created, in part, by the peptide.](image-url)
tion due to its bulky indole ring, which is partially exposed on the TCR binding surface. As for the rest of the peptide, the backbone of the P–2 residue is engaged in a mini parallel β sheet with the MHC molecule as discussed above, whereas the P–3 and the COOH-terminal three residues (P11 to P13) have no contacts with MHC or TCR whatsoever, although well defined by unambiguous densities.

The P2 residue is an Arg. It forms multiple salt bridges with both Asp104b and I-Ak Glu74, respectively. The same TCR Asp104b also interacts with I-Ak Arg80. Moreover, the upward-pointing P2-Arg is within van der Waals contacts to backbone of CDR3α Gly99a and CDR1α Thr28a (21). This knitted local structure packs closely onto the side chain of Ile at the P5 position from the NH2-terminal side of the peptide. The P5 residue is important structurally and biologically. Alteration of this residue adversely affects D10-TCR recognition of CA/I-Ak (19). The side chain of Ile at P5 fits extremely well into a hydrophobic pocket. Apart from the neutralized network discussed above, on the COOH-terminal peptide side is the indole ring of the Trp at P7 stacking onto the isobutyl group of the P5-terminally located Arg. On the top, from the TCR direction, the P5-Ile contacts the backbone of the tip of CDR3α, which consists of Gly99a-Ser100a-Phe101a. The phenolic ring of Phe101a bends toward the P7-Trp position. The exposed tip of the indole ring of P7-Trp makes contacts with the Phe101a aromatic ring. The other peptide residue engaged in recognition is the P8-Glu residue. P8-Glu forms bifurcated hydrogen bonds to side chains of Tyr108b and Tyr70b of the I-Ak molecule. Only the aliphatic portion of the P8-Glu side chain makes van der Waals interaction with CDR3α Phe101a and the aliphatic part of CDR3β Gly79b. In addition, there is one hydrogen bond between the carbonyl oxygen of Gly79b and the side chain of P8-Glu. Together, it appears that the TCR recognition of the particular antigenic peptide in question is largely hydrophobic and involves a number of backbone associations with the TCR molecule. Although scD10 TCR recognition of CA/I-Ak is centered at the P5 position, it is also coordinately involved with interactions to peptide residues at P2, P7, and P8. The observed contacts are consistent with studies mapping the D10 footprint onto CA/I-Ak (20, 34).

**Implications for Class II MHC-based Immune T Cell Recognition**

The current scD10-CA/I-Ak complex offers several insights into immune recognition of other pMHC class II ligands by other TCRs. First, the size of a TCR footprint on the MHC covers maximally nine peptide residues (~25 Å). Hence, while MHC class II molecules capture peptides of substantially larger length, only a subset of residues is “read out” by the bound TCR. Second, the P5 residue of the MHC-bound peptide occupies the central position (corresponding to the P4 position of the MHC class I-bound peptide (32)). As such, this central, solvent-exposed residue is critically important for the TCR binding process. Therefore, even a minor conservative substitution at this residue can destroy binding (that is, null ligand) or lead to altered peptide ligands with very weak agonist or in fact, antagonist activity (35–37). Third, for all class II molecules examined, there appear to be three to four pMHC binding pockets (at P1, P4, P6, and P9 for I-Ak, I-Ek, and DR and P1, P4, and P9 for I-Aβ). Only several upward-pointing peptide residues can serve as direct TCR contacts. On the basis of the observed molecular envelope of the TCR and the observed orthogonal orientation for class II MHC–restricted TCR interaction, it is likely that these basic principles apply in a general way to recognition of multiple pMHCII ligands including HLA-DR6/2/I-Aβ (12, 38), HLA-A2/I-Eα (39), moth cytochrome C (MCC)93-103/I-Eα (35), and DR2-restricted myelin basic protein (MBP)55-69 (40, 41). Moreover, it has been suggested that a single TCR can recognize multiple pMHCII ligands (40, 41). As the class II–specific TCR focuses on the central P5 residue, mutations that affect non-P5 positions may be less detrimental to the recognition process.

Despite overall structural similarity, CDR3 conformations appear to differ between free and complexed scD10. In the x-ray structure of the complex, the CDR3 loops are close to one another. Packing among the side chain of Gin106b, Ala104b, and Leu104a forms a hydrophobic core between the CDR3 loops. In contrast, there is no evidence that CDR3α packs with CDR3β in unligated scD10. Numerous nuclear Overhauser enhancements (NOEs) are observed between the methyl group of Ala104b and other CDR3β residues (Gly106, Gin105, Arg104, Glu105), but contacts to Leu104a or other CDR3α residues are not observed. In all of the calculated NMR structures, the CDR3α and CDR3β loops are well separated. The backbones of both CDR3α are also highly mobile on the picosecond time scale in the free protein (23), suggesting that they are not tightly packed. Hence, during immune recognition, the mobile CDR3 loops of scD10 assume their pMHCII binding conformation, clamping down on the central peptide region.

**Structural Basis of Alloreactivity**

About 1 to 10% of peripheral T cells are able to recognize allogeneic MHC molecules to which they were never exposed (42). The precise molecular basis of alloreactivity is yet to be fully defined. In this regard, the complex of scD10-CA/I-Ak is informative because the D10 TCR not only recognizes the antigenic CA peptide bound to I-Ak but also responds to all MHC class II molecules whose I-A β chain contains the sequence PEI at positions 65 to 67, including I-Akβ, I-Akβ, and I-Akβ (19). In comparison, MHC class II molecules having a Tyr at this position such as I-Akβ, I-Akβ, and I-Akβ, cannot stimulate D10 cells in the absence of the CA peptide. Various mutagenesis studies conducted on D10 showed that a hybrid I-Akα/I-Akβ MHCII molecule can stimulate D10 cells in the absence of exogenous antigen, suggesting that polymorphic residues critical for alloreactivity are located on the I-A β chain.

In order to elucidate this source of alloreactivity from the structural perspective, we compared the CA/I-Ak ligand and the alloreactive I-Ak molecule. The latter was taken from the recently solved x-ray structure of I-Ad. CDR3 γ1a and CDR3 β1 are shown. The side chains and main-chain interactions displayed on the CDR1 and CDR2 loops of D10 Vα make no hydrogen bonds or salt bridges to I-Ak β1 helix residues. (B) Model of scD10 bound to I-Ak based on superposition of I-Aα and I-Ak. The α1 H2 helix and the β1 region between and including H2α-H2β of the two class II MHC molecules were superimposed (46 Cα atoms, rmsd = 0.55 Å). The potential interactions between Glu66 from the PEI65-67 motif of I-Akβ to Tyr31 of CDR1 and Ala48, Ser50, and Lys56 of CDR2 of D10 Vα are indicated. The hydrogen bonds are drawn as magenta dashed lines. The salt bridge is drawn as a brown dashed line.
ovalbumin (323–339) complexed with I-A\textsuperscript{d} (13). Because all residues from I-A\textsuperscript{d} involved in the interaction with D10 as listed in the supplementary table are conserved in I-A\textsuperscript{k}, with the exception of \(\beta\) residues Tyr\textsuperscript{67} in I-A\textsuperscript{k} and Pro\textsuperscript{55}, Glu\textsuperscript{66}, Ile\textsuperscript{67} (PEI) in I-A\textsuperscript{d}, it is likely that D10 docks onto I-A\textsuperscript{k} in the same way as onto I-A\textsuperscript{d}. A model was constructed with the I-A\textsuperscript{k} superimposed onto the scD10-CA/I-A\textsuperscript{k} complex (Fig. 5). The major structural difference involves the \(\beta\)-chain residues Pro\textsuperscript{65}, Glu\textsuperscript{66}, and Ile\textsuperscript{67} in I-A\textsuperscript{d}, which form a protrusion interrupting the \(\beta\)-chain \(\alpha\) helix. As a consequence, in I-A\textsuperscript{k}, residue Ile\textsuperscript{67} assumes a similar position to residue Tyr\textsuperscript{67} in I-A\textsuperscript{d} (Fig. 5, A and B). The aliphatic side chain of the Ile\textsuperscript{67} in I-A\textsuperscript{d} can replace the aromatic ring on the side chain of Tyr\textsuperscript{67} in I-A\textsuperscript{k}, forming van der Waal’s contacts with the \(\beta\) CDR3 loop. To avoid steric clashes, side chains from residue Arg\textsuperscript{99} of D10 V\textsubscript{\(\beta\)} and residue Glu\textsuperscript{66} of I-A\textsuperscript{d} \(\alpha\) helix are rotated and the main-chain conformation around PEI on the \(\alpha\) helix is not preserved. Therefore, despite loss of one hydrogen bond of Tyr\textsuperscript{67} to D10 V\textsubscript{\(\beta\)}, these potential additional contacts between Gln\textsuperscript{64} and Arg\textsuperscript{99} from CDR3 of V\textsubscript{\(\beta\)} can form multiple potential interactions with CDR1 and CDR2 of D10 V\textsubscript{\(\alpha\)} (Fig. 5B). Additionally, the hydrogen bond between Glu\textsuperscript{49} and Arg\textsuperscript{99} from CDR3 of V\textsubscript{\(\beta\)} is preserved. Therefore, despite loss of one hydrogen bond of Tyr\textsuperscript{67} to D10 V\textsubscript{\(\beta\)} Gly\textsuperscript{28} (Fig. 5A), these potential additional contacts between the CDR loops of D10 V\textsubscript{\(\alpha\)} and the inserted PEI residues can enhance the affinity between MHC and D10. Other TCR allo-pMHCII interactions cannot be excluded. Consistent with this view, it has also been demonstrated that the case of the 2C allo-

MHCI response (L\textsuperscript{d}), allore cognition results from increased interaction between the 2C TCR V\textsubscript{\(\beta\)} domain and the allostimulus (43). The ability of exposed MHC helical polymorphic residues to permute the number and nature of contacts with the TCR is a feature of other class II MHC–restricted allogeneic responses (44, 45). For example, the naturally occurring I-A\textsuperscript{b} mutant H-2\textsuperscript{bm}\textsuperscript{12} generates a strong alloresponse in H-2\textsuperscript{b} mice. This molecule differs from I-A\textsuperscript{a} at only three positions: 67\(\beta\), 70\(\beta\), and 71\(\beta\).

While self peptides bound to MHC have been shown to play a critical role in allore cognition against MHC class I molecules (45), less is known about the nature of peptide ligands in class II MHC-based allore cognition (46). Given the additional contacts between the D10 TCR and I-A\textsuperscript{d}, it is possible that there are fewer interactions required between the peptide (or peptides) associated with I-A\textsuperscript{d} molecules and the D10 TCR. However, a peptide (or peptides) must also be involved because replacement of the PEI sequence in the 14.3.d V\textsubscript{\(\beta\)}-SEB complex (Fig. 6B) has been replaced with I-A\textsuperscript{d} on the basis of structural alignment of residues of the two helices of each MHC molecule (43 C atoms, \textit{rmsd} = 1.02 Å).

**Research Articles**

**Superantigen Binding**

Superantigens (SAGs) are a family of immunostimulatory and disease-causing proteins derived from bacterial or endogenous retroviral genes that are capable of activating a large fraction of the T cell population (47). In general, the activation appears to require a bridging interaction between the \(\beta\) domain of the TCR and an MHC class II molecule. Although crystal structures (48, 49) showing the detailed interactions between SEB, a representative bacterial SAG, and a TCR V\textsubscript{\(\beta\)} chain or SEB and the HLA-DR1 class II MHC molecule have been determined, the physiologically relevant tripartite TCR-SAG-pMHC complex has not yet been characterized. A structural model of TCR-SAG-pMHC complex was previously generated (48, 49) based on least-squares superposition of (i) the 14.3.d V\textsubscript{\(\beta\)}C\textsubscript{\(\beta\)}-SEB complex, (ii) the SEB-HLA-DR1 complex, and (iii) the 2C TCR \(\alpha\)\(\beta\) heterodimer. However, because the docking mode of TCR on the class II MHC was structurally unknown and presumed to be similar to the observed diagonal mode of TCR on class I MHC, it was noted that the rotational orientation of the TCR and MHC molecules in the predicted TCR-SEB-pMHC complex was substantially different (~40°) from the 2C-dEV8/K\textsubscript{\(\alpha\)} complex. The structural determination of the D10-CA/I-A\textsuperscript{d} complex reported here enables us to offer additional insight into the nature of the SAG binding to TCR and pMHC.

Fig. 6A shows the 14.3.d V\textsubscript{\(\beta\)}B\textsubscript{\(\beta\)}-SEB complex superimposed onto the D10-CA/I-A\textsuperscript{d} complex. Because the TCR docks on the MHC molecule in a nearly perpendicular manner, the SEB directly interacts with the MHC \(\alpha\) helix without any requirement for TCR rotation. However, certain segments of SEB and the \(\alpha\) helix collide. Because there is no significant conformational change observed for either of the component domains involved in this interaction, we reasoned that a relative domain movement could alleviate any steric clash. To test this idea, we removed I-A\textsuperscript{d} from the complex and then superimposed the SEB HLA-DR1 complex onto the 14.3.d V\textsubscript{\(\beta\)}C\textsubscript{\(\beta\)}-SEB complex (Fig. 6B). From
this second model, we observed that the direct interaction between Vβ and the MHC α1 helix is disrupted by SAG. The key interaction site for SEB involves CDR2 (Tyr35, Ala45, Gly53, Ser54, Thr55) and certain other Vβ residues (Glu56, Lys57, Thr58, Lys59, Ala60) as reported by Li et al. (49). In this way, the superantigen wedges itself between Vβ and the MHC class II α1 helix, forcing the MHC to swing away from Vβ and toward Vα while preserving the direct interaction between the Vα domain of the TCR and the MHC class II β1 helix. This latter interaction has been proposed to be critical in stabilizing the TCR-SAG-pMHC complex. In fact, T cell activation by SAG is believed to be dependent on the interaction between a given TCR Vα domain and the MHC class II β1 helix (50). In Fig. 6C, we have replaced the HLA-DR with the I-A<sup>+</sup> molecule on the basis of the structural alignment, thereby creating a third model. From the latter, we estimate that the relative swing angle between TCR and MHC in the TCR-SAG-pMHCII complex compared with the TCR-pMHCII complex is ~17°.

Differential TCR Binding and Coreceptor Selection in the Thymus

Given that there are no intrinsic structural differences between class I versus class II MHC–restricted TCR V modules as shown above, what directs expression of a TCR to the proper CD4 or CD8 subset? During thymocyte development, progenitor cells transit from a CD4<sup>+</sup>CD8<sup>+</sup> double negative (DN) stage through a CD4<sup>+</sup>CD8<sup>+</sup> double positive (DP) stage and then into a CD4<sup>+</sup>CD8<sup>+</sup> or CD4<sup>+</sup>CD8<sup>+</sup> single positive (SP) stage (51). Selection for maturation occurs upon the interaction of thymocytes with stromal cells expressing self-pMHC1 or self-pMHCII ligands within the thymus, beginning at the DP stage where the TCR first appears. Differentiation to the SP thymocyte stage, however, requires a match between the MHC class specificity of the TCR that a thymocyte bears and the CD4 or CD8 coreceptor it expresses. To explain how a thymocyte precisely coordinates coreceptor expression and TCR specificity, two models have been proposed (52). The “instruction model” argues that coengagement of TCR and CD4 or CD8 on a DP thymocyte specifically signals the cell to move down one pathway while extinguishing the expression of the inappropriate coreceptor. On the other hand, the “selection model” postulates that cells initiate stochastically or otherwise a process that terminates expression of one of the two coreceptors. If the correct match was chosen, then the cell further differentiates, but if not, differentiation is stalled.

Distinctions between class I versus II pMHC complexes and variation in TCR docking observed here offer strong backing for the notion of the “instruction model.” We suggest that depending on the degree of complementarity of a given TCR recognition surface and a self-pMHC1 or self-pMHCII complex, binding occurs and a diagonal docking mode with substantial variability onto pMHCII is a preferred orthogonal docking mode onto pMHCII is established. Subsequently, CD8<sup>+</sup> differentially coengages with the former and CD4 with the latter. Expression of the irrelevant coreceptor is then extinguished. On the basis of CD8<sup>+</sup>-MHC class I crystal structures and on mapping of MHC class II residues involved in CD4 binding, the two coreceptors likely occupy an “homologous” orientation relative to the TCR (17, 18). Thus, we postulate that the differential TCR docking to self-pMHC1 versus self-pMHCII contributes specifically for coordination of appropriate coreceptor selection.

References and Notes

7. The supplemental Web table is available at www.sciencemag.org/data/1045623.shl.
16. E. L. Reinherz et al., unpublished results.
20. Results from specific-CDR3<sup>3</sup> T cell hybridomas derived from mice carrying a given V<sup>α</sup>2 or Vβ1.2 D10 TCR transgene immunized with altered peptide ligands of CA (20) are consistent with the structural informations hypothesized for example, immunization of D10 TCR V<sup>α</sup>2 transgenic (tg) mice with the Glu<sup>α</sup>2-<sup>ε</sup>AA<sup>β</sup> peptide variant gives rise to T cell hybridomas, all of which use Vβ1.2 with variation in CDR3<sup>β</sup>. This suggests that the view that CDR3<sup>β</sup> is involved in recognition of the P8 position. Immunization of D10 TCR α tg mice or D10 TCR β tg mice with the Glu<sup>α</sup>2-<sup>ε</sup>LS<sup>β</sup>-CA variant both failed to generate specificity biasing that CDRL3<sup>α</sup> or CDRL3<sup>β</sup> (or both) may be important for ile<sup>ε</sup> recognition. Immunization of D10 TCR β tg mice with the Arg<sup>α</sup>2-<sup>ε</sup>Asp<sup>β</sup> variant resulted in a switch in V<sup>α</sup> usage from V<sup>α</sup>2 to V<sup>α</sup>8, suggesting that the germ
line CDRI or CDRII loops (or both) interact with this peptide residue.


44. H. Li, T. S. Jardetzky et al.


46. H. Li, T. S. Jardetzky et al.


49. H. Li et al., Immunity 8, 353 (1998).


60. Production and purification of scD10 TCR: The scD10 TCR, constructed by polymerase chain reaction, consists of 237 residues and is organized from NH2- to COOH-terminus as follows: [residue 3 to 110]-linker-[CAADDKXADKDK]-[V-D (225)]-[residue 1 to 112] with a Cys5-> Ser mutation. This linker was modified from that previously used for NMR studies (23) because the longer linker failed to give rise to I-Ak/cyclotides of diffraction quality. For bacterial expression, we used the T7 promoter expression vector pET-11a. An overnight preculture for bacterial expression, we used the T7 promoter expression vector pET-11a. An overnight preculture

61. Antigenic drift due to mutations in the hemagglutinin gene necessitates frequent replacement of influenza A strains in the human vaccine. Antibodies against hemagglutinin are a primary determinant of susceptibility to infection. However, the effects on antigenicity of specific mutations in the hemagglutinin gene are not well understood because multiple amino acid changes often occur together in important antigenic variants. Changes in antigenicity depend not only on the nature and position of the amino acid replacements but also on the amino acids currently encoded at other key positions in the HA1. We have developed a method for predicting the evolution of the virus that makes use of genetic data in the absence of specific knowledge.

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