Self-reactive T cells that escape elimination in the thymus can cause autoimmune pathology, and it is therefore important to understand the structural mechanisms of self-antigen recognition. We report the crystal structure of a T cell receptor (TCR) from a patient with relapsing–remitting multiple sclerosis that engages its self–peptide–major histocompatibility complex (pMHC) ligand in an unusual manner. The TCR is bound in a highly tilted orientation that prevents interaction of the TCR-α chain with the MHC class II β chain helix. In this structure, only a single germline-encoded TCR loop engages the MHC protein, whereas in most other TCR–pMHC structures all four germline-encoded TCR loops bind to the MHC helices. The tilted binding mode also prevents peptide contacts by the short complementarity-determining region (CDR) 3β loop, and interactions that contribute to peptide side chain specificity are focused on the CDR3α loop. This structure is the first example in which only a single germline-encoded TCR loop contacts the MHC helices. Furthermore, the reduced interaction surface with the peptide may facilitate TCR cross-reactivity. The structural alterations in the trimolecular complex are distinct from previously characterized self-reactive TCRs, indicating that there are multiple unusual ways for self-reactive TCRs to bind their pMHC ligand.

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Supplemental material can be found at:
http://doi.org/10.1084/jem.20100725
Cite by DOI: 10.1084/jem.20100725
it only partially occupied the binding groove. These results suggest that the observed structural defects in these trimolecular recognition units are caused by selection events that eliminated self-reactive T cells with optimal TCR binding properties. T cells that expressed the three EAE TCRs were pathogenic (Goverman et al., 1993; Lafaille et al., 1994; Pearson et al., 1997), and transgenic mice that expressed the human Ob.1A12 TCR and the relevant human MHC molecule developed spontaneous CNS inflammation and demyelination (Madsen et al., 1999; Ellmerich et al., 2005). These TCRs thus have altered binding properties that apparently enabled escape from negative selection but still allowed them to bind to their target peptide–MHC (pMHC) complex with sufficient strength to cause disease. The vast majority of antimicrobial TCRs are positioned over the center of the pMHC surface. An N-terminal shift has so far been observed in one case, apparently also as a result of self-tolerance mechanisms (Gras et al., 2009). This TCR (CF34) was specific for an HLA-B8–bound EBV peptide and originated from a person who was HLA-B8 and HLA-B44 heterozygous. The N-terminal shift by this TCR appeared to prevent self-reactivity to HLA-B44. In contrast, the LC13 TCR was alloreactive for HLA-B44 because it was isolated from a person who expressed HLA-B8 but not HLA-B44. This TCR bound over the C-terminal part of the HLA-B8–bound EBV peptide (Gras et al., 2009).

However, the structural database is still too small to adequately describe the recognition properties of self-reactive T cells at a repertoire level, and a substantially larger number of complexes needs to be crystallized to determine to what extent self-reactive TCRs deviate from the rules followed by most antimicrobial TCRs. In particular, the range of possible binding solutions by human autoimmune T cells remains largely unexplored. A large number of crystal structures, as well as functional studies, have shown that antimicrobial TCRs use a diagonal binding mode that positions the four germline-encoded TCR loops (complementarity-determining region [CDR] 1 and CDR2 of TCR-α and -β) over the MHC helices (Sun et al., 1995; Garboczi et al., 1996; Garcia et al., 1996; Garcia and Adams, 2005; Rudolph et al., 2006; Marrack et al., 2008). In most structures, the CDR3 loops also contribute to MHC binding. This binding mode allows the two hypervariable CDR3 loops to make extensive contacts with the bound peptide. In addition, peptide contacts are frequently made by the CDR1α and β loops to N-terminal and C-terminal peptide residues, respectively. A substantial body of recent work has suggested that the observed placement of the germline-encoded CDR1 and CDR2 loops on the MHC helices is the product of coevolution of MHC and TCR genes (Turner et al., 2006; Feng et al., 2007; Dai et al., 2008; Garcia et al., 2009).

Previous studies on human autoimmune TCRs focused on two DR-restricted T cell clones specific for MBP from MS patients. We now report the crystal structure of a human TCR (Hy.1B11) from a relapsing-remitting MS patient that recognizes the same MBP peptide (residues 85–99) as Ob.1A12 TCR but presented by a different MHC molecule, HLA-DQ1 (abbreviated as DQ1). The T cell response to MBP in this patient was dominated by three in vivo expanded T cell clones specific for the MBP<sub>85-99</sub> peptide that persisted over time. Two of these clones were DR restricted (DRA, DRB1*1602) and one was DQ1 restricted (DQA1*0102, DQB1*0502; Wucherpfennig et al., 1994a, b). A total of three clones with the Hy.1B11 TCR sequence (TRAV13-1*020, TRAJ48*01, and TRBV7-3*01, TRBD2*01, TRBJ2-3*01) were isolated from independent cultures, two from the initial time point and a third 13 mo later (Wucherpfennig et al., 1994b). Furthermore, this T cell clone was activated by four microbial peptides (from herpes simplex virus, adenovirus, human papillomavirus, and pseudomonas) that had limited sequence similarity to the MBP<sub>85-99</sub> peptide (Wucherpfennig and Strominger, 1995). The structure showed that only one of the two CDR3 loops contacted the bound peptide, explaining why specificity was limited to few peptide residues. This self-reactive TCR thus followed some of the rules established for antimicrobial TCRs (such as binding of the TCR-β chain to the MHC α1 helix) while clearly violating other rules (no MHC helix contacts by TCR-α germline–encoded loops and peptide contacts by only one CDR3 loop). The structure thereby reveals a novel way of self-pMHC recognition by a TCR from a patient with a chronic inflammatory disease.

RESULTS

Unusual features of Hy.1B11 TCR binding to the self-pMHC complex

The complex of Hy.1B11 TCR and DQ1-MBP<sub>85-99</sub> peptide crystallized in the space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, and one of the crystals diffracted to a resolution of 2.55 Å. The structure was determined by molecular replacement with one molecule in the asymmetric unit and refined to R<sub>work</sub>/R<sub>f</sub> values of 23.2 and 25.8%, respectively. Crystal data and refinement statistics for the structure are shown in Table S1. Excellent electron density was observed at the interface for the CDR loops of the TCR, the peptide, and the DQ1 helices (Fig. S1). The structure showed a strong tilt in TCR binding toward the DQ1 α1 helix, which prevented interaction of the TCR V<sub>α</sub> chain with the DQ1 β1 helix (Fig. 1, a, c, and e). The tilt was 14.5° compared with the influenza hemagglutinin (HA<sub>306-319</sub>)–specific HA1.7 TCR (Hennecke et al., 2000; Fig. 1, b, d, and f), measured using a vector through the centers of mass of the V<sub>α</sub> and V<sub>β</sub> domains. In addition, a small crossing angle (40°) of the TCR over the pMHC surface was observed which apparently prevented MHC engagement by the CDR1B loop. This crossing angle was smaller than for any other studied MHC class II restricted TCR (Fig. S2), but a similar crossing angle had been observed for the MHC class I restricted 2C TCR (Garcia et al., 1998; Rudolph et al., 2006). As a result of the tilt and the small crossing angle, the center of mass of the V<sub>α</sub> domain was shifted toward the peptide by ~6 Å compared with HA1.7, and the center of mass of the V<sub>β</sub> domain was also shifted by ~6.5 Å (Fig. S2). The tilted position resulted in limited Hy.1B11 TCR interaction with the DQ1 B1 helix (Table I) and also substantially reduced TCR interactions...
Table I. Contacts of Hy.1B11 TCR with HLA-DQ1

<table>
<thead>
<tr>
<th>CDR TCR residue</th>
<th>DQ1 residue</th>
<th>Number of contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR3α G96</td>
<td>Q57</td>
<td>2</td>
</tr>
<tr>
<td>CDR3α G96</td>
<td>G58*</td>
<td>2</td>
</tr>
<tr>
<td>CDR3α N97</td>
<td>D55</td>
<td>2</td>
</tr>
<tr>
<td>CDR3α N97</td>
<td>Q57</td>
<td>3</td>
</tr>
<tr>
<td>CDR3α E98</td>
<td>R61**</td>
<td>11</td>
</tr>
<tr>
<td>CDR2β Y46</td>
<td>R61</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β Q48</td>
<td>R61</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β Q48</td>
<td>A64</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β Q48</td>
<td>V65</td>
<td>4</td>
</tr>
<tr>
<td>CDR2β G49</td>
<td>H68</td>
<td>3</td>
</tr>
<tr>
<td>CDR2β T50</td>
<td>H68*</td>
<td>4</td>
</tr>
<tr>
<td>CDR2β A52</td>
<td>A64</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β A53</td>
<td>A64</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β A53</td>
<td>R61</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β A53</td>
<td>Q57*</td>
<td>2</td>
</tr>
<tr>
<td>CDR2β A53</td>
<td>L60</td>
<td>1</td>
</tr>
<tr>
<td>CDR2β D54</td>
<td>Q57</td>
<td>4</td>
</tr>
<tr>
<td>CDR3β L95</td>
<td>R61</td>
<td>1</td>
</tr>
<tr>
<td>CDR3β A94</td>
<td>E66</td>
<td>3</td>
</tr>
<tr>
<td>CDR3β L95</td>
<td>E66</td>
<td>2</td>
</tr>
</tbody>
</table>

* Putative hydrogen bond; **, two putative hydrogen bonds.

Only a single germline-encoded Hy.1B11 TCR loop binds to HLA-DQ1

Four germline-encoded TCR loops, CDR1 and CDR2 of both TCR chains (Fig. 2 b, yellow), and both CDR3 loops (Fig. 2 b, red) typically contact the MHC helices, as illustrated for the human HA1.7 TCR (Hennecke et al., 2000). In stark contrast, only a single germline-encoded loop of Hy.1B11 TCR (CDR2β) bound to DQ1 (Fig. 2 a). There were no contacts between the TCR Vα domain and the DQ1 β1 helix, and only a single residue on the DQ1 β1 helix (E66) was contacted by Hy.1B11 TCR (through CDR3β and Table I). Hy.1B11 TCR thus formed extensive interactions with the DQ1 α1 helix but only limited contacts with the DQ1 β1 helix (Fig. 2, c and e), whereas HA1.7 TCR had extensive interactions with both MHC helices (Fig. 2, d and f). The absence of MHC binding by three of the germline-encoded TCR loops was partially compensated by both CDR3 loops, in particular CDR3α, which made a substantial number of contacts with the DQ1 α1 helix (Table I; Fig. 2, c and e). Two distinctive features of Hy.1B11 TCR binding were responsible for this highly unusual interaction with the MHC molecule: the tilt that prevented DQ1 binding by the germline-encoded TCR-α loops and a small crossing angle.

The highly tilted Hy.1B11 TCR binding mode prevents MHC contact by the germline-encoded TCR-α chain loops. The self-reactive Hy.1B11 TCR (a, c, and e) is compared with the influenza HA-specific HA1.7 TCR (b, d, and f). The TCR-α and -β variable domains are colored in yellow and red, respectively, the MHC molecules in blue, and peptides in green. TCR and MHC constant domains have been omitted for clarity. The trimolecular complexes formed by Hy.1B11 and HA1.7 TCRs are viewed from the peptide C terminus (a and b) and rotated by 90° (c and d). The CDR1α and CDR2α loops of Hy.1B11 TCR are labeled (c). CDR1α (α1) is colored orange, CDR2α (α2) cyan, and CDR3α purple. Hy.1B11 (e) and HA1.7 (f) TCR residues that contact the respective pMHC complex are colored yellow for TCR-α and red for TCR-β.

with the peptide (Fig. 1 e), as discussed below in greater detail. In contrast, HA1.7 TCR made a large number of contacts with both MHC helices and the HA peptide (Fig. 1 f). The total buried surface area of the complex was 1645 Å², of which approximately half was contributed by TCR and pMHC, respectively. Overall, the peptide contributed 36% of the pMHC buried surface area, with the remainder contributed by the MHC protein. The TCR-α chain contributed almost half (44%) of the total buried surface area, but 85% of the surface area buried by the TCR-α chain was contributed by the CDR3α loop because the CDR1α and CDR2α loops did not bind to DQ1.
Altered self-pMHC engagement by an autoreactive TCR | Sethi et al.

The tilt prevented engagement of CDR1 and CDR2β, and the small crossing angle shifted CDR3 towards the DQ1 α1 helix. This TCR thus represents the first example in which the MHC molecule is recognized by only one of the four germline-encoded TCR loops.

**Positioning of the Hy.1B11 CDR2β loop on DQ1**

Despite these highly unusual features, the Hy.1B11 TCR was positioned diagonally on the pMHC complex similar to other TCRs. The CDR2β loop contributed >70% of the surface area buried by the TCR-β chain, and we therefore examined its interaction with the DQ1 α1 helix in detail. Crystallographic studies of TCRs that used VB8.2 identified a common binding mode of the CDR2β loop on the MHC class II α1 chain helix (Fig. 3 d, showing B3K506 TCR as an example; Feng et al., 2007; Dai et al., 2008; Garcia et al., 2009). Furthermore, mutation of this loop in TCR-β single chain transgenic mice interfered with T cell development (Scott-Browne et al., 2009). Comparison of Hy.1B11 TCR, two other human TCRs (HA1.7 and 3A6), and the mouse B3K506 TCR (as well as four other investigated TCRs; not depicted) showed a similar overall positioning of this CDR2β loop on the MHC class II α1 helix (Fig. 3, a–d; Marrack et al., 2008). Among all four TCRs, CDR2β residues 48 and 54 contacted the same positions on the MHC class II α1 helix (positions 61 and 57, respectively; Fig. 3 e), even though many of the interacting MHC and TCR side chains were chemically different. In addition, CDR2β residue 46 of Hy.1B11 and B3K506 TCRs interacted with residue 61 on the MHC α1 helix, whereas CDR2β residue 53 of both Hy.1B11 and 3A6 TCR bound to positions 57 and 61 on the MHC. Although the

Figure 2. Only a single germline-encoded loop of Hy.1B11 TCR interacts with MHC. The CDR loops that contact the MHC helices are shown for two human MHC class II restricted TCRs (Hy.1B11 and HA1.7; a and b, respectively). The germline-encoded CDR1 and CDR2 loops which contact MHC are colored yellow, hypervariable CDR3 loops red, and CDR1/ CDR2 loops contacting only peptide gray. Only loops making contact with MHC or peptide are shown. MHC class II molecules are rendered as a surface colored in blue. Peptides are colored teal. TCR-α CDR loops are labeled as α1, α2, and α3, and TCR-β loops as β1, β2, and β3. Footprint of TCR Hy.1B11 (c) and HA1.7 (d) on their pMHC ligand is shown. MHC is rendered as a surface. TCR contacts with the MHC α1 helix are colored yellow, and MHC β1 helix orange. Peptide surfaces are colored light green. Peptide residues contacted by TCR are colored dark green. Contacts with the MHC helices made by Hy.1B11 (e) and HA1.7 TCR (f) are colored yellow for the MHC class II α1 helix and orange for the MHC class II β1 helix.

Figure 3. The overall placement of the Hy.1B11 CDR2β loop on the MHC class II α1 helix is similar to other structures. The placement of the CDR2β loop of Hy.1B11 TCR (a) on the MHC class II α1 helix is compared with two other human TCRs (HA1.7 and 3A6; b and c, respectively) and a murine TCR (B3K506, d). MHC class II residues on the α1 helix contacted by multiple TCRs are highlighted as yellow spheres. The CDR2β loop is colored red and only side chains making contact to the MHC molecule are shown. CDR2β residues, as well as interacting MHC α1 helix residues, are listed (e). Contacts made by multiple TCRs are highlighted in yellow.
overall positioning of the β2 loops on the MHC class II α1 helix was similar, the molecular details of the interactions were distinct as a result of differences in docking angles, MHC polymorphisms, and the actual sequences of the TCR. CDR2β loops.

Only one of the CDR3 loops interacts with the MBP peptide

In most previously examined complexes of TCR and pMHC, both CDR3 loops contacted the bound peptide (as exemplified by HA1.7 TCR; Fig. 4b). CDR3β apparently could not contact the DQ1-bound peptide because of its short length and the unusual tilt of Hy.1B11 TCR. The Hy.1B11 CDRβ3 loop was 10 amino acids in length compared with 9–16 amino acids in other TCR structures (Table S2). The majority of peptide contacts were instead made by the CDR3α loop, in particular F95α (TRAJ48), which was inserted deeply between the P2 His and P3 Phe side chains of the peptide (Fig. 4a and Table II). Furthermore, the main chain carbonyl of F95α formed a hydrogen bond with P5 Lys of the peptide. Only one other residue of this CDR3 loop, E98α, contributed to peptide recognition through two contacts with P5 Lys. Functional experiments using a set of single amino acid analogue peptides confirmed that peptide specificity of the Hy.1B11 T cell clone was limited to the P2 to P5 peptide segment to which the CDR3α loop bound. All alanine analogues within this segment showed a substantial reduction in T cell proliferation (100 nM; Fig. 5a). However, only the alanine substitution of P5 Lys resulted in a complete loss of activity for large peptide concentration (1 µM; Fig. 5b), sug-

Table II. Contacts of Hy.1B11 TCR with the MBP85-99 peptide

<table>
<thead>
<tr>
<th>CDR TCR residue</th>
<th>Residue of peptide</th>
<th>Number of contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR1α S28*</td>
<td>P-3 Asn</td>
<td>1</td>
</tr>
<tr>
<td>CDR3α F95</td>
<td>P2 His</td>
<td>15</td>
</tr>
<tr>
<td>CDR3α F95</td>
<td>P3 Phe</td>
<td>7</td>
</tr>
<tr>
<td>CDR3α F95*</td>
<td>P5 Lys</td>
<td>3</td>
</tr>
<tr>
<td>CDR3α E98</td>
<td>P5 Lys</td>
<td>2</td>
</tr>
<tr>
<td>CDR1β Thr28</td>
<td>P8 Val</td>
<td>2</td>
</tr>
<tr>
<td>CDR2β Gln48</td>
<td>P8 Val</td>
<td>2</td>
</tr>
<tr>
<td>CDR2β Gly49</td>
<td>P8 Val</td>
<td>3</td>
</tr>
</tbody>
</table>

Putative hydrogen bond to peptide sidechain (*) or to peptide backbone (#).

Peptide positions in the P2 to P5 segment was further investigated using a panel of single amino acid analogues for each position that represented all naturally occurring amino acids except cysteine (Fig. 5, c–f). Consistent with the structural data, the highest degree of specificity was observed for P3 Phe and P5 Lys, which showed a preference for hydrophobic and basic amino acids, respectively (Fig. 5, d and f). In contrast, more than half of the analogues of P2 His showed stimulatory activity (Fig. 5 c). An analogue with a phenylalanine–to-glycine substitution at P3 was active, possibly as a result of new contacts enabled by the well-established flexibility of CDR3 loops (Fig. 5d; Garcia et al., 1998). P4 Phe represented an anchor residue for DQ1 binding that was deeply buried in the DQ1 binding groove (Fig. S3). This pocket had a preference for large hydrophobic residues, but smaller hydrophobic residues and histidine were also tolerated (Fig. 5 e). In the structure, a few contacts were also made to peptide positions P-3 Asn (by CDR1α) and P8 Val (by CDR1β and CDR2β). These contacts were mediated through the peptide backbone. Consistent with this fact, substitution of these peptide residues by alanine did not reduce activity. These data thus support the functional relevance of the structural data and demonstrate that the majority of functionally important peptide contacts are made by a single TCR loop, CDR3α.

Intermediate binding affinity of Hy.1B11 TCR

Surface plasmon resonance measurements were performed to compare the binding affinity of the MBP85-99–specific Hy.1B11 and Ob.1A12 TCRs for their ligands (DQ1–MBP85-99 and DR2–MBP85-99, respectively). Monobiotinylated pMHC complexes were captured on a streptavidin sensor chip and soluble Hy.1B11 or Ob.1A12 TCR was injected over these surfaces. Hy.1B11 TCR bound to DQ1–MBP85-99 with a Kd of 14.3 µM based on equilibrium binding data (Fig. 6b and Fig. 7b). In contrast, the affinity of Ob.1A12 TCR for DR2/MBP85-99 was lower, at 100 µM (our measurements; Cole et al., 2007). Hy.1B11 TCR binding was specific because a control TCR (Ob.1A12) showed no binding to DQ1–MBP85-99.
the site of negative selection, whereas HLA-DR protein could be readily detected (Ishikura et al., 1987). It is also well known that HLA-DQ molecules are expressed at substantially lower (≥10-fold) than HLA-DR molecules on peripheral antigen-presenting cells (Roucard et al., 1996). Direct comparison of Hy.1B11 and Ob.1A12 T cell clones showed that Hy.1B11 T cells required higher concentrations of MBP85-99 peptide for stimulation, despite the higher affinity of Hy.1B11 TCR for its pMHC ligand (Wucherpfennig et al., 1994a). The higher affinity of Hy.1B11 compared with Ob.1A12 TCR may therefore compensate for the substantially lower level of HLA-DQ than HLA-DR expression.

Mutagenesis of MHC and peptide contacts

Given this unusual binding topology, alanine scanning mutagenesis was performed to validate the structure and to define the site of negative selection, whereas HLA-DR protein could be readily detected (Ishikura et al., 1987). It is also well known that HLA-DQ molecules are expressed at substantially lower levels (~10-fold) than HLA-DR molecules on peripheral antigen-presenting cells (Roucard et al., 1996). Direct comparison of Hy.1B11 and Ob.1A12 T cell clones showed that Hy.1B11 T cells required higher concentrations of MBP85-99 peptide for stimulation, despite the higher affinity of Hy.1B11 TCR for its pMHC ligand (Wucherpfennig et al., 1994a). The higher affinity of Hy.1B11 compared with Ob.1A12 TCR may therefore compensate for the substantially lower level of HLA-DQ than HLA-DR expression.

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key TCR contact residues for DQ1 and the MBP85-99 peptide. All TCR residues that contacted DQ1 were mutated to alanine, except native alanine or glycine residues. In addition, CDR3α F95 was mutated to alanine, given its central role in peptide recognition (Fig. 7, a and b). For each mutant, nine concentrations were tested under equilibrium binding conditions to obtain reliable affinity measurements (Fig. S5). As a control, a double mutant of two noncontacting residues in CDR1α and CDR2α (N31A and S52A) was generated. This double mutant showed an affinity to DQ1-MBP85-99 close to wild-type Hy.1B11 TCR (13.8 and 14.3 µM, respectively). Four of the nine mutants had greatly reduced affinities for DQ1-MBP85-99, and these targeted TCR residues made contacts solely to DQ1 (CDR3β L95A, 110 µM), to both DQ1 and MBP85-99 peptide (CDR2β Q48A, 108 µM; and CDR3α E98A, >250 µM), or only the peptide (CDR3α F95A, >250 µM). These mutagenesis data also showed that the unusual TCR binding mode was not a crystallization artifact.

Binding by the CDR2β loop involved a combination of side chain and main chain contacts (Table I). The Q48A mutant showed substantially reduced DQ1-MBP85-99 binding (108 µM; Fig. 7 b). However, the binding contribution from two main chain hydrogen bonds (CDR2β Thr 50–DQ1α His 68 and CDR2β Ala 53–DQ1α Gln 57) could not be assessed with these mutants (Table I), which made it difficult to determine the total contribution of this TCR loop to DQ1 binding.

In contrast, CDR3α contacts were predominantly made by side chains. CDR3α E98A and F95A mutations resulted in a severe reduction in binding (>250 µM for both mutants). CDR3α F95 formed the majority of peptide contacts, and mutation to alanine greatly reduced TCR binding affinity for DQ1-MBP85-99. Nevertheless, this mutation did not assess the entire contribution by F95 because it did not eliminate the hydrogen bond between the main chain of F95 and the peptide side chain P5 Lys. The CDR3α E98A mutation resulted in the most substantial reduction in DQ1-MBP85-99 binding because of loss of two hydrogen bonds to DQ1α R61 and 11 other contacts, including two contacts to P5 Lys of the peptide.

CDR3β made no contacts to the peptide and only limited contact to the DQ1β helix. CDR3β L95 contacted DQ1β E66, and the L95A mutant showed a substantial reduction in affinity (110 µM). The interaction of CDR3β with the DQ1β helix also involved water-mediated hydrogen bonds. DQ1β E66 hydrogen bonded with a water molecule, which, in turn, hydrogen bonded with CDR3β main chain (S93, A94, L95, and E96) and side chain (D97) residues. It is possible that this hydrogen bonding network was destabilized by the CDR3β L95A mutation.

The mutagenesis data showed that all three TCR loops that contacted the DQ1 helices in the crystal structure contributed to DQ1 binding in this functional assay. The data also highlighted the critical energetic role of the CDR3α loop in binding to the DQ1-MBP85-99 complex, in particular the important contributions by CDR3α F95 and E98, which made many contacts to the peptide (CDR3α F95) and DQ1 (CDR3α E98) in the structure.

**DISCUSSION**

Autoaggressive T cells have to meet two competing requirements: they have to escape negative selection in the thymus, yet they need to be capable of initiating TCR signals of sufficient strength upon recognition of the self-antigen in the target organ of the disease. The prior structural characterization of two human TCRs from MS patients (Hahn et al., 2005; Li et al., 2005) and of three mouse TCRs from the EAE model (Maynard et al., 2005; Feng et al., 2007) showed in each case significant structural alterations that impaired TCR binding to the MHC-bound self-peptide (Wucherpfennig et al., 2009). The human TCRs bound with an altered topology, whereas the mouse TCRs bound with a normal topology. However, in all cases the interaction with the peptide was compromised either because of a shift in TCR binding (human TCRs) or partial occupancy of the peptide binding groove (murine TCRs). The structure of the self-reactive Hy.1B11 TCR
represents the first case in which only one of the four germline-encoded TCR loops interacts with the MHC helices. Furthermore, only one of the CDR3 loops interacts with the peptide, limiting TCR specificity to a short peptide segment (P2 His, P3 Phe, and P5 Lys). The structure thus explains how Hy.1B11 TCR responds to multiple microbial peptides that share limited sequence similarity with MBP85–99, in particular P3 Phe, a basic residue at P5, and hydrophobic anchors at P1 and P4 (Fig. S6; Wucherpfennig and Strominger, 1995).

The Hy.1B11 and Ob.1A12 TCRs recognize the MBP85–99 peptide bound in the same register to DQ1 and DR2, respectively (Fig. S3). The structures of the bound peptides are thus quite similar, except that the P4 Phe side chain is positioned deeper in the hydrophobic P4 pocket of DQ1 (Fig. S3c). Interestingly, specificity of both TCRs is focused on the P2, P3, and P5 peptide sidechains, but through different structural mechanisms; Ob.1A12 TCR is shifted toward the peptide N terminus, which centers the CDR3 loops over P2 His, whereas Hy.1B11 is positioned over the center of the peptide binding groove but recognizes peptide side chains only through CDR3α and not CDR3β. It is possible that both TCRs bind to this peptide segment because it is more firmly anchored in the peptide binding groove, whereas the C-terminal part of the MBP85–99 peptide is raised in both structures (Fig. S3c). In both cases, the TCR contacts one of the MHC helices in the typical location, either the MHC class II α1 chain helix (Hy.1B11 TCR–β chain with CDR2β loop) or the MHC class II β1 helix (Ob.1A12 TCR–α chain with CDR1α and CDR2α loops; Fig. S7). However, the germline-encoded loops of the other TCR chain either do not engage the MHC molecule (Hy.1B11) or bind in a highly unusual location (Ob.1A12).

In the majority of previously determined structures, all four germline-encoded loops contact the MHC helices (21 of 29 structures, with each TCR counted only once using the structure with the principal pMHC ligand; Rudolphi et al., 2006; Marrack et al., 2008; Garcia et al., 2009). These include 12 of 17 structures involving MHC class I and 9 of 12 structures involving MHC class II molecules. In seven structures, three germline-encoded TCR loops interact with the MHC helices (four MHC class I and three MHC class II restricted TCRs). These seven include two human self-reactive TCRs (Ob.1A12, 3A6) with a shifted binding topology toward to the peptide N terminus (Hahn et al., 2005; Li et al., 2005). In each of these seven cases, one of the CDR3 loops contacts both MHC helices. The A6 TCR, which recognizes a HLA–A2–bound HTLV-1 Tax peptide uses the CDR1α and CDR2α loops to bind to the HLA–A2 α2 helix, but the CDR1β and CDR2β loops do not contact the HLA–A2 α1 helix (Garboczi et al., 1996). However, the CDR3β loop has a large footprint and interacts with both MHC α1 and α2 helices. In addition, both MHC helices are contacted by the CDR1α loop. This large structural database permits the conclusion that the interaction of Hy.1B11 TCR with its MHC molecule is unusual.

There are now several examples in which there are unusual features of peptide recognition, such as TCR binding to peptides bulging out of the MHC class I binding groove. A super-bulged 13-amino peptide bound to HLA–B*3508 is recognized by SB27 TCR and the interface is dominated by TCR–peptide interactions. Nevertheless, each of the four germline-encoded TCR loops contacts the MHC protein, even though these interactions are limited (Tynan et al., 2005). In another example, the ELS4 TCR flattens a peptide bulging out of the groove of HLA–B*3501, which enables more extensive MHC contacts. Again, all four germline-encoded loops are involved in MHC binding (Tynan et al., 2007). The BM3.3 TCR shows cross-reactivity between VSV8 and pBMI peptides bound to H-2Kd, and there are large differences in the contribution of the Vα and Vβ chains to the interface between the two structures (Reiser et al., 2000). Nevertheless, all four germline-encoded TCR loops contribute to MHC recognition in both complexes.

The YAe62 TCR was isolated from mice in which negative selection was severely limited by expression of a single MHC class II–peptide complex in the thymus (Huseby et al., 2005). Even though YAe62 TCR contacts both MHC helices, it has a substantially larger interaction surface with the α1 than the β1 helix of the MHC molecule because of a tilt in TCR binding (Dai et al., 2008). Unbalanced TCR interactions with the two MHC helices can thus occur in T cells that were either not subjected to negative selection (YAe62 TCR) or escaped elimination in the thymus (Hy.1B11 TCR). Furthermore, there are now three TCRs that recognize the bound peptide using only one CDR3 loop: YAe62 and BM3.3 TCRs with their CDR3β loops and Hy.1B11 with its CDR3α loop (Reiser et al., 2000; Dai et al., 2008). The YAe62 TCR is extremely cross-reactive to both peptide and MHC variants, and the BM3.3 TCR was shown to cross react with a viral octapeptide presented by H-2Kd. Peptide recognition by a single CDR3 loop is therefore also unusual within the large structural database that is now available.

The importance of the germline loops of both TCR chains in MHC restriction has been repeatedly demonstrated using mutagenesis approaches and analysis of natural MHC micropolymorphisms (Sim et al., 1996; Manning et al., 1998; Wu et al., 2002; Huseby et al., 2006). Recent structural and functional studies strongly support the hypothesis that the conventional diagonal TCR binding mode is the result of coevolution between MHC and TCR genes (Turner et al., 2006; Feng et al., 2007; Dai et al., 2008; Marrack et al., 2008; Garcia et al., 2009). The diagonal binding orientation on the pMHC surface is similar among most crystallized αβ TCRs, but because of variation in the binding angle it has not been possible to identify conserved MHC residues contacted by all TCRs (Baker and Wiley, 2001; Rudolphi et al., 2006). However, seven crystal structures involving six different Vβ8.2 TCRs and one Vβ8.1 TCR that bound mouse I-A molecules showed a close convergence of CDR1β and CDR2β contacts with the I-A α1 helix (Garcia et al., 2009). These results suggest that particular V domains have preferred binding sites on the MHC helices. In mice with a single rearranged TCR–β chain, mutation of the CDR2β residues Tyr46 or Tyr48 to
alanine caused a substantial reduction in the number of thy-
mocytes. Furthermore, these mutations changed Vα usage, which is apparently a compensatory mechanism to enable positive selection of some T cells despite reduced MHC bind-
ing by the Vβ chain (Scott-Browne et al., 2009). Studies on MHC class I restricted TCRs have also identified MHC residues that are frequently recognized by TCRs, but the contribution of these MHC residues to binding differs among individual TCRs (Burrows et al., 2010).

CDR2β residues of Hy.1B11 were found to interact with a similar set of MHC positions as other MHC class II re-
stricted TCRs. Given that CDR2β is the only germline-
encoded TCR loop that contacts DQ1, it is possible that the placement of this loop on the DQ1 α1 helix contributed to the diagonal position of this TCR over the center of the DQ1-MBP85-99 surface. Alanine scanning mutagenesis showed that the two CDR3 loops contributed significantly to DQ1 binding. CDR3 loops have been shown to be flexible (Garcia et al., 1998; Hare et al., 1999; Armstrong et al., 2008) and, in addition to contacting the peptide, they can make important contributions to MHC binding (Garbozzi et al., 1996; Borg et al., 2005). The orientation of Hy.1B11 TCR on the DQ1-MBP85-99 surface therefore appears to result from the combined binding contributions of the CDR2β and both CDR3 loops.

The unusual structure of the Hy.1B11 TCR–DQ1-
MBP85-99 complex also raises the important question of how the corresponding human T cell escaped from negative selec-
tion in the thymus, but caution has to be used to extrapolate from structural data to complex in vivo events. This TCR has a higher affinity for its pMHC target than the previously crystallized self-reactive TCRs Ob.1A12 and 3A6 (Hahn et al., 2005; Li et al., 2005). However, the Hy.1B11 TCR is HLA-DQ restricted, whereas the other two TCRs are HLA-DR restricted. HLA-DQ molecules are expressed at ~10-fold lower levels than HLA-DR molecules (Roucard et al., 1996), and the higher affinity of Hy.1B11 TCR may therefore be re-
quired for this TCR to adequately respond to the self-peptide on peripheral antigen-presenting cells. HLA-DQ molecules are expressed at very low levels in the medulla of the thymus (Ishikura et al., 1987), which may have facilitated escape of negative selection by the Hy.1B11 T cell. It is also possible that the tilted binding mode of Hy.1B11 TCR binding affects formation of higher order structures among TCRs and/or other proteins involved in T cell activation at the immuno-
logical synapse.

In summary, this self-reactive TCR interacts in an un-
usual manner with both the self-peptide and the MHC molecule. The binding mode differs substantially from the other two autoimmune TCRs that have been crystallized, demonstrating that there are multiple unusual ways for self-reactive TCRs to bind their pMHC ligand. Particularly intriguing is the limited interaction surface with the self-peptide and the functional relevance of this finding for the activation of this self-reactive T cell clone by micro-
bial peptides.

**MATERIALS AND METHODS**

**Protein expression and complex formation.** Hy.1B11 TCR used the gene segments TRAV13-1*02, TRAJ4*01 (non-nucleotide–encoded sequence, g) CDR3 protein sequence, AASSFGNEKLT and TRBV7-3*01, TRB62*01, TRBJ2-3*01 (non-nucleotide–encoded sequence, ccctggccct; CD83 protein sequence. ATALNDTQQY. In the expression construct, the MBP85-99 peptide was attached to the N terminus of the TCR-β chain through a flexible octapeptide linker (GGGSGGPPP), as reported by Hennecke et al. (2000). The interchain disulfide bond located at the C terminus of the Cα and Cβ Ig domains was moved to the N-terminal part of these domains (replacement of CαThr48 and CβSer57 with cysteines) to enhance refolding of TCR heterodimer (Boulter et al., 2003). The chains were sepa-

ratedly cloned into pET-22b vector (Novagen), and inclusion bodies produced in BL21 (DE3) E. coli cells (Novagen) were dissolved in 6 M guanidine hydro-
chloride, 10 mM dithiotheitol, and 10 mM EDTA. To initiate refolding, TCR-α and -β chains were diluted at a 1:1 molar ratio to a concentration of 25 µg/ml of each chain in a refolding buffer containing 4.5 M urea, 0.35 M t-arginine–HCl, 100 mM Tris–HCl, pH 8.2, 1 mM of reduced glutathione (GSH), and 0.1 mM of oxidized glutathione (GSSH). After 1–24 h, the refolding mixture was dialyzed twice against denatured water and twice against 10 mM Tris–HCl, pH 8.0. Refolded TCR was purified by anion exchange chromatography using Poros PI (Applied Biosystems) and MonoQ (GE Healthcare) columns. TCR mutants were generated by overlapping PCR and cloned into the pET-22b vector (Novagen). These mutant proteins were re-

folded and purified using the same procedure as wild-type TCR Hy.1B11. DQ1 was produced in glycosylation-deficient Lec3.2.8.1 cells (Stanley, 1989). The CLIP peptide was attached to the N terminus of the DQ1β chain using a linker with a thiomercapto cleavage site, and the two chains were cloned into a vector that drives expression of glutamine synthetase to enable selec-
tion of transfected clones in glutamine-deficient media (Day et al., 2003). Stable clones were produced under methionine sulfoximine selection and tested for DQ1 secretion by Western blotting. The clone with the highest DQ1 production level was expanded in a hollow fiber bioreactor (AccuSyst miniMax; Biorevest International) and secreted DQ1 was affinity-purified using mAb 9.3.F10 (American Type Culture Collection). Fos and Jun leucine zipper dimerization domains at the C terminus used to facilitate DQ1 hetero-
dimer formation were removed by V8 protease cleavage.

After cleavage of the CLIP peptide linker, complexes were formed by permitting binding of the TCR-β chain–linked MBP85-99 peptide to the DQ1 binding site. TCR, DQ1, and HLA-DM were incubated at a molar ratio of 6:4:1 for 18 h at 25°C in a pH of 5.4, and the complex was separated from components by gel filtration (Superdex S-200 column; GE Healthcare) and anion-exchange chromatography (MonoQ; GE Healthcare).

**Crystallization and data collection.** The complex was determined to be pure by SDS-PAGE and isoelectric focusing PAGE. The complex was con-

centrated to 7.5 mg/ml in 10 mM Hepes, pH 7.2. A crystallization matrix based on conditions in which other TCR–MHC complex crystals were ob-

tained was used as a starting point for screening. Crystals were obtained in multiple conditions. The final crystals for data collection were grown by the hanging-drop vapor-diffusion method against a reservoir of 0.1 M ammonium sulfate, 8%–10% PEG 8000, and 50 mM sodium citrate, pH 6.1, at 24°C. The crystals were cryoprotected by the addition of ethylene glycol to 25%. Data were collected at 100 K at the National Synchrotron Light Source at Brookhaven National Laboratories (Upton, New York) using beamline X29 at a wave-

length of 1.0 Å by participating in the mail-in program. The data were pro-

cessed with the HKL2000 program (Otwinowski and Minor, 1997).

**Structure determination and refinement.** The structure of the complex was determined by molecular replacement using PHASER software (McCoy et al., 2007). A BLAST (Altschul et al., 1990) search of the sequences of TCR Hy.1B11 and DQ1 against the PDB database was used to find the best model for molecular replacement. Separate BLAST searches for TCR-α and -β chains were performed and the TCR with the highest consensus score (PDB accession code 3HG1) was used for molecular replacement. DQ6 (PDB accession code 3HG1) was used as the starting model for molecular replacement.
Online supplemental material is available at http://www.jem.org/cgi/Ob.1A12 TCR and other MHC class II restricted TCRs. Table S1 shows data on germline-encoded loops by the human autoimmune disease-associated TCR Hy.1B11. Fig. S3 shows that the MBP85-99 peptide binds in the binding pocket of HLA-DQ1 with a relative tilt of 5.0°.

**Affinity measurements.** The interaction of Hy.1B11 TCR with the DQ1–MBP85-99 complex was assessed by surface plasmon resonance using a Biacore 3000 instrument (GE Healthcare). DQ1 with a biotinylated C-terminal BirA tag was captured on a Biacore streptavidin chip. After immobilization of ∼700, 1,000, or 3,000 resonance units for analysis of TCR binding, solutions containing different concentrations of soluble monomeric wild-type or mutant TCR, Hy.1B11 in 10 mM Hepes, 150 mM NaCl, and 0.005% Tween 20 were injected at 15 µl/min at 25°C. Flow cells with DQ1-CLIP or DR2/MBP85-99 complexes were used as specificity controls. BIaEavaluation version 4.1 was used for all analysis. Equilibrium $K_D$ values were obtained by nonlinear curve fitting of subtracted curves using the steady-state affinity fitting mode in BIaEavaluation version 4.1. $K_D$ values are reported as mean and standard deviation.

**Analysis of peptide analogues.** Proliferation assays with the Hy.1B11 T cell clone were performed using EBV-transformed B cell line 9209 (DQA*1012, DQB*0602) as antigen-presenting cells. B cells were irradiated (5,000 rad) and treated with 50 µg/ml mitomycin C (EMD) for 30 min at 37°C. Assays were set up in 96-well U bottom plates with 5 × 10⁴ T cells and 10³ B cells in 0.2 ml of AIM-V media supplemented with 10% FCS, 2 mM GlutaMAX. Peptides were tested in triplicates at concentrations of 0.1 and 1 µM. After 72 h of co-culture, T cell proliferation was determined by [³H]-thymidine incorporation.

**PDB accession no.** The coordinates of the Hy.1B11–DQ1–MBP85-99 complex have been deposited under PDB accession no. 3P6L.

**Online supplemental material.** Fig. S1 shows electron density for critical parts of the crystal structure. Fig. S2 shows the center of mass of αVα and ββ domains of TCR, Hy.1B11. Fig. S3 shows that the MBP85-99 peptide binds in the same register to DQ1 and DR2. Fig. S4 shows a specificity control for TCR binding to DQ1 and DR2. Fig. S5 shows surface plasmon resonance experiments, and Fig. S6 shows equilibrium binding data for the TCR mutants. Fig. S6 shows the angle as it intersects a line drawn between the P1 and P9 peptide anchor residues. Relative tilt was calculated as in Teng et al. (1998).


