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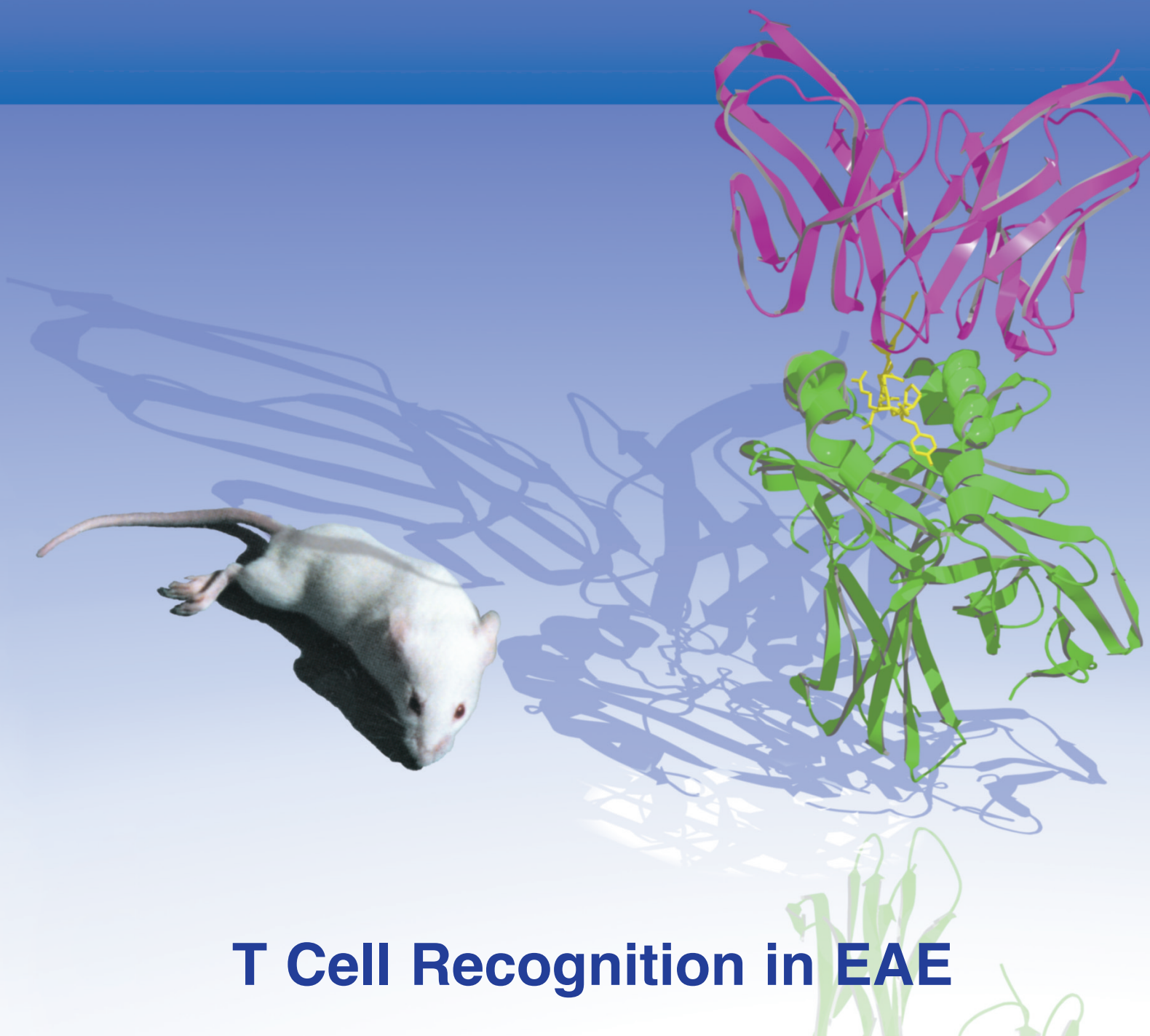

T Cell Recognition in EAE

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Structure of an Autoimmune T Cell Receptor Complexed with Class II Peptide-MHC: Insights into MHC Bias and Antigen Specificity

Jennifer Maynard,[1,3] Karin Petersson,[1,3] Dianne H. Wilson,[2,3] Erin J. Adams,[1] Sylvie E. Blondelle,[2] Marty J. Boulanger,[1] Darcy B. Wilson,[2,3] and K. Christopher Garcia[1,*]

[1]Departments of Microbiology and Immunology, and Structural Biology
Stanford University School of Medicine
Stanford, California 94305

[2]Torrey Pines Institute for Molecular Studies
San Diego, California 92121

Summary

T cell receptor crossreactivity with different peptide ligands and biased recognition of MHC are coupled features of antigen recognition that are necessary for the T cell's diverse functional repertoire. In the crystal structure between an autoreactive, EAE T cell clone 172.10 and myelin basic protein (1–11) presented by class II MHC I-A^u, recognition of the MHC is dominated by the Vβ domain of the TCR, which interacts with the MHC α chain in a manner suggestive of a germline-encoded TCR/MHC "anchor point." Strikingly, there are few specific contacts between the TCR CDR3 loops and the MBP peptide. We also find that over 1,000,000 different peptides derived from combinatorial libraries can activate 172.10, yet the TCR strongly prefers the native MBP contact residues. We suggest that while TCR scanning of pMHC may be degenerate due to the TCR germline bias for MHC, recognition of structurally distinct agonist peptides is not indicative of TCR promiscuity, but rather highly specific alternative solutions to TCR engagement.

Introduction

The engagement of the T cell receptor by peptide-MHC is the central antigen-specific event mediating the cellular immune response. The concept of an inherent TCR degeneracy has emerged to explain how a TCR is able to recognize the diverse peptide antigens it encounters during the processes of thymic education and peripheral surveillance (Ignatowicz et al., 1997; Nikolic-Zugic and Bevan, 1990; Hemmer et al., 1998b; Holler and Kranz, 2004; Kersh and Allen, 1996a; Mason, 1998; Wucherpfennig, 2004). This concept has been buttressed by biophysical studies of TCR/MHC interactions (Rudolph and Wilson, 2002), which indicate that flexibility in the central CDR3 loops of the TCR may serve as an adaptation mechanism to "read out" different peptide antigens during TCR "scanning" of the universe of peptide-MHC (Garcia et al., 1998; Willcox et al., 1999; Wu et al., 2002). The fact that peptide comprises a fraction (~25%–30%) of the composite pMHC surface, while the majority of the helical surface (~70%–75%) is conserved and evolved for biased recognition by the TCR, suggests that TCR/pMHC interactions are tuned for the sampling of different antigens (Daniel et al., 1998; Davis and Bjorkman, 1988; Germain, 1990; Housset and Malissen, 2003; Madden, 1995).

In support of this hypothesis, examples exist of structurally diverse, crossreactive peptides to a single T cell clone (Bhardwaj et al., 1993; Crawford et al., 2004; Hagerty and Allen, 1995; Hemmer et al., 1998a; Ignatowicz et al., 1997; Kersh and Allen, 1996b; Krogsgaard et al., 2003; Loftus et al., 1999; Reiser et al., 2003; Sykulev et al., 1994; Wucherpfennig and Strominger, 1995). While some of these peptides retain recognizable sequence similarities with the cognate peptides (Crawford et al., 2004; Krogsgaard et al., 2003; Sykulev et al., 1998), other crossreactive peptides have been shown to be minimally homologous in sequence and therefore presumably engaging the TCR though a unique structural solution (Lang et al., 2002; Loftus et al., 1999; Reiser et al., 2003; Wucherpfennig, 2004).

In contradiction to the notion of a promiscuous TCR, most T cell clones are exquisitely sensitive to mutations in the peptide (Shih and Allen, 2004). Some of the most extensively studied TCR/MHC systems, such as 2B4/I-E^k (Krogsgaard et al., 2003), 3L.2/I-E^k (Kersh et al., 1998; Shih and Allen, 2004), KRN/I-A^{g7} (Basu et al., 2000), and 2C/H-2K^b (Degano et al., 2000; Sykulev et al., 1996), exhibit extremely specific peptide recognition and are largely intolerant of amino acid changes in the TCR contacts. In most reported cases of degenerate TCR recognition, the TCR contact residues of the crossreactive peptides are similar (Basu et al., 2000; Crawford et al., 2004; Grogan et al., 1999; Sykulev et al., 1998; Wilson et al., 1999). Indeed, a single centrally located peptide residue is sufficient to produce tight selectivity by a TCR (Degano et al., 2000; Ding et al., 1998; Krogsgaard et al., 2003; Shih and Allen, 2004). Thus, the idea of degenerate T cell recognition is difficult to reconcile with experimental observations of T cell specificity.

To address these questions, we have been studying TCR/pMHC interactions in murine experimental allergic encephalomyelitis (EAE), an intensively studied model system, to understand autoimmunity to neural self-antigens, such as myelin basic protein (MBP) (Zamvil and Steinman, 1990). The immunodominant encephalitogenic T cell epitope of MBP, recognized by T cells in mice of the H-2^u haplotype (PL/J or B10.PL), is the acetylated N-terminal 11-mer (Ac1-11) (Zamvil et al., 1987). The Ac1-11 epitope in the context of class II MHC I-A^u has a number of unusual features such as a very short half-life (<15 min.) and a requirement for an N-terminal acetylation, and MBP peptides as short as Ac1-6 can still activate EAE T cell clones (Fairchild et al., 1993; Fugger et al., 1996; Gautam et al., 1994; Mason et al., 1995; Wraith et al., 1992). A crystal structure of I-A^u complexed with MBP1-11 provided a rationale for these properties by finding that the peptide sits in an unusual shifted register in the groove, which results in empty p1 and p2 pockets, the MBP N terminus in the p3 pocket,

*Correspondence: kcgarcia@stanford.edu
[3]These authors contributed equally to this work.

and the peptide exiting the C-terminal end of the I-A^u groove at the P7 position (He et al., 2002). The T cell response to Ac1-11 has been extensively studied (Goverman, 1999) and exhibits highly biased TCR Vβ8.2 chain usage (Acha-Orbea et al., 1988; Urban et al., 1988; Fairchild et al., 1993; Fugger et al., 1996; Gautam et al., 1994; Mason et al., 1995; Wraith et al., 1992).

Here, we focus on the 172.10 T cell clone, which is derived from encephalitogenic T cells isolated from MBP-immunized H-2^u mice (Goverman et al., 1993). We previously measured the affinity of 172.10 for I-A^u-Ac1-11 and found it to be within the ranges of normal cognate TCR/pMHC interactions ($K_D \sim 5\ \mu M$) (Garcia et al., 2001). Companion thermodynamic measurements suggested flexibility in the TCR binding loops while binding to I-A^u-Ac1-11, which may be favorable for degenerate recognition of structurally diverse peptides. In the present study, we analyze the structure of the 172.10 TCR in complex with I-A^u-MBP1-11. In parallel, we used the 172.10 T cell clone to scan a combinatorial decapeptide library to identify an extensive panel of peptide mimotopes with a wide range of activities on the 172.10 clone. The complementary results of these experiments have implications for our current understanding of TCR cross-reactivity and biased recognition of MHC.

Results

Overall Structure

The 2.4 Å structure (see Experimental Procedures, and Supplemental Table S1 http://www.immunity.com/cgi/content/full/22/1/81/DC1/) of the 172.10 single-chain VαVβ heterodimer complexed to I-A^u-MBP1-11 is reminiscent of previously determined class I and class II TCR/pMHC complexes (Figure 1) (Hennecke et al., 2000; Housset and Malissen, 2003; Reinherz et al., 1999; Rudolph and Wilson, 2002). The TCR is oriented with the Vα domain largely overlaying the N-terminal region of the peptide and β1 helix and the Vβ domain overlaying the C-terminal end of the peptide and the α1 helix (Figures 1B and 1C). This docking orientation falls within the $\sim 60^\circ$ diagonal range seen in previous class I and class II TCR/pMHC complexes (Hennecke and Wiley, 2001). An interesting deviation from previous TCR/pMHC complexes is due to the unusual binding register of the MBP peptide in the I-A^u groove, where the peptide P1 residue (ala) occupies the p3 pocket, resulting in a two-pocket shift of the entire peptide toward the C-terminal end of the MHC groove (Figure 1B) (He et al., 2002). As a result, the N terminus of the peptide sits underneath the CDR3α, which results in the majority of the MBP peptide residing underneath the 172.10 Vβ footprint (Figure 1C). In our recombinant protein, the MBP N-terminal acetylation is mimicked by a P-0-Gly residue (Radu et al., 1998), and three residual N-terminal peptide residues (colored in gray in Figure 1) remain from the *Drosophila* leader peptide, making a number of van der Waals interactions with the TCR Vα (Supplemental Table S2).

172.10 Interface with I-A^u-MBP-1-11: Recognition of the MHC Helices

Collectively, the TCR/pMHC interface (Supplemental Table S2) is striking in two respects: (1) the predominance of the TCR Vβ in both the number and specificity of interactions with the MHC (Figure 2), and (2) an unusual chemistry of peptide recognition that exhibits poor structural complementarity and few specific interactions between either CDR3α or β and MBP1-11 peptide residues (Supplemental Table S2) (Figure 3). In the structure, the 172.10 footprint buries approximately 1160$Å^2$ of surface area distributed roughly equally between Vα (54%) and Vβ (46%). On I-A^u, a total of 1100$Å^2$ of surface area is buried, with 867$Å^2$ attributable to the MHC helices (79%) and only 233$Å^2$ to the peptide (21%). There are a total of 44 amino acid contacts involving 127 atoms (van der Waals plus hydrogen bonds) between the TCR and I-A^u-MBP1-11, with 35 interesidue contacts (92 atoms) between the TCR and the MHC helices (17 from Vα, 27 from Vβ), and 9 inter-residue contacts between the TCR and MBP peptide (six van der Waals, 3 H bonds) involving 35 atoms. Overall, the 172.10/I-A^u-MBP1-11 interaction has a shape complementarity (Sc) value of 0.62, which is at the low end for protein-protein interactions and indicates a moderately poorly packed TCR/pMHC interface.

A striking observation is that the α chain contact with the MHC helices is limited to 11 inter-residue van der Waals contacts (47 atoms) (Figure 2A), while the β chain forms 24 helical contacts, 8 of which are hydrogen bonds (totaling 80 atoms) (Figure 2B). Even though the 172.10 Vα (containing CDR1α and CDR2α) buries a little more than half of the total interface, it does not form a single hydrogen bond with the MHC helices or any contacts with the MBP peptide. The lack of peptide contacts is clearly due to the register shift translating the MBP peptide out of range for CDR1α or 2α contacts (Figures 1 and 3). While there is excellent knob-in-hole complementarity between Vα and the β1 helix resulting from van der Waals contacts (Figure 2A), the absence of any hydrogen bonds between the CDR1α and 2α and the MHC suggests a lack of tight specificity in these interactions. This characteristic is unexpectedly different from the majority of TCR/pMHC complexes, in which the Vα domain dominates the pMHC interaction with respect to both buried surface and numbers of interatomic contacts (Rudolph and Wilson, 2002).

The 172.10 Vβ domain shows much greater structural complementarity to the MHC surface than the Vα, forming more than twice as many contacts (Supplemental Table S2) (Figure 2B). As 8 of these 24 amino acid contacts with the MHC are hydrogen bonds, this suggests that the specificity of recognition of the I-A^u helices is largely dictated by the Vβ, which is consistent with the well-documented observation that the EAE T cell response is strongly biased toward Vβ8.2 (Zamvil and Steinman, 1990). Residues unique to the Vβ8.2 CDR1 and 2 form abundant specific contacts with residues on the I-A^u helices. For example, Asn31β of the Vβ8.2 CDR1 makes a hydrogen bond with Gln61α on the I-A^u α1 helix. Additionally, CDR3β is "braced" on both sides of the helical groove by main chain hydrogen bonds (Gly98β N and Gly100β O) to side chains on the α1 and β1 helices (Gln61α and Arg70β, respectively) (Figure 2B). These interactions position the CDR3β Tyr-104β hydroxyl group for formation of a hydrogen bond with the P5-Arg Nη1 atom of the MBP peptide. Thus, the explanation for the Vβ8.2 bias seems to be that the interactions

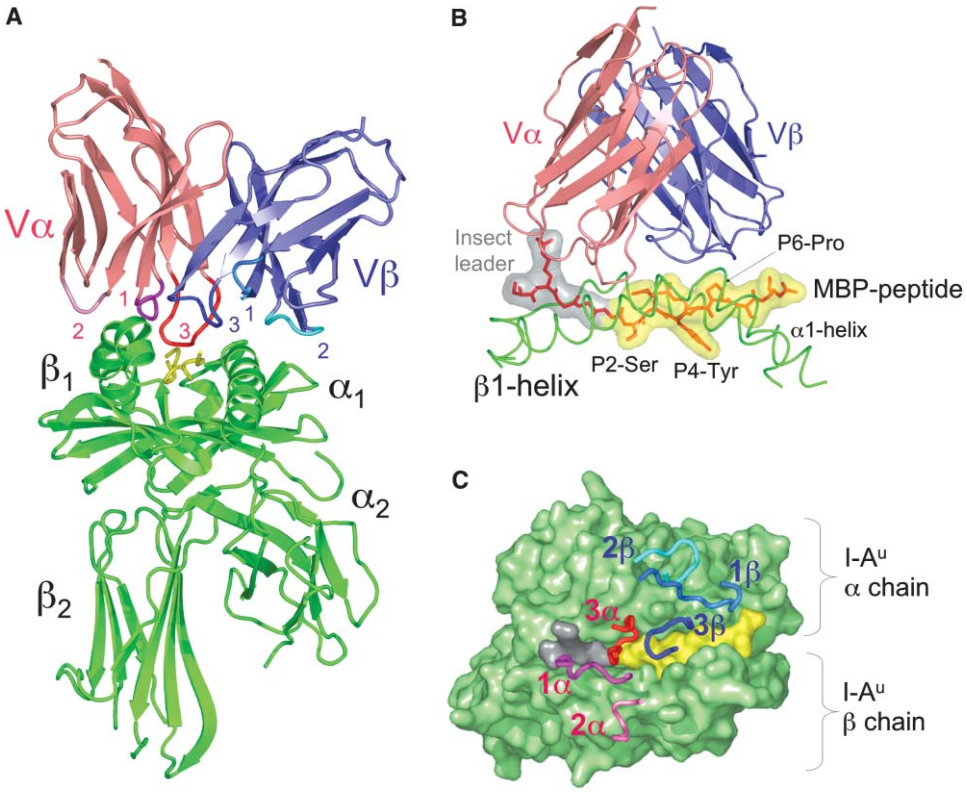

Figure 1. Structure of TCR 172.10 in Complex with I-A^u/MBP1-11

(A) Ribbon representation of the complex including 172.10Vα (red), 172.10Vβ (blue), I-A^u (green), and MBP residues 1–8 (yellow). The CDR loops from 172.10 are colored as follows: CDR1α (magenta), CDR2α (pink), CDR3α (red), CDR1β (maroon), CDR2β (cyan), and CDR3β (blue).

(B) Side view (turned 90° from [A]) of the complex. MBP peptide is shown as a transparent molecular surface (yellow) with ball-and-stick representations of amino acids inside, including the leader peptide (gray). The β1 helix from I-A^u is in the front, and the α1 helix is behind the peptide.

(C) 172.10 footprint showing docking orientation on I-A^u/MBP1-11. Peptide-MHC is shown as a green surface (peptide in yellow, leader residues in gray), and the 172.10 CDR loops are drawn as tubes and labeled. This and all subsequent figures were produced with PyMol (Delano, 2002).

between Vβ8.2 and I-A^u helices are optimal in the *context* of specific MBP interactions with the CDR3β.

172.10 Interface with I-A^u-MBP-1-11: Recognition of the MBP Peptide

The CDR3α and β loops overlay the central region of the I-A^u groove, containing the amino-terminal region (P1 to P6) of the 11 residue MBP peptide (Figure 3). There is no interaction from either germline-encoded Vα or Vβ with MBP, which is unique for TCR/pMHC complex structures, which generally show some interaction between the TCR CDR1α and β with peptide (Rudolph and Wilson, 2002).

The interface chemistry of 172.10 is notable in that it appears rather structurally degenerate in its recognition of the MBP peptide, as evidenced by the paucity of side-chain-specific hydrogen bonds (1) and salt bridges (0) and the use of main-chain hydrogen bonds between the CDR3 loops and peptide (Figure 3). CDR3α has a main-chain hydrogen bond between the carbonyl oxygen of position Asn99α to the main-chain amide nitrogen of P1-Ala of the MBP peptide. There is another main-chain hydrogen bond from the CDR3α Gly101α amide nitrogen to the side chain of the MBP P3-Gln (Figures 3A and 3C). CDR3β, which contains a Gly-Gly-Gly sequence at its apex, forms the only side-chain-specific hydrogen bond from the TCR to the MBP peptide in the complex structure (Figures 3B and 3C). The hydroxyl group of CDR3β residue Tyr-104β forms a hydrogen bond with the terminal Nη1 group of P5-Arg (Figures 3B and 3C). Finally, P6-Pro sits in a pocket underneath CDR3β, forming van der Waals contacts with the side chain of Asp-96β (Figure 3C). The structure of the long CDR3β appears compressed back into the TCR binding site by the presence of the peptide. Given the thermodynamic data indicating unfavorable binding entropy of 172.10 for I-A^u-MBP1-11 (Garcia et al., 2001), it appears likely that this loop has undergone conformational rearrangement upon binding. The peptide contacts with the TCR rationalize the structure-function data of Ac1-11 recognition in the context of I-A^u, which indicate that P3-Gln, P5-Arg, and P6-Pro comprise the main recognition epitope (Anderton et al., 1998; Gautam et al., 1994).

Comparison of 172.10/I-A^u-MBP1-11 with D10/I-A^k-CA

The closest overall structural comparison that can be made with the 172.10/I-A^u-MBP1-11 complex (determined to 2.4 Å) is with the D10/I-A^k-CA (conalbumin) complex (determined to 3.2 Å) (Reinherz et al., 1999)

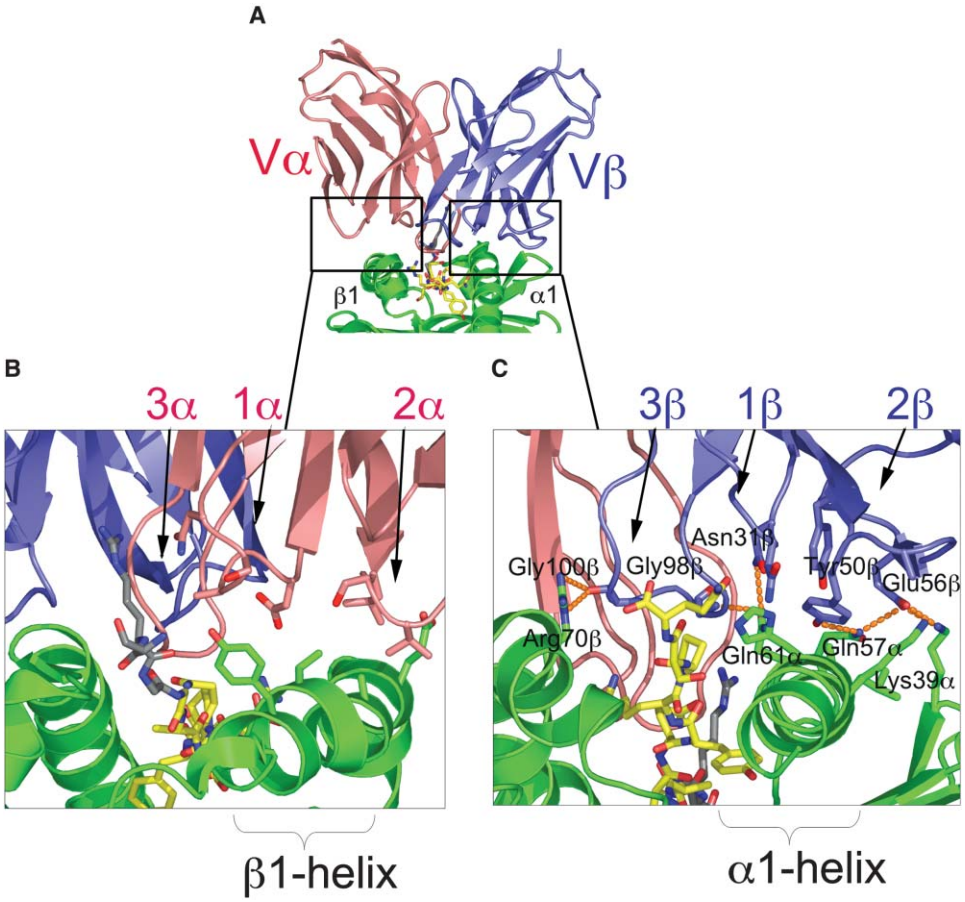

Figure 2. 172.10 Vα and Vβ Interactions with β1 and α1 Helices of I-A^u

(A) Overview of the interface between 172.10 Vα (red) and Vβ (blue) and I-A^u (green)/MBP-peptide (yellow).

(B) Contact interface between the 172.10 Vα and the I-Au β1 helix. All contacts are van der Waals.

(C) Contact interface between the 172.10 Vβ and the I-Au α1 helix showing both hydrogen bonds and van der Waals interactions. Labeled residues are those involved in the hydrogen-bonding network.

(Figure 4). I-A^u and I-A^k have eight and fifteen amino acid differences in the α and β chains, respectively, and D10 and 172.10 both use Vβ8.2. Therefore, CDR1 and CDR2 of Vβ are identical in the two TCRs. D10 uses Vα2 and 172.10 uses the subgroup Vα2.3, which are highly similar (84% sequence identity) but contain amino acid differences in the CDR1α and 2α loops. An overlay of the footprints of 172.10 and D10 reveals that the Vβ domains superimpose almost identically in an "orthogonal" orientation (discussed below) (Reinherz et al., 1999), but the 172.10 Vα domain is rotated ~15°, shifting the 172.10 Vα footprint toward the end of the groove by approximately 6 Å relative to D10 (Figure 4B). In 172.10, there is no MBP contact by the Vα, whereas in D10 the majority of peptide contact is by the Vα, which is likely playing a role in the divergence of the Vα footprints.

A TCR/MHC Anchor Point

Although all TCR/pMHC complex structures have roughly similar docking orientations, each complex shows unique sets of TCR V domain contacts to MHC, confounding the idea that there may exist "rules" for TCR docking onto the MHC consisting of particular TCR germline residues interacting with conserved residues on the MHC helices (Bankovich and Garcia, 2003; Housset and Malissen, 2003). However, in the case of the 172.10/I-A^u-MBP1-11 and D10/I-A^k-CA complexes, the amino acids and the atomic structure of the contact patch between CDR1β, CDR2β, and the α1 helix are essentially identical with respect to both van der Waals interactions and hydrogen bonds (Figures 4B–4D). The CDR1β and CDR2β residues Asn31β, Tyr50β, and Glu56β share four hydrogen bonds and one salt bridge with MHC α chain residues Gln-57α, Gln61α, and Lys-39α (Figures 4C and 4D), respectively. In addition, numerous residues on the TCR and MHC form van der Waals interactions that are shared in both complexes (Figures 4C and 4D). The preservation of these interatomic contacts is the result of conserved interacting residues on the I-A^u and I-A^k α1 helices and CDR1β and CDR2β (Figure 4D). This convergence of Vβ docking solutions has the important implication that there may be a limited set of binding solutions for Vβ8.2 onto I-A MHC haplotypes (see Discussion).

Scanning the 172.10 T Cell Clone with Combinatorial Peptide Libraries

Given the apparent dearth of specific contacts between 172.10 and MBP1-11, we asked whether this TCR may

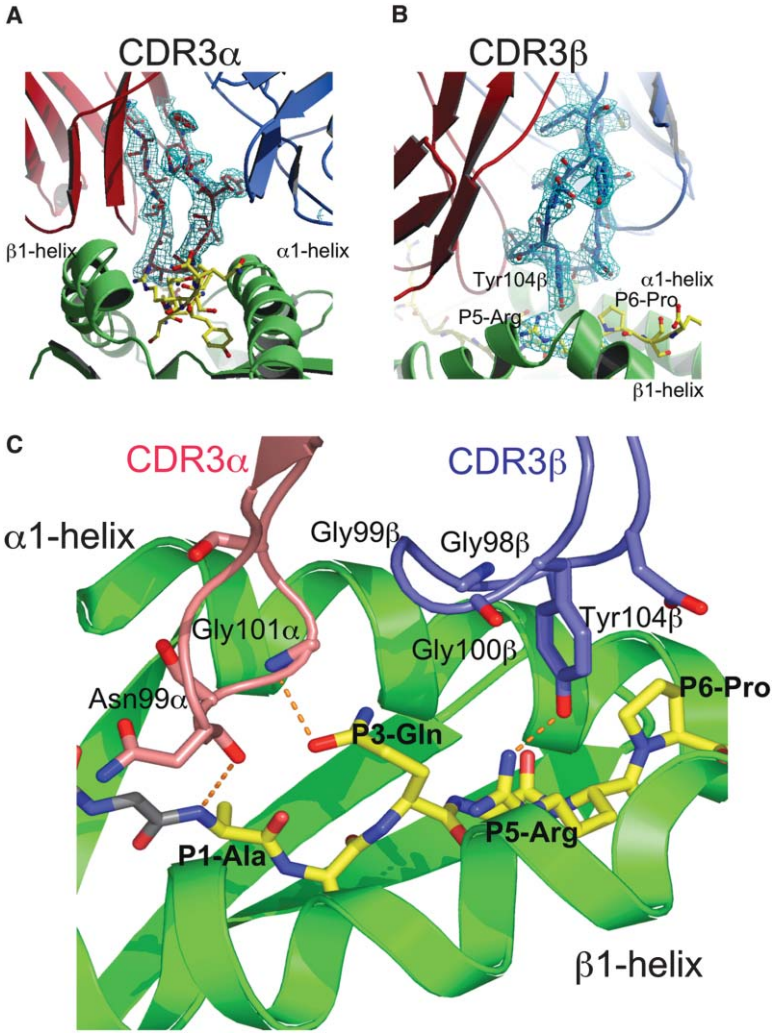

Figure 3. 172.10 CDR3 Interactions with the MBP Peptide

(A and B) Front view (A) with electron density of the 172.10 CDR3α loop from the final 2.4 Å $2F_o$-F_c map and (B) side view of the 172.10 CDR3β loop. The 172.10Vα is in red, 172.10Vβ in blue, I-A^u in green, and the MBP peptide in yellow.

(C) The interface between the CDR3 loops and the peptide binding groove, including ribbon representation of the α1 and β1 helix in I-A^u as well as the MBP peptide in ball and stick. Hydrogen bonds between the loops and the peptide are indicated as orange dots. Not all residues shown are in contact (see Supplemental Table S2).

be capable of recognition of other peptides in association with I-A^u. We carried out a peptide library scan of the 172.10 T cell hybridoma using a synthetic combinatorial peptide library containing all possible combinations of N-acetylated decapeptides. Such library scans of a given T cell clone lead to definition of a large number of peptide analogs (>100) of varying activity on the selecting clone (Pinilla et al., 1999; Wilson et al., 1999). In this study, a decamer peptide library is arrayed in a positional scanning format and consists of 200 different peptide mixtures, each one having one of the 20 amino acids (O) in a defined position and all of the natural L-amino acids except cysteine (A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y) in stoichiometric amounts in each of the remaining positions (OX_9). For example, the first mixture has alanine (A) fixed in position 1 (AX_9), i.e, it contains all peptides with Ala at the N terminus, while mixture 200 has tyrosine (Y) fixed in position 10 (X_9Y), i.e, it contains peptides with Tyr at the C terminus. Each OX_9 mixture consists of 3.2×10^{11} (19^9) different peptides. Given an average molecular weight of 1200 Da for a decapeptide mixture, when used at a concentration of 100 μg/ml, the concentration of each individual peptide in an OX_9 mixture is 2.6×10^{-16} M.

The extent of degeneracy of the 172.10 clone is shown in Figure 5, which shows the IL-2 response of the 172.10 hybridoma, to the 200 different library mixtures. Note that the first library mixture with alanine fixed at position 1 (AX_9) generates a signal substantially above background, reflecting a strong preference for P1-Ala. The AX_9 mixture used at a concentration of 100 μg/ml contains 320×10^9 different peptides, each at approximately a concentration of 3×10^{-7} nM. A minimal estimate of the degeneracy of the 172.10 clone can be calculated by comparing the peptide concentration required to trigger a detectable response ($EC_{10} \sim 3 \times 10^{-10}$ M) with the concentration of each peptide in the AX_9 mixture (3×10^{-16} M). Such a comparison yields as many as 10^6 ($3 \times 10^{-10}/3 \times 10^{-16}$) active peptides in the AX_9 mixture that could have contributed to provide a detectable signal. In addition to P1-Ala, P5-Arg is clearly dominant. In contrast, the two other TCR contact residues, P3-Gln and P6-Pro, could possibly be substituted with semiconservative or nonconservative residues (Figure 6).

The scan information in Figure 5 provides aggregate data that are an experimentally derived estimate of the TCR degeneracy, but it does not yield individual peptide sequences to test, as there are far too many active candidates. Therefore, to reduce the number of candidate mimotopes to a manageable size which could be

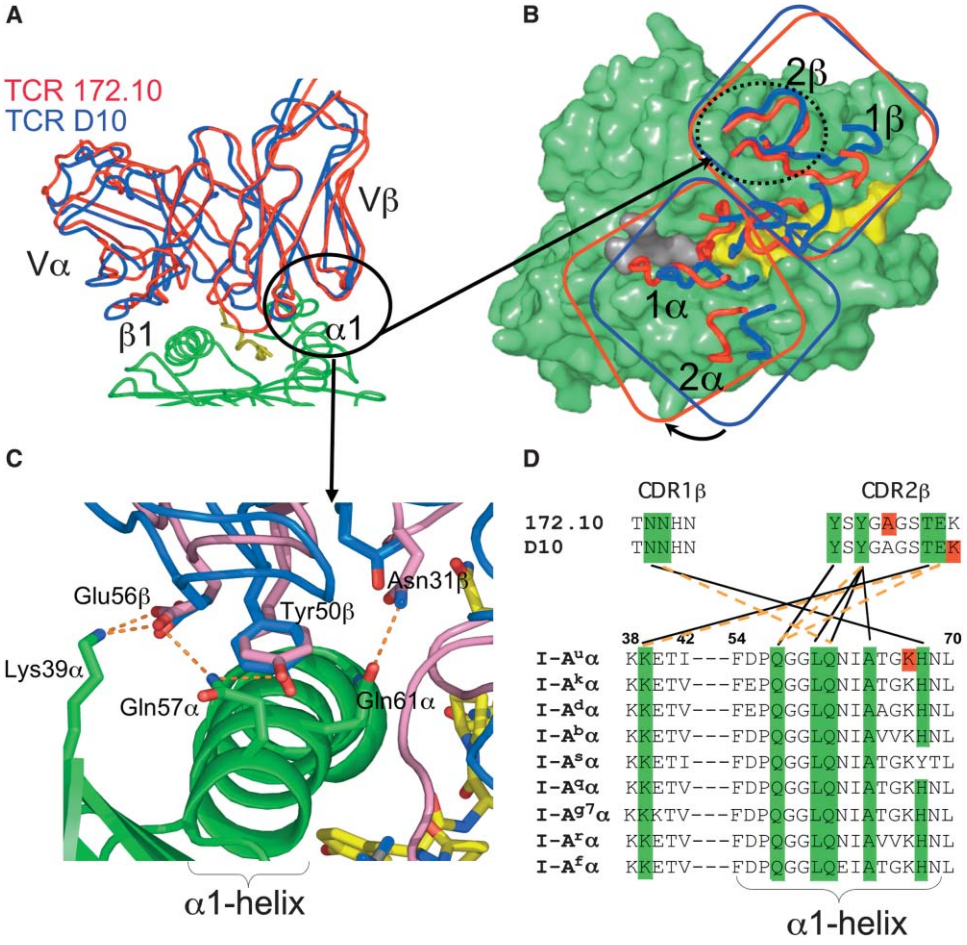

Figure 4. Structural Comparisons between 172.10/I-A^u and D10/I-A^k Complexes

(A) The I-A^k molecule from the D10/I-A^k complex (PDBID 1D9K) was superimposed on I-A^u in the 172.10/I-A^u complex. The superposition is represented as tubes; 172.10 is colored red, and D10 is colored blue, I-A^u is colored green, and the MBP peptide is yellow. The region of closest superposition (CDR1β and CDR2β) is highlighted with a circle.

(B) Top view of the superposition with I-A^u/MBP in surface representation including the CDR loops from 172.10 and D10 as tubes. The differences in footprint for 172.10 and D10 on the I-A are shown as color-coded squares (red for 172.10, blue for D10).

(C) Shared hydrogen-bonding residues in the interface between the α1-helix (I-A^u) and the CDR1β and 2β loops from 172.10 and D10. Hydrogen bonds are indicated as orange dots and drawn for the 172.10/I-A^u complex.

(D) Shared overall contacts between the Vβ8.2 in both 172.10 and D10 with I-A^u and I-A^k, respectively. Sequence alignments are between the recognition surface of I-A α chains from residues 38 to 70, as well as between the CDR1β and 2β loops from 172.10 and D10. Residues shaded in green are making contacts in both the 172.10/I-A^u and D10/I-A^k complexes, while residues shaded red are only making contacts in one of the complexes. Black lines are drawn between residues with conserved van der Waals contacts and orange dot lines for conserved hydrogen bonds.

individually synthesized and tested, we screened a biased library (Figure 6A) with only those most prominent amino acids that emerged from the library scans in Figure 5. We included those amino acids corresponding to the active mixtures causing a SI $\geq$ mean + 1 SD of all of the mixtures for a given position and that ranked among the four most active mixtures in three of four different experiments.

The results of the biased library scan (Figure 6A) clearly show a preferred set of residues that activate the 172.10 clone containing P1-Ala, P3-Gln, P5-Arg, and P6-Pro, which are the wild-type MBP TCR contacts in the structure. As predicted from the library screen (Figure 5), the TCR contact residue P5-Arg is intolerant of substitution. However, the biased library data indicate that TCR contact residues P3-Gln and P6-Pro also cannot be replaced by others in the set chosen based on the aforementioned criteria, but do not rule out alternative allowed substitutions not tested in this experiment (Anderton et al., 1998). In contrast, MHC binding residues at P4 and P8 can utilize up to four residues, and P7 can substitute lysine for alanine. The true extent of 172.10 degeneracy is larger than this experiment indicates because we tested a restricted subset of amino acids so as to have a manageable library size that would ultimately yield individual sequences.

From this biased library, we individually synthesized and tested 128 peptides for activity (Figure 6B), of which over half were more potent than MBP1-11. In all 128 peptides, the P1-Ala, P3-Gln, P5-Arg, and P6-Pro are

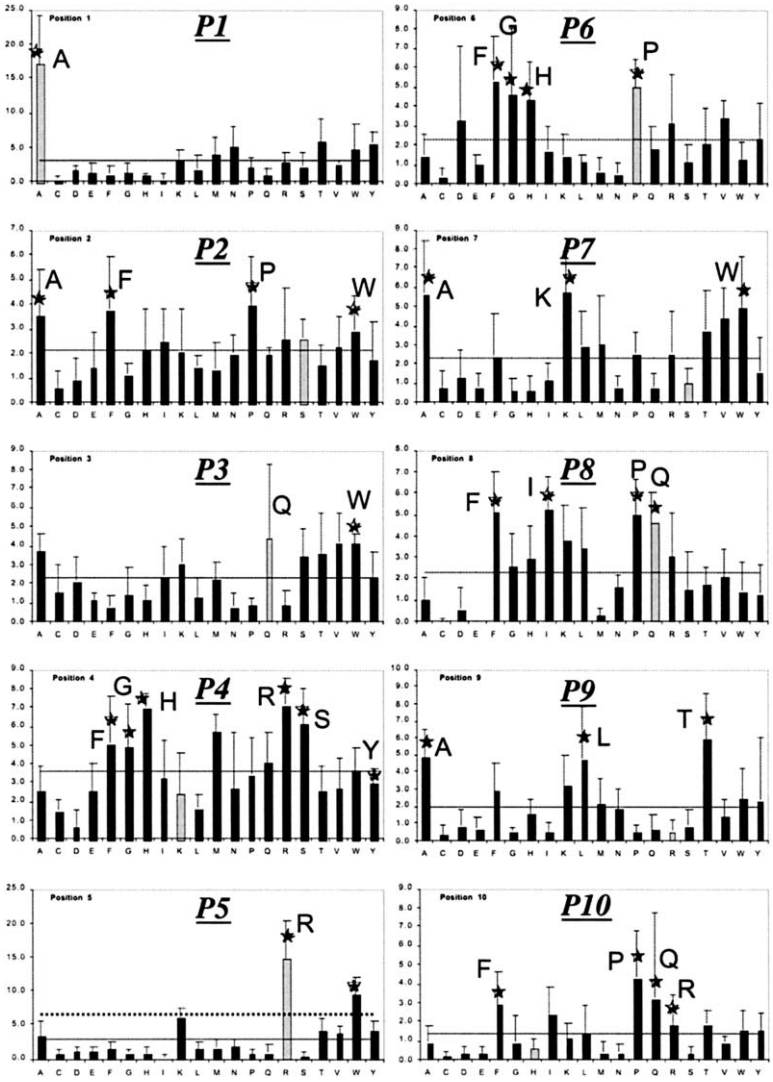

Figure 5. Library Scan of the 172.10 Clone

Each panel represents each position of the peptide in counterclockwise order. The panels are labeled for position (P1, P2, etc.), and the top hits at each position are labeled in single-letter amino acid code. The x axis in each panel indicates each of the 20 fixed amino acids. The y axis indicates the IL-2 response of 172.10 cells to each library mixture in terms of stimulation index (SI) determined in secondary cultures of HT2 cells with supernatants from library-stimulated wells. Solid horizontal lines indicate the mean SI for all 20 mixtures within the panel; dashed horizontal lines indicate mean SI plus 1 SD. The gray bars indicate the amino acids of the native Ac1-10 sequence. Stars indicate amino acids selected at each position to generate candidate sequences. Amino acids selected for synthesis of candidate mimic sequences, indicated at the top of Figure 6, were those from fixed amino acid mixtures with SIs greater than mean plus 1 SD. In some instances, amino acids were also selected on the basis that the corresponding mixtures ranked among the four most active (of 20) in three or four different experiments.

identical to that of MBP in the structure, which are the primary TCR contacts (Figure 3C; Supplemental Table S2). The peptides span a 6-log range in activity through only varying residues other than TCR contacts, with most of the degeneracy in the C-terminal region of the peptide, which is likely outside of the groove (the molecular basis of this is unclear).

Discussion

TCR degeneracy has been extensively discussed in order to rationalize the plurality of recognition requirements inherent in the life of the T cell (Kersh and Allen, 1996a; Mason, 1998; Wilson et al., 2004; Wucherpfennig, 2004). In the case of the 172.10 TCR, we see a TCR interface with peptide that appears to lack robust peptide-specific interactions and is surrounded by a framework of interactions between the TCR and MHC helices, many of which are conserved. This led us to question whether other peptides could form more complementary interactions with 172.10 CDR3s than the MBP peptide. However, the large peptide libraries we tested revealed that the TCR contacts are highly focused on the native MBP amino acids. Although the peptide libraries do not rule out the existence of alternative TCR contacts, the clear trend is a preference for the native MBP residues in the strong agonist ligands, contrary to the notion of a promiscuous TCR.

We have also carried out a peptide library scan against another Ac1-11-specific T cell clone called 1934.4 (Gautam et al., 1994), which has a similar focus on P5-Arg, and P6-Pro, which are the presumed TCR contacts (D.B.W., data not shown). A peptide scan was also carried out on the unrelated 2B4 TCR, which is specific for I-E^k and moth cytochrome C and whose TCR and MHC contact residues have been precisely mapped by mutagenesis (Krogsgaard et al., 2003). Similar to 172.10 and 1934.4, there was an almost complete intolerance to mutation of the TCR contacts and a strong modulatory effect of alterations in flanking amino acids (Krogsgaard et al., 2003).

Defining Crossreactivity

The terms “crossreactive” TCR or “molecular mimic” peptides have been used rather vaguely and often with-

A Biased library scan.

Native seq.	A	S	Q	K	R	P	S	Q	R	H
Position	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Composition of biased library mixtures	A	A F P W	Q W	F G H R S Y	R W	F G H P	A K W	F I P Q	A L T	F P Q R
AA predictions from biased library scan	A	A	Q	F H S Y	R	P	A K	F I P Q	T L	P Q

B Subset of 128 agonist peptides.

Ac1-10	A	S	Q	K	R	P	S	Q	R	H	EC_{50} (nM) 137.2
104	-	A	-	Y	-	-	A	I	T	Q	<0.05
13	-	A	-	F	-	-	A	-	L	P	<0.05
3	-	A	-	F	-	-	A	F	T	P	<0.1
101	-	A	-	Y	-	-	A	I	L	P	0.1
15	-	A	-	F	-	-	A	-	T	P	0.7
66	-	A	-	S	-	-	A	F	L	Q	2.2
72	-	A	-	S	-	-	A	I	T	Q	2.2
100	-	A	-	Y	-	-	A	F	T	Q	3.6
65	-	A	-	S	-	-	A	F	L	P	4
108	-	A	-	Y	-	-	A	P	T	Q	7.7

Figure 6. Biased Library Scan and Activities of Individual Peptides

(A) The active fixed amino acid mixtures selected from Figure 5 are indicated at the top. We included those amino acids corresponding to the active mixtures causing a SI $\geq$ mean + 1 SD of all of the mixtures for a given position and that ranked among the four most active mixtures in three of four different experiments. The results of this scan are shown summarized at the bottom of the figure and indicated 128 different possible different candidate peptides, each of which was synthesized and an EC50 was determined.

(B) Activity of selected candidate peptide analogs, out of 128, of Ac1-10 predicted by the biased library showing limited degeneracy tolerated in the MBP sequence, and the effect of the C-terminal flanking residues on activity. For comparison, we also show the EC50 of wild-type MBP Ac-1–10 (4Lys). The EC50 (nM) for each peptide is at the right.

out regard to whether the peptide sequence variations reflected direct TCR interactions or were simply alternative MHC anchors. Here, we can make a precise distinction between alternative TCR contacts that would be indicative of TCR crossreactivity versus peptides with only variation in MHC binding residues. We suggest that in many examples of TCR crossreactivity, it is probable that the peptide-TCR contacts are similar to the cognate peptide and are therefore not truly indicative of TCR degeneracy. As one example, in the KRN system a crossreactive peptide was shown, using modeling and peptide substitution assays, to precisely recapitulate, rather than mimic in an alternative fashion, the TCR contacts of the cognate ligand (Basu et al., 2000). Similarly, Lang et al. showed that a human myelin basic protein peptide and an Epstein-Barr virus mimic presented structurally equivalent peptide-MHC surfaces that would be recognized by the TCR, despite a lack of sequence similarity (Lang et al., 2002). Therefore, the mimicry was achieved by preservation of specific TCR contacts, without the need for TCR degeneracy. In the murine system, molecular mimic peptides have been reported that are broadly active against MBP1-11-specific T cell clones; however, these mimic sequences all contain the residues Arg-Pro, which are identical to two of the MBP residues directly contacting 172.10 in our complex (Gautam et al., 1998; Grogan et al., 1999).

There are clear functionally relevant examples of TCRs which recognize unrelated peptide sequences that are potent agonists compared to the cognate antigen (Loftus et al., 1997; Reiser et al., 2003; Sykulev et al., 1994; Wucherpfennig, 2004). In these cases, the TCR is undoubtedly recognizing unique structural epitopes. However, these cases are probably not the result of a generalized TCR promiscuity, but rather represent highly specific, alternative structural solutions to engagement of the TCR. For instance, for 172.10 the peptide scans did not identify individual peptides with different TCR contacts, but the calculations of numbers of activating peptides, based on the aggregate 172.10 stimulation data, were far too many for us to individually test. Certainly, within the initial large library pool, structurally unrelated peptides do exist that activate 172.10 yet are present in too low abundance or are too weak agonists to emerge from the background.

We suggest that TCR crossreactivity be defined based on thresholds (Figure 7). In their normal functions, TCRs scan an enormous array of pMHC complexes, largely driven by biased TCR recognition of the MHC helices (Ignatowicz et al., 1997). It has been shown that T cells require weak activation signals from self-pMHC in the periphery; therefore, low-threshold crossreactivity is probably required for the basal stimulation of the T cell (Stefanova et al., 2002). Hence, the baseline for TCR crossreactivity is elevated in comparison to antibody/antigen interactions, which have no functional requirement for basal or low-threshold binding to diverse antigens (Figure 7). The antibody molecule has high-threshold (i.e., affinity) and narrow specificity recognition properties, as shown by a general intolerance to ligand structural alterations exemplified by viral escape mutants (Mateu, 1995). We propose that TCRs share a simi-

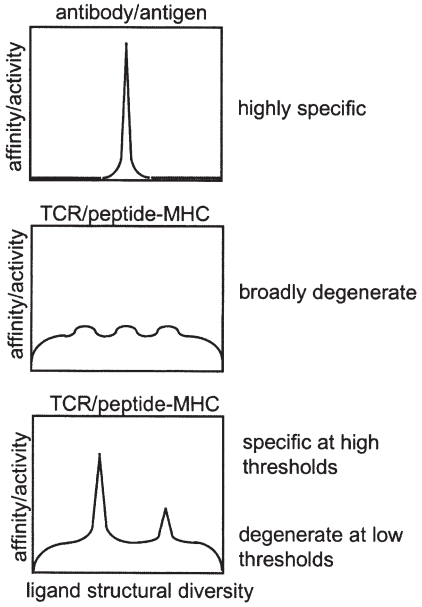

Figure 7. Specificity versus Degeneracy in Antigen Recognition

For each panel, the y axis represents an arbitrary measure of affinity or activity, and the x axis denotes a unitless measure of ligand structural diversity. An antibody generally exhibits high specificity and affinity with narrow tolerances for mutation (top panel). TCRs are sometimes considered broadly degenerate (middle panel). We suggest that TCRs are highly specific for activating peptide ligands, with a narrow tolerance for mutation, but also possess a low-threshold background level of crossreactivity consistent with pMHC scanning (bottom panel).

lar narrow specificity for structurally unrelated peptides at the higher thresholds that lead to full T cell activation, rather than being part of a simple continuum of cross-reactive TCR ligands.

TCR Bias for MHC

One of the puzzling generalizations that has emerged from the TCR/pMHC complex structures so far is a roughly convergent docking orientation ($\pm 45^\circ$ diagonal), in spite of a lack of common sets of interatomic contacts between TCR and MHC (Housset and Malissen, 2003). For example, in structures of different Vβ2-containing TCRs in complex with H-2K^b, the CDR1β and CDR2β have different binding registers when docked to the H-2K^b α1 helix (Housset and Malissen, 2003). The question has arisen, then, of whether the TCR V-gene repertoire encodes the biased docking orientation of MHC in the amino acids on the CDR1 and 2 loops, or whether external factors such as coreceptors (CD8, CD4) may influence the orientation of the TCR through steric effects during thymic selection (Buslepp et al., 2003).

In our structure, we see that Vβ8.2 of the 172.10 and D10 TCRs superimpose essentially identically at a large multipoint contact patch between CDR1β and CDR2β and the MHC α1 helix (Figure 4). This shared contact patch is composed of five shared TCR residues interacting with five shared MHC residues and comprises $\sim$73% of the total Vβ interaction surface with the I-A^u α1 helix. (Figures 4C and 4D). We also aligned the sequences of I-A haplotypes d, b, s, q, g7, r, and f and found that the five shared MHC contact residues between I-A^k and I-A^u are also conserved (Figure 4D). This is further suggestive that the Vβ8.2/I-A contact residues in the structures may be a generalized I-A recognition motif.

The fact that the Vβ2 complexes with class I MHC H-2K^b have not revealed a similar conservation of contacts may be due to class I TCR/pMHC complexes allowing more variation in TCR orientation relative to class II (Reinherz et al., 1999). There is a broader "peak" on the surface of class II MHC that more narrowly constrains the rotational freedom of the TCR bound to class II MHC (Reinherz et al., 1999). Second, the Vα in 172.10 (Vα2.3) and D10 (Vα2) are closely related, perhaps facilitating the Vβ convergence we are seeing. Similar to the "sets" of peptide anchor residues used by MHC (Rammensee et al., 1995), it is likely that sets of TCR interaction motifs will exist for each TCR chain type (both α and β chain) with each MHC haplotype.

Autoimmunity

Mice transgenic for the 172.10 TCR spontaneously develop EAE at a relatively high frequency (Goverman, 1999). An important question is how the binding chemistry of this autoreactive TCR compares to normal cognate TCR/self-pMHC interactions. The most conspicuous structural feature of the 172.10/I-A^u-MBP1-11 complex is the recognition of the frame-shifted MBP peptide. Wild-type MBP (P4-Lys) has a very short half-life ($<$15 min) bound to I-A^u, which may predispose it to a short lifetime in the thymus, leading to ineffective deletion of autoreactive TCRs (Fairchild et al., 1993). In vivo, the focus of 172.10 on contacts with the transiently bound wild-type MBP peptide would prevent formation of TCR/pMHC complexes of sufficient half-life for signaling, resulting in inefficient negative selection. Consistent with this interpretation, the stable P4-Tyr MBP variant has been shown to block development of EAE, presumably through deletion of MBP-reactive T cells (Anderton et al., 2001). In the periphery, 172.10 T cells will ultimately lead to development of EAE in mice transgenic for this TCR (Goverman, 1999). Perhaps over time and many collective encounters, the TCR germline-encoded interactions with the surrounding MHC helices provide the stabilizing influence necessary to overcome the transient peptide occupancy for priming of MBP-specific TCR, leading to full activation and autoimmunity. An additional factor to consider in this complex is that, due to the peptide frame shifting, the N-terminal third of the peptide groove is empty when Ac1-11 is bound, which is the region of the groove overlaid by the Vα domain. The empty pockets in the groove may prevent obstructionist peptide contacts that would normally interfere with binding of MBP-specific TCR carrying certain α chains. Finally, our initial supposition that a highly degenerate peptide repertoire might further facilitate the autoimmunity of 172.10 has not been supported by our peptide library results. On the contrary, it may be that the highly specific recognition of I-A^u-Ac1-11 might serve to focus T cell activation on the MBP peptide without competition from crossreactive ligands.

Experimental Procedures

Expression

The 172.10 TCR was expressed as a single-chain Fv (scTCR). The variable domains of the 172.10 T cell receptor were amplified by PCR and joined with a cassette encoding a 20 amino (Gly4Ser)4 linker, which proved to be the optimal linker length after testing a number of versions. Four amino acid substitutions were introduced into the Vβ framework that had been shown to increase expression levels and solubility in Vβ8.2 TCR (G17E, H47Y, I75T, and L78S) in a series of pilot studies (Garcia et al., 2001; Shusta et al., 1999; J.M. et al., unpublished data). Linker and amino acid substitutions were introduced via oligonucleotide-directed mutagenesis and overlap PCR prior to ligating the modified gene into the bacterial expression vector pAK400 (Krebber et al., 1997) via directional SfiI-SfiI restriction sites and sequencing. Protein was expressed in the periplasm of *E. coli* strain BL21, extracted by a modified osmotic shock procedure, and purified to >95% by sequential immobilized metal affinity, S75 size exclusion, and anion exchange chromatographic steps. The C-terminal histidine tag was removed by overnight digestion at 4°C with a 1:100 mass ratio of carboxypeptidase A (Calbiochem) prior to the size-exclusion step. As a final step, the proteins were concentrated by Centricon-10 (Millipore) to ~35 mg/ml in HBS, sterile filtered, and stored at 4°C. I-A^u was expressed and purified from a stably transfected *Drosophila* cell line as described (He et al., 2002).

Crystallization

Crystals of 172.10 scTCR and I-A^u MHC (1:1 molar ratio; 35 mg/ml total protein concentration) were grown from 21% PEG-3350, 0.1 M HEPES (pH 7.5), and 0.2 M lithium sulfate in the sitting drop format to ~0.2 × 0.4 μm size and were cryo-cooled for X-ray data collection in the presence of 25% glycerol. A complete data set was collected from one crystal at beamline 8.2.1 at the Berkeley Advanced Light Source (ALS, Berkeley, CA) using 1.08 Å wavelength X-rays and an ADSC Quantum 210 CCD detector. A total of 120° of 1° oscillation images were collected under cryogenic conditions (T = 100 K). The data were indexed, integrated, and scaled with HKL2000 in space group C2221, with unit cell dimensions a = 87.84 Å, b = 327.16 Å, c = 127.16 Å, α = β = γ = 90°, and two complexes per asymmetric unit (mosaicity = 0.5°). The crystal diffracted to 2.4 Å resolution, and the final data set had an overall R_{sym} of 6.1% and completeness of 96%. Details of the data and refinement statistics are given in Supplemental Table S1.

Structure Determination and Refinement

The structure was determined by molecular replacement using the MOLREP program as implemented in the CCP4 package (CCP4, 1994). The D10 TCR from theI-A^k/D10-CA (PDB ID: 1d9k) complex was used as the TCR model, and our previous I-A^u-MBP1-11 structure was used as the starting model for the MHC (He et al., 2002). Model building and visual inspection of the electron density maps were done with the program O (Jones et al., 1991), and the refinement was performed using conjugate gradient and simulated annealing (with maximum likelihood target function) in CNS (Brunger et al., 1998), combined with omit and SIGMAA-weighted electron density maps. Water molecules were automatically located by CNS and manually edited with $F_o - F_c$ electron density maps. The final model included A2-A116 (the α chain of TCR), B3-B117 (the β chain of TCR), C1-C181 (the α chain of I-A^u), D1-D190 (the β chain of I-A^u), and P-3-P8 (p-3-P0 is part of the linker and P1-P8 is the MBP peptide). Repeated iterations between manual rebuilding and minimization as well as B factor refinement resulted in a model with R factors of 24.3% and R_{free} of 27.4%. The stereochemistry of the structure was analyzed with PROCHECK (Laskowski et al., 1993). Details of the refinement statistics are given in Supplemental Table S1.

Libraries and Peptides

Libraries and biased sublibrary mixtures were prepared at Mixture Sciences, Inc. (San Diego, CA) as described previously (Pinilla et al., 1994). PCL 97-4 is a synthetic N-acetylated, C-terminal amide, L-amino acid combinatorial decapeptide library arrayed in a positional scanning format. It consists of 200 mixtures in the OX_9 format, where O represents one each of the 20 natural L-amino acids in a defined position, and X represents all of the natural amino acids, with the exception of cystein (C), in each of the remaining positions. For example, the first mixture has alanine (A) in position 1 (A_1X_9), whereas mixture number 200 has tyrosine (Y) in position 10 (X_9Y_{10}). Each OX_9 mixture consists of 3.2×10^{11} (19^9) different decamer peptides in approximate equimolar concentration, and the total X_{10} library consists of 6.4×10^{12} (20×10^{19}) different peptides. Assuming an average molecular weight of 1200 Da for a decapeptide mixture and a concentration of 100 μg/ml (83 μM), the concentration of each individual peptide is 2.6×10^{-16} M.

Small (milligram quantities) of individual candidate peptides were synthesized by solid-phase synthesis using standard Fmoc chemistry and a Spyder multiple-peptide synthesizer. The Spyder synthesizer is formatted to synthesize up to 192 individual peptide sequences in two 96-well plates. This formatting is designed so that the synthesized peptides can be cleaved from their solid support while still in the plates and then extracted robotically into two new sets. The first set was placed in an automated LC-MS system for analysis, and the second set was lyophilized and used for biological screening.

Larger, bulk synthesis quantities of individual peptides was accomplished by the simultaneous multiple-peptide synthesis method (Houghten, 1985) or with an automated peptide synthesizer (ABI, Foster City, CA). Purity and identity of each peptide was characterized using an electrospray mass spectrometer interfaced with a liquid chromatography system.

Culture Conditions

The 172.10 T cell hybridoma cells, reactive to MBP Ac1-9 (Goverman et al., 1993), were cultured in triplicate in round-bottom microwells (50×10^3 cells/well) with irradiated (3000 R) ammonium chloride Tris-buffer-treated B10.PL splenic cells as APCs (250×10^3 cells per well) and peptide library mixtures (100 μg/ml) or with varying concentrations of individual peptides for 24 hr. IL-2 responses were assessed in recovered supernatants (50 μl) added to secondary, 24 hr cultures of IL-2-addicted HT2 cells (2000 per well). The extent of IL-2-dependent HT2 cell proliferation was determined with ^{3}H-Tdr added during the last 4 hr.

Determination of EC_{50} Values

T cell populations were cultured using conditions described previously with varying dilutions of peptides (Judkowski et al., 2001; Wilson et al., 1999). The concentration of peptide causing a half-maximal IL-2 response (EC_{50}) was determined by curve fitting, using a scientific graphics software program (GraphPad Prism; Graph Pad Software, San Diego, CA). The maximal IL-2 response was fixed using the mean of the highest values obtained in each experiment.

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References

Acha-Orbea, H., Mitchell, D.J., Timmermann, L., Wraith, D.C., Tausch, G.S., Waldor, M.K., Zamvil, S.S., McDevitt, H.O., and Steinman, L. (1988). Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell *54*, 263–273.

Anderton, S.M., Manickasingham, S.P., Burkhart, C., Luckcuck, T.A., Holland, S.J., Lamont, A.G., and Wraith, D.C. (1998). Fine specificity

of the myelin-reactive T cell repertoire: implications for TCR antagonism in autoimmunity. J. Immunol. *161*, 3357–3364.

Anderton, S.M., Radu, C.G., Lowrey, P.A., Ward, E.S., and Wraith, D.C. (2001). Negative selection during the peripheral immune response to antigen. J. Exp. Med. *193*, 1–11.

Bankovich, A.J., and Garcia, K.C. (2003). Not just any T cell receptor will do. Immunity *18*, 7–11.

Basu, D., Horvath, S., Matsumoto, I., Fremont, D.H., and Allen, P.M. (2000). Molecular basis for recognition of an arthritic peptide and a foreign epitope on distinct MHC molecules by a single TCR. J. Immunol. *164*, 5788–5796.

Bhardwaj, V., Kumar, V., Geysen, H.M., and Sercarz, E.E. (1993). Degenerate recognition of a dissimilar antigenic peptide by myelin basic protein-reactive T cells. Implications for thymic education and autoimmunity. J. Immunol. *151*, 5000–5010.

Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S., et al. (1998). Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr. D Biol. Crystallogr. *54*, 905–921.

Buslepp, J., Wang, H., Biddison, W.E., Appella, E., and Collins, E.J. (2003). A correlation between TCR Valpha docking on MHC and CD8 dependence: implications for T cell selection. Immunity *19*, 595–606.

CCP4 (Collaborative Computational Project, Number 4) (1994). The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. *50*, 760–763.

Crawford, F., Huseby, E., White, J., Marrack, P., and Kappler, J.W. (2004). Mimotopes for alloreactive and conventional T cells in a peptide-MHC display library. PLoS Biol. 2(4): e90 DOI: 10.1371/journal.pbio.0020090.

Daniel, C., Horvath, S., and Allen, P.M. (1998). A basis for alloreactivity: MHC helical residues broaden peptide recognition by the TCR. Immunity *8*, 543–552.

Davis, M.M., and Bjorkman, P.J. (1988). T-cell antigen receptor genes and T-cell recognition. Nature *334*, 395–402.

Degano, M., Garcia, K.C., Apostolopoulos, V., Rudolph, M.G., Teyton, L., and Wilson, I.A. (2000). A functional hot spot for antigen recognition in a superagonist TCR/MHC complex. Immunity *12*, 251–261.

Delano, W.L. (2002). The PyMOL Molecular Graphics System (San Carlos, CA: DeLano Scientific).

Ding, Y.H., Smith, K.J., Garboczi, D.N., Utz, U., Biddison, W.E., and Wiley, D.C. (1998). Two human T cell receptors bind in a similar diagonal mode to the HLA- A2/Tax peptide complex using different TCR amino acids. Immunity *8*, 403–411.

Fairchild, P.J., Wildgoose, R., Atherton, E., Webb, S., and Wraith, D.C. (1993). An autoantigenic T cell epitope forms unstable complexes with class II MHC: a novel route for escape from tolerance induction. Int. Immunol. *5*, 1151–1158.

Fugger, L., Liang, J., Gautam, A., Rothbard, J.B., and McDevitt, H.O. (1996). Quantitative analysis of peptides from myelin basic protein binding to the MHC class II protein, I-Au, which confers susceptibility to experimental allergic encephalomyelitis. Mol. Med. *2*, 181–188.

Garcia, K.C., Degano, M., Pease, L.R., Huang, M., Peterson, P.A., Teyton, L., and Wilson, I.A. (1998). Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. Science *279*, 1166–1172.

Garcia, K.C., Radu, C.G., Ho, J., Ober, R.J., and Ward, E.S. (2001). Kinetics and thermodynamics of T cell receptor-autoantigen interactions in murine experimental autoimmune encephalomyelitis. Proc. Natl. Acad. Sci. USA *98*, 6818–6823.

Gautam, A.M., Lock, C.B., Smilek, D.E., Pearson, C.I., Steinman, L., and McDevitt, H.O. (1994). Minimum structural requirements for peptide presentation by major histocompatibility complex class II molecules: implications in induction of autoimmunity. Proc. Natl. Acad. Sci. USA *91*, 767–771.

Gautam, A.M., Liblau, R., Chelvanayagam, G., Steinman, L., and Boston, T. (1998). A viral peptide with limited homology to a self peptide can induce clinical signs of experimental autoimmune encephalomyelitis. J. Immunol. *161*, 60–64.

Germain, R.N. (1990). Immunology. Making a molecular match. Nature *344*, 19–22.

Goverman, J. (1999). Tolerance and autoimmunity in TCR transgenic mice specific for myelin basic protein. Immunol. Rev. *169*, 147–159.

Goverman, J., Woods, A., Larson, L., Weiner, L.P., Hood, L., and Zaller, D.M. (1993). Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. Cell *72*, 551–560.

Grogan, J.L., Kramer, A., Nogai, A., Dong, L., Ohde, M., Schneider-Mergener, J., and Kamradt, T. (1999). Cross-reactivity of myelin basic protein-specific T cells with multiple microbial peptides: experimental autoimmune encephalomyelitis induction in TCR transgenic mice. J. Immunol. *163*, 3764–3770.

Hagerty, D.T., and Allen, P.M. (1995). Intramolecular mimicry. Identification and analysis of two cross- reactive T cell epitopes within a single protein. J. Immunol. *155*, 2993–3001.

He, X.L., Radu, C., Sidney, J., Sette, A., Ward, E.S., and Garcia, K.C. (2002). Structural snapshot of aberrant antigen presentation linked to autoimmunity: the immunodominant epitope of MBP complexed with I-Au. Immunity *17*, 83–94.

Hemmer, B., Vergelli, M., Gran, B., Ling, N., Conlon, P., Pinilla, C., Houghten, R., McFarland, H.F., and Martin, R. (1998a). Predictable TCR antigen recognition based on peptide scans leads to the identification of agonist ligands with no sequence homology. J. Immunol. *160*, 3631–3636.

Hemmer, B., Vergelli, M., Pinilla, C., Houghten, R., and Martin, R. (1998b). Probing degeneracy in T-cell recognition using peptide combinatorial libraries. Immunol. Today *19*, 163–168.

Hennecke, J., and Wiley, D.C. (2001). T cell receptor-MHC interactions up close. Cell *104*, 1–4.

Hennecke, J., Carfi, A., and Wiley, D.C. (2000). Structure of a covalently stabilized complex of a human alphabeta T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. EMBO J. *19*, 5611–5624.

Holler, P.D., and Kranz, D.M. (2004). T cell receptors: affinities, cross-reactivities, and a conformer model. Mol. Immunol. *40*, 1027–1031.

Houghten, R.A. (1985). General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA *82*, 5131–5135.

Housset, D., and Malissen, B. (2003). What do TCR-pMHC crystal structures teach us about MHC restriction and alloreactivity? Trends Immunol. *24*, 429–437.

Ignatowicz, L., Rees, W., Pacholczyk, R., Ignatowicz, H., Kushnir, E., Kappler, J., and Marrack, P. (1997). T cells can be activated by peptides that are unrelated in sequence to their selecting peptide. Immunity *7*, 179–186.

Jones, T.A., Zou, J.Y., Cowan, S.W., and Kjeldgaard (1991). Improved methods for binding protein models in electron density maps and the location of errors in these models. Acta Crystallogr. A *47*, 110–119.

Judkowski, V., Pinilla, C., Schroder, K., Tucker, L., Sarvetnick, N., and Wilson, D.B. (2001). Identification of MHC class II-restricted peptide ligands, including a glutamic acid decarboxylase 65 sequence, that stimulate diabetogenic T cells from transgenic BDC2.5 nonobese diabetic mice. J. Immunol. *166*, 908–917.

Kersh, G.J., and Allen, P.M. (1996a). Essential flexibility in the T-cell recognition of antigen. Nature *380*, 495–498.

Kersh, G.J., and Allen, P.M. (1996b). Structural basis for T cell recognition of altered peptide ligands: a single T cell receptor can productively recognize a large continuum of related ligands. J. Exp. Med. *184*, 1259–1268.

Kersh, G.J., Kersh, E.N., Fremont, D.H., and Allen, P.M. (1998). High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. Immunity *9*, 817–826.

Krebber, A., Bornhauser, S., Burmester, J., Honegger, A., Willuda, J., Bosshard, H.R., and Pluckthun, A. (1997). Reliable cloning of

functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system. J. Immunol. Methods *201*, 35–55.

Krogsgaard, M., Prado, N., Adams, E.J., He, X.L., Chow, D.C., Wilson, D.B., Garcia, K.C., and Davis, M.M. (2003). Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T cell activation. Mol. Cell *12*, 1367–1378.

Lang, H.L., Jacobsen, H., Ikemizu, S., Andersson, C., Harlos, K., Madsen, L., Hjorth, P., Sondergaard, L., Svejgaard, A., Wucherpfennig, K., et al. (2002). A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat. Immunol. *3*, 940–943.

Laskowski, R.A., MacArthur, M.W., Moss, D.S., and Thornton, J.M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Crystallogr. *26*, 283–291.

Loftus, D.J., Chen, Y., Covell, D.G., Engelhard, V.H., and Appella, E. (1997). Differential contact of disparate class I/peptide complexes as the basis for epitope cross-recognition by a single T cell receptor. J. Immunol. *158*, 3651–3658.

Loftus, C., Huseby, E., Gopaul, P., Beeson, C., and Goverman, J. (1999). Highly cross-reactive T cell responses to myelin basic protein epitopes reveal a nonpredictable form of TCR degeneracy. J. Immunol. *162*, 6451–6457.

Madden, D. (1995). The three-dimensional structure of peptide-MHC complexes. Annu. Rev. Immunol. *13*, 587–622.

Mason, D. (1998). A very high level of crossreactivity is an essential feature of the T-cell receptor. Immunol. Today *19*, 395–404.

Mason, K., Denney, D.W., Jr., and McConnell, H.M. (1995). Kinetics of the reaction of a myelin basic protein peptide with soluble IAu. Biochemistry *34*, 14874–14878.Mateu, M.G. (1995). Antibody recognition of picornaviruses and escape from neutralization: a structural view. Virus Res. *1*, 1–24.

Nikolic-Zugic, J., and Bevan, M.J. (1990). Role of self-peptides in positively selecting the T-cell repertoire. Nature *344*, 65–67.

Pinilla, C., Appel, J.R., and Houghten, R.A. (1994). Investigation of antigen-antibody interactions using a soluble, non-support-bound synthetic decapeptide library composed of four trillion (4 x 10(12) sequences. Biochem. J. *301*, 847–853.

Pinilla, C., Martin, R., Gran, B., Appel, J.R., Boggiano, C., Wilson, D.B., and Houghten, R.A. (1999). Exploring immunological specificity using synthetic peptide combinatorial libraries. Curr. Opin. Immunol. *11*, 193–202.

Radu, C.G., Ober, B.T., Colantonio, L., Qadri, A., and Ward, E.S. (1998). Expression and characterization of recombinant soluble peptide: I-A complexes associated with murine experimental autoimmune diseases. J. Immunol. *160*, 5915–5921.

Rammensee, H.G., Friede, T., and Stevanoviic, S. (1995). MHC ligands and peptide motifs: first listing. Immunogenetics *41*, 178–228.

Reinherz, E.L., Tan, K., Tang, L., Kern, P., Liu, J., Xiong, Y., Hussey, R.E., Smolyar, A., Hare, B., Zhang, R., et al. (1999). The crystal structure of a T cell receptor in complex with peptide and MHC class II. Science *286*, 1913–1921.

Reiser, J.B., Darnault, C., Gregoire, C., Mosser, T., Mazza, G., Kearney, A., van der Merwe, P.A., Fontecilla-Camps, J.C., Housset, D., and Malissen, B. (2003). CDR3 loop flexibility contributes to the degeneracy of TCR recognition. Nat. Immunol. *4*, 241–247.

Rudolph, M.G., and Wilson, I.A. (2002). The specificity of TCR/pMHC interaction. Curr. Opin. Immunol. *14*, 52–65.

Shih, F.F., and Allen, P.M. (2004). T cells are not as degenerate as you think, once you get to know them. Mol. Immunol. *40*, 1041–1046.

Shusta, E.V., Kieke, M.C., Parke, E., Kranz, D.M., and Wittrup, K.D. (1999). Yeast polypeptide fusion surface display levels predict thermal stability and soluble secretion efficiency. J. Mol. Biol. *292*, 949–956.

Stefanova, I., Dorfman, J.R., and Germain, R.N. (2002). Self-recognition promotes the foreign antigen sensitivity of naive T lymphocytes. Nature *420*, 429–434.

Sykulev, Y., Brunmark, A., Tsomides, T.J., Kageyama, S., Jackson, M., Peterson, P.A., and Eisen, H.N. (1994). High-affinity reactions between antigen-specific T-cell receptors and peptides associated with allogeneic and syngeneic major histocompatibility complex class I proteins. Proc. Natl. Acad. Sci. USA *91*, 11487–11491.

Sykulev, Y., Joo, M., Vturina, I., Tsomides, T.J., and Eisen, H.N. (1996). Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. Immunity *4*, 565–571.

Sykulev, Y., Vugmeyster, Y., Brunmark, A., Ploegh, H.L., and Eisen, H.N. (1998). Peptide antagonism and T cell receptor interactions with peptide-MHC complexes. Immunity *9*, 475–483.

Urban, J.L., Kumar, V., Kono, D.H., Gomez, C., Horvath, S.J., Clayton, J., Ando, D.G., Sercarz, E.E., and Hood, L. (1988). Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell *54*, 577–592.

Willcox, B.E., Gao, G.F., Wyer, J.R., Ladbury, J.E., Bell, J.I., Jakobsen, B.K., and Anton van der Merwe, P. (1999). TCR binding to peptide-MHC stabilizes a flexible recognition interface. Immunity *10*, 357–365.

Wilson, D.B., Pinilla, C., Wilson, D.H., Schroder, K., Boggiano, C., Judkowski, V., Kaye, J., Hemmer, B., Martin, R., and Houghten, R.A. (1999). Immunogenicity. I. Use of peptide libraries to identify epitopes that activate clonotypic CD4+ T cells and induce T cell responses to native peptide ligands. J. Immunol. *163*, 6424–6434.

Wilson, D.B., Wilson, D.H., Schroder, K., Pinilla, C., Blondelle, S., Houghten, R.A., and Garcia, K.C. (2004). Specificity and degeneracy of T cells. Mol. Immunol. *40*, 1047–1055.

Wraith, D.C., Smilek, D.E., Mitchell, D.J., Steinman, L., and McDevitt, H.O. (1989). Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. Cell *59*, 247–255.

Wraith, D.C., Bruun, B., and Fairchild, P.J. (1992). Cross-reactive antigen recognition by an encephalitogenic T cell receptor. Implications for T cell biology and autoimmunity. J. Immunol. *149*, 3765–3770.

Wu, L.C., Tuot, D.S., Lyons, D.S., Garcia, K.C., and Davis, M.M. (2002). Two-step binding mechanism for T-cell receptor recognition of peptide MHC. Nature *418*, 552–556.

Wucherpfennig, K.W. (2004). T cell receptor crossreactivity as a general property of T cell recognition. Mol. Immunol. *40*, 1009–1017.

Wucherpfennig, K.W., and Strominger, J.L. (1995). Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell *80*, 695–705.

Zamvil, S.S., and Steinman, L. (1990). The T lymphocyte in experimental allergic encephalomyelitis. Annu. Rev. Immunol. *8*, 579–621.

Zamvil, S.S., Mitchell, D.J., Moore, A.C., Schwarz, A.J., Stiefel, W., Nelson, P.A., Rothbard, J.B., and Steinman, L. (1987). T cell specificity for class II (I-A) and the encephalitogenic N- terminal epitope of the autoantigen myelin basic protein. J. Immunol. *139*, 1075–1079.

Accession Numbers

The coordinates have been deposited in the Protein Data Bank under accession number 1U3H.