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T cell receptor crossreactivity as a general property of T cell recognition

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Abstract

TCR recognition of MHC/peptide complexes directs many aspects of T cell biology, including thymic selection, survival of naïve T cells and differentiation into effector and memory T cells. It was widely thought that TCR recognition is highly specific, with an individual T cell being capable of only recognizing a particular peptide and closely related sequence variants. By considering the structural requirements for peptide binding to MHC molecules and TCR recognition of MHC/peptide complexes, we demonstrated that T cell clones could recognize a number of peptides from different organisms that are remarkably distinct in their primary sequence. These peptides are particularly diverse at those sequence positions buried in pockets of the MHC binding site, while a higher degree of similarity is present at a limited number of peptide residues that create the interface with the TCR. Many examples have now been documented for human and murine T cells, indicating that TCR crossreactivity represents a general feature of TCR recognition.

Keywords: T cell receptor; Crossreactivity; Molecular mimicry; Autoimmunity

1. How specific is TCR recognition of MHC/peptide complexes?

The experimental practice of isolating "antigen-specific" T cells by in vivo or in vitro selection with a particular antigen led to the widely held notion that TCR recognition is highly specific since T cell clones selected in such a fashion are typically not activated by control antigens. Within this conceptual framework it was thought that rare microbial antigens in which the peptide sequences were closely related to a self-peptide could represent mimics and be responsible for the induction of autoimmune diseases. Since it was assumed that such peptides would have to possess substantial sequence identity with the self-antigen, sequence alignments with self-peptides were used to identify candidate sequences. This approach was used to identify a peptide from the hepatitis B virus DNA polymerase that induced histological signs of experimental autoimmune encephalomyelitis (EAE) following immunization of rabbits (Fujinami and Oldstone, 1985). However, a large number of groups used this method to identify potential mimicry peptides with largely disappointing results since the vast majority of peptides had no biological activity. It thus appeared that TCR crossreactivity might be a very rare event. Given the paucity of definitive experimental data, the biological relevance of TCR crossreactivity was questioned by many basic scientists.

However, structural studies on the interaction of MHC molecules with peptides and on TCR recognition of MHC/peptide complexes suggested that this view of TCR recognition might have to be reconsidered. Peptide elution studies demonstrated that a single MHC molecule could bind a very large and diverse set of peptides since several hundred distinct peptide masses could be defined with mass spectrometry techniques (Hunt et al., 1992; Chicz et al., 1993). Investigation of the structural requirements for peptide binding by MHC class II molecules demonstrated that five peptide side chains contributed to binding (Stern et al., 1994). However, each of these 'anchor residues' could typically be substituted by a number of other amino acids so that the resulting peptide binding motifs were highly degenerate (Hammer et al., 1993). For the multiple sclerosis (MS) associated HLA-DR2 molecule (DRA, DRB1*1501) that has been the focus of our studies, three of these five peptide positions (P6, P7 and P9 pockets) could be substituted by many different amino acids. Even though a higher degree of specificity was observed for the P1 and P4 anchor residues, substitutions by structurally related amino acids were permitted (Wucherpfennig et al., 1994a). The crystal structure of HLA-DR1 with a bound peptide from influenza hemagglutinin (HA, 306–318) elucidated how peptides are

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bound with high affinity, despite such degenerate sequence motifs: a significant fraction of the binding energy is derived from interactions between the backbone of the peptide and conserved residues of the MHC class II binding cleft. This structure also demonstrated that the peptide is buried in the binding site such that substitutions of peptide side chains located in deep pockets might not interfere with TCR recognition of the MHC/peptide surface (Stern et al., 1994).

Analysis of TCR recognition of MHC-bound peptides in light of this structural information demonstrated that specificity was typically confined to a small number of peptide side chains. For the myelin basic protein (MBP) specific T cell clones that we have studied, three peptide side chains in the core of the MBP peptide (P2 His, P3 Phe and P5 Lys) were particularly relevant for TCR recognition (Wucherpfennig et al., 1994a; Wucherpfennig and Strominger, 1995). This observation appeared to be general since similar findings were made for murine TCR reactive with microbial or self-antigens. Global amino acid replacements in the moth cytochrome C (93–103) peptide recognized by murine I-E^k restricted T cells demonstrated a strong preference for a particular amino acid at three peptide positions (Reay et al., 1994). For the Ac(1–11) peptide of MBP that is encephalitogenic in PL/J mice, only four native MBP residues were required for activation of MBP-specific T cells (Gautam et al., 1994).

Based on these considerations, we developed the hypothesis that TCR recognition is characterized by a considerable degree of crossreactivity and that a TCR could recognize a number of different peptides that may be rather distinct in their sequence. This hypothesis was supported by a reported case of crossreactivity where no obvious sequence similarity was present between the two peptides, as well as the observation that many T cell clones recognized alloreactive MHC/peptide complexes (Bhardwaj et al., 1993; Burrows et al., 1994).

2. Development of a strategy to systematically examine TCR crossreactivity

The challenge therefore was to develop a systematic approach that would allow us to identify such peptides even though their structural similarity might not be evident by conventional sequence alignments. Formation of the trimolecular complex of MHC, peptide and TCR is based on two independent binding events: high affinity binding of peptide to an MHC molecule and the more short-lived association of TCR with this MHC/peptide surface. We decided to base our strategy on the minimal structural requirements for each of these two binding events. This approach took advantage of the fact that T cell epitopes can be mapped to short peptide segments and that the contribution of individual peptide side chains to MHC binding and TCR recognition can be evaluated with a series of peptide analogs. Experimentally, this work focused on T cell recognition of a peptide from human MBP (residues 85–99) that is bound with high affinity by the MS-associated HLA-DR2 molecule (DRA, DRB1*1501) (Wucherpfennig et al., 1994a). Analysis of TCR crossreactivity for T cell clones activated by this HLA-DR2/MBP peptide complex could thus provide insights into disease mechanisms in MS. We defined the minimal structural requirements for binding of the MBP peptide to HLA-DR2 and for TCR recognition of this MHC/peptide complex and searched available sequence databases with this motif information. This approach permitted the identification of many examples of TCR crossreactivity, not only for the human MBP specific T cell clones that we have studied but also for CD4 and CD8 T cells of both human and murine origin, as described in some of the other contributions to this issue. A more recent variant of this approach has been to determine the search motif with peptide analogs in which all neighboring positions carry mixtures of amino acids (so called combinatorial peptide libraries/positional scanning libraries) (Hemmer et al., 1997). These libraries provide less detailed motif information, but can be used on any MHC class II restricted T cell clone.

3. T cell clones can recognize multiple peptides with limited sequence similarity

The search criteria focused on the two major HLA-DR2 anchor residues of the peptide (P1 and P4) and four putative TCR contact residues (at P-1, P2, P3 and P5), all of which were located in a six amino acid core segment of the peptide. In the initial study, we synthesized a panel of 129 microbial peptides that matched these criteria and tested them for their ability to activate human MBP specific T cell clones that had been isolated from the peripheral blood of two patients with MS. Even though we only analyzed T cell clones that recognized a single self-peptide, we could identify eight microbial peptides that activated MBP reactive T cell clones (Wucherpfennig and Strominger, 1995). These peptides were remarkably distinct in their sequence from each other and the MBP peptide and only one of the eight peptides had obvious sequence similarity with the MBP peptide in the core segment. The motif-based strategy was thus essential for the identification of these peptides. In the initial study, such peptides were identified for three of the seven T cell clones that we studied. In order to determine whether microbial peptides could activate the majority of these clones, we examined the recognition motif for two of the four remaining clones in detail and synthesized a panel of peptides that included sequences from recently characterized microbial genomes. A total of five bacterial peptides were identified in this set of experiments (Hausmann et al., 1999a). Thus, we have identified a total of 13 microbial peptides that can activate human T cell clones specific for a single myelin peptide. Since we could only synthesize a subset of peptides identified in each search and a number of microbial genomes have not yet been sequenced, it is evident that a

substantial number of microbial peptides can activate T cell clones that recognize this MBP peptide. These experiments thus demonstrated that T cell clones could recognize a variety of different peptides with limited sequence similarity.

A number of different terms have been used to describe this property of TCR recognition. TCR crossreactivity describes the basic biological observation, while plasticity and degeneracy suggest structural mechanisms—mobility of TCR CDR loops (plasticity) or a relatively poor fit of the TCR on the MHC/peptide surface (degeneracy). Molecular mimicry has been widely utilized to describe the specialized case where TCR crossreactivity involves peptides from an infectious agent and a self-antigen.

4. Activation of MBP specific T cells by naturally processed viral antigen

One of the viral peptides was derived from EBV DNA polymerase and we examined whether the viral peptide is presented by infected antigen presenting cells to MBP specific T cells. The EBV DNA polymerase gene is part of the lytic cycle and is therefore not transcribed in EBV transformed B cells. However, the lytic cycle and expression of the EBV DNA polymerase gene can be induced by treatment of EBV transformed B cells with phorbol esters (Datta et al., 1980). A HLA-DR2^{+} B cell line that had been treated with a phorbol ester activated a MBP specific T cell clone that recognized both MBP and EBV DNA polymerase peptides. T cell activation was blocked by a mAb specific for HLA-DR but not by a control mAb against HLA-DQ, and was not observed when MHC-mismatched EBV transformed B cells were used as antigen presenting cells. These results demonstrated that the MBP specific T cell clone recognized not only the EBV peptide but also antigen presenting cells in which the viral gene was transcribed (Wucherpfennig and Strominger, 1995).

5. Crystal structure of the HLA-DR2/MBP peptide complex

Since the peptides that can be recognized by the same TCR are quite distinct in their primary sequence, an important question relates to the structural basis of TCR crossreactivity. Structural information is also required to determine

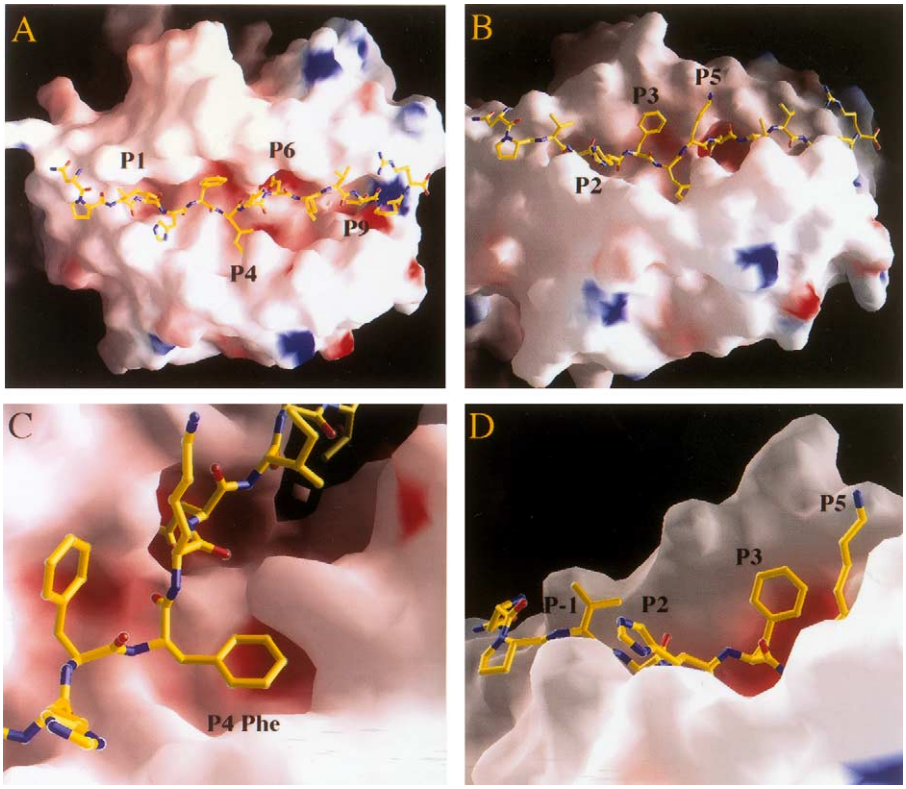

Fig. 1. Crystal structure of the complex of HLA-DR2 (DRA, DRB1*1501) and the MBP (85–99) peptide. (A) Overview of the structure. MBP peptide side chains that are located in four pockets of the binding site are indicated, P1 Val, P4 Phe, P6 Asn and P9 Thr. (B) Solvent exposed residues that are important for TCR recognition of the MBP (85–99) peptide, P2 His, P3 Phe and P5 Lys. (C) P4 pocket of the HLA-DR2 binding site. This large and hydrophobic pocket is occupied by P4 Phe of the MBP peptide. The necessary room for this aromatic side chain is created by the presence of a small residue (Ala) at the polymorphic DRβ 71 position. (D) Close-up view of MBP peptide residues recognized by the TCR of MBP reactive T cell clones, P-1 Val, P2 His, P3 Phe and P5 Lys. Preferences at these positions were considered in the search criteria for the identification of microbial peptides that activate MBP reactive T cell clones. Reprinted from *The Journal of Experimental Medicine* (Smith et al., 1998) with permission of the publisher.

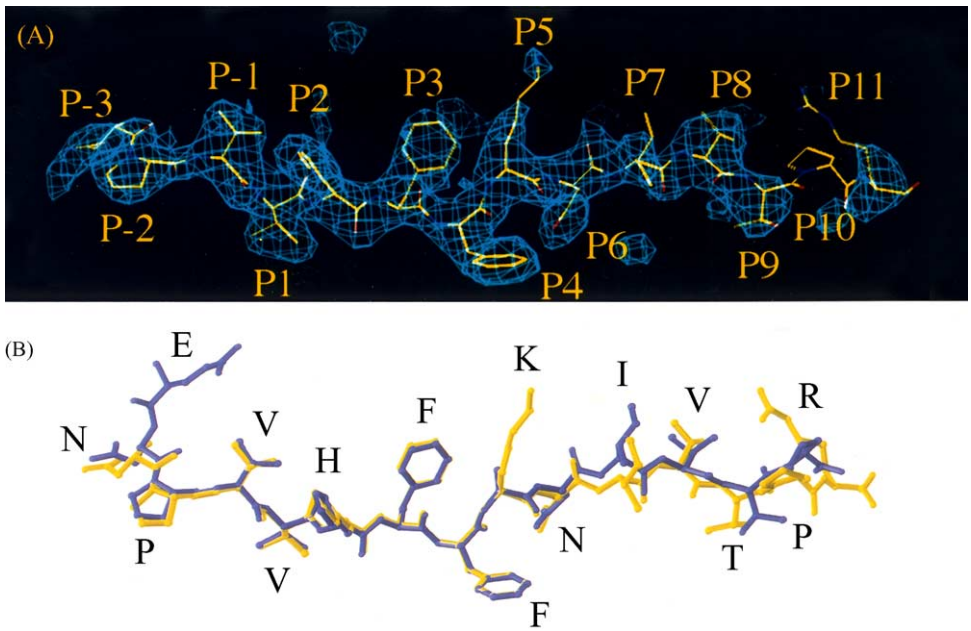

Fig. 2. Electron density and model of the MBP peptide in the binding site of HLA-DR2. (A) Experimental electron density of the MBP peptide bound to HLA-DR2. (B) Model of the MBP peptide based on the electron density. The DR2/MBP peptide complex crystallized as a dimer of dimers, as other HLA-DR molecules (Brown et al., 1993; Stern et al., 1994), and both peptide copies are shown (blue and yellow). The peptide backbones superimpose in the P1–P4 segment and are more divergent in the C-terminal segment due to different crystal contacts. Reprinted from *The Journal of Experimental Medicine* (Smith et al., 1998) with permission of the publisher.

why only a subset of peptides that match the MHC binding/TCR recognition motif activate the appropriate T cell clones. We determined the crystal structure of HLA-DR2 (DRA, DRB1*1501) with the bound MBP peptide as a step towards defining molecular mimicry at a structural level (Smith et al., 1998). Fig. 1 gives an overview of the structure and illustrates features of HLA-DR2 that are important for MBP peptide binding as well as TCR recognition of the HLA-DR2/MBP peptide complex. The MBP peptide is bound in an extended conformation as a type II polyproline helix and MBP peptide side chains occupy the P1, P4, P6 and P9 pockets of the binding groove (Fig. 1a). The two major anchor residues of the MBP peptide (Val and Phe) occupy the P1 and P4 pockets of HLA-DR2. The P4 pocket of HLA-DR2 is distinct from DR molecules associated with other autoimmune diseases. In HLA-DR2, this pocket is large and predominantly hydrophobic due to the presence of a small residue (Ala) at a key polymorphic position (DRβ 71), which permits an aromatic side chain of the MBP peptide to be accommodated (Phe) (Fig. 1c). The peptide residues shown to be important for TCR recognition of the MBP peptide (P2 His, P3 Phe and P5 Lys) are solvent exposed in the structure of the HLA-DR2/MBP peptide complex (Fig. 1b and d; Fig. 2). Comparison with the published high resolution crystal structures of human MHC class I/peptide/TCR complexes (Garboczi et al., 1996; Garcia et al., 1998; Ding et al., 1998) suggests that P5 Lys binds in a TCR pocket formed by the CDR3 loops of the α and β chains.

6. Common features of microbial peptides that activate MBP specific T cells

The structural information on the HLA-DR2/MBP peptide complex was used to dissect the crossreactive peptides identified for the MBP specific T cell clone (Ob.1A12) for which the largest number of stimulatory microbial peptides were identified (Table 1). The crossreactive peptides were aligned with the MBP peptide in order to determine residues that may be located in pockets of the HLA-DR2 binding site (Table 2). This analysis shows that the HLA-DR2 contact surface of this set of peptides is highly diverse. No sequence identity with the MBP peptide is required on the HLA-DR2 binding surface, as illustrated by comparison of the MBP and *Bacillus subtilis* peptides. Common features of putative HLA-DR2 contact residues are the presence of an aliphatic residue in the P1 pocket and a large hydrophobic residue in the P4 pocket. At P6, a preference for asparagine is observed, while the residues that occupy the P7 and P9 positions are diverse. These data are in agreement with HLA-DR2 binding studies, which indicated that only two positions of the peptide (P1 and P4) were critical for binding and that they could be substituted with other aliphatic residues or phenylalanine (P1 pocket) or other hydrophobic residues (P4 pocket).

Analysis of the residues that may be solvent exposed and that could interact with the TCR shows a higher degree of sequence similarity/identity, in particular in the center of the epitope (Table 2). All peptides that activate this T cell clone carry the two primary TCR contact residues of the MBP

Table 1
Sequences of microbial peptides that activate human MBP specific T cell clones

Peptides that activate T cell clone Ob.1A12 (HLA-DR2 restricted)

Organism	Source Protein	Sequence
Homo sapiens	Myelin basic protein	**ENPVVHFFKNIVTPR**
Staphylococcus aureus	VgaB	VLARL**HF**YR**N**DVHKE
Mycobacterium avium	Transposase	QRCR**VHF**LR**N**VLAQV
Mycobacterium tuberculosis	Transposase	QRCR**VHF**MR**N**LY**T**AV
Bacillus subtilis	YqeE	ALA**V**L**HF**YPDKGAKN
Haemophilus influenzae/E. coli	HI0136/ORF	DFAR**VHF**ISALHGSG

Peptides that activate T cell clone Hy.1B11 (HLA-DQ1 restricted)

Organism	Source Protein	Sequence
Homo sapiens	Myelin basic protein	**ENPVVHFFKNIVTPR**
Herpes simplex virus type 1	UL15 protein	FRQL**VHF**VRDFAQLL
Adenovirus type 12	ORF	DFE**VV**T**F**L**K**DVLPEF
Human papillomavirus type 7	L2 protein	IGGR**VHFFK**D**I**SPIA
Pseudomonas aeruginosa	Phosphomannomutase	DRLLML**FA**K**D**V**V**SRN

Microbial peptides that activate two human T cell clones reactive with the MBP (85–99) peptide are shown. Clone Ob.1A12 is restricted by HLA-DR2 (DRA, DRB1*1501) and five microbial peptides have been identified that activate this T cell clone. Clone Hy.1B11 is HLA-DQ1 restricted and activated by four microbial peptides that are quite distinct in their primary sequence. The peptide from human papillomavirus type 7 is the only peptide with obvious sequence similarity to the MBP peptide within the entire set of identified microbial peptides. Residues that are identical between the MBP peptide and microbial peptides are highlighted. The HLA-DQ1 restricted T cell clone recognizes the same core segment of the MBP peptide as HLA-DR2 restricted clones (Wucherpfennig & Strominger, 1995; Wucherpfennig et al., 1994a; Hausmann et al., 1999a). ORF, open reading frame.

peptide (His and Phe at P2 and P3). Also, a preference for a positively charged residue (Lys and Arg) is observed at P5. A high degree of sequence diversity is observed in the N- and C-terminal flanking segments, even though they are required for efficient T cell stimulation.

The data also indicate that combinatorial effects shape the peptide surface that can be recognized by a TCR. Analysis of double-amino acid substitutions of the MBP peptide demonstrated that certain combinations of amino acids at TCR contact residues were stimulatory, even though the individual analogs had no activity (Ausubel et al., 1996). This notion is supported by the observation that the majority of microbial peptides that match the MHC binding/TCR recognition motif did not stimulate the MBP specific T cell clones. Identification of a complete set of peptide sequences that represent agonists for a TCR will therefore require analysis of complex peptide libraries. At present, such analyses represent a technical challenge since a large number of peptides may need to be sequenced from phage display libraries or peptide libraries on beads. However, the complexity of the peptide repertoire that is recognized by an individual TCR may be underestimated unless the combinatorial nature of peptide recognition by the TCR is taken into consideration.

7. Structural features of autoreactive TCRs that contribute to the degree of crossreactivity

Comparison of the MBP (85–99) reactive T cell clones demonstrated obvious differences in the degree of specificity/crossreactivity. For some of the T cell clones, such as Ob.A12, amino acid identity was required at two TCR contact residues of the MBP peptide while every TCR contact residue of the MBP peptide could be substituted by at least one structurally related amino acid for other T cell clones.

Two of the T cell clones (Ob.1A12 and Ob.2F3) differed only in the CDR3 loops of TCR α and β since they had identical Vα-Jα and Vβ-Jβ rearrangements (Wucherpfennig et al., 1994b). Nevertheless, the clones differed in the level of crossreactivity since only three of the five microbial peptides identified for clone Ob.1A12 activated the other clone. We examined the basis for the different level of crossreactivity and found that the clones had a very similar fine specificity for the MBP peptide, except for the P5 position of the peptide (P5 Lys). The microbial peptides that activated clone Ob.1A12 were characterized by conservative or non-conservative changes at P5 (Lys to Arg, Ser or Pro). In contrast, clone Ob.2F3 was only stimulated by the

Table 2
Alignment of microbial peptides based on the crystal structure of the HLA-DR2/MBP peptide complex

Residues located in HLA-DR2 binding pockets	
	1 4 67 9
Myelin basic protein	**----V--F-NI-T--**
Staphylococcus aureus	----L--Y-**N**D-H--
Mycobacterium avium	----**V**--L-**N**V-A--
Mycobacterium tuberculosis	----**V**--M-**N**L-**T**--
Bacillus subtilis	----L--Y-DK-A--
Haemophilus influenzae/E. coli	----**V**--I-AL-G--
Solvent exposed residues	
	23 5 8
Myelin basic protein	**ENPV-HF-K--V-PR**
Staphylococcus aureus	VLAR-**HF**-R--**V**-KE
Mycobacterium avium	QRCR-**HF**-R--L-QV
Mycobacterium tuberculosis	QRCR-**HF**-R--Y-AV
Bacillus subtilis	ALA**V**-**HF**-P--G-KN
Haemophilus influenzae/E. coli	DFAR-**HF**-S--H-SG

Peptide sequences were dissected in terms of putative HLA-DR2 anchor residues and solvent accessible residues that are available for interaction with the TCR. The HLA-DR2 contact surface of these peptides is highly diverse, and no sequence identity is observed at these five positions between the MBP and *B. subtilis* peptides. A higher degree of sequence similarity/identity is observed for peptide residues that are likely to interact with the TCR (P2, P3 and P5). Numbers above the sequences represent positions in a nine-amino acid peptide core, starting with the anchor residue for the P1 pocket of the binding site.

peptides that had a conservative lysine to arginine substitution. The degree of specificity in recognition of the P5 side chain was the key difference between these TCR since the *Haemophilus influenzae*/*E. coli* peptide stimulated both clones when the P5 position was substituted from serine to arginine (Hausmann et al., 1999a). In the crystal structure of the HLA-DR2/MBP peptide complex, P5 Lys was a prominent, solvent exposed residue in the center of the DR2/MBP peptide surface (Fig. 1). Structures of MHC class I/peptide complexes have shown that this peptide side chain occupies a central TCR pocket created by the CDR3 loops of TCRα and β (Garboczi et al., 1996; Ding et al., 1998). This suggests that the two MBP specific TCR differ in the size and shape of the TCR pocket that accomodates the central lysine of the MBP peptide.

Similar findings were made for MHC class I restricted T cell clones that recognize the HTLV-1 Tax (11–19) peptide bound to HLA-A2. The crystal structure of the MHC/peptide/TCR complex has been determined for both of these TCRs (A6 and B7), which demonstrated major differences in the shape and charge of the TCR pocket for the P5 peptide residue (Garboczi et al., 1996; Ding et al., 1998). The B7 TCR was exquisitely specific for P5 tyrosine of Tax (11–19) since only aromatic substitutions were tolerated. In contrast, the A6 TCR was much more degenerate at the P5 position since 10 of 17 analog peptides induced lysis of target cells at low peptide concentrations (Hausmann et al., 1999b). The absolute requirement for an aromatic side chain by the B7 T cell clone could be explained based on the structure of the P5 pocket: the P5 Tyr represented a tight fit for this TCR pocket and a favorable stacking interaction between the aromatic ring of P5 Tyr and an aromatic residue of the CDR3 loop of the TCR β chain (Y104 β) was observed. In contrast, the A6 TCR had a larger P5 pocket and the P5 Tyr did not interact with an aromatic TCR residue. These data demonstrate that the TCR CDR3 loops can determine the degree of specificity and degeneracy of the central TCR pocket.

Further characterization of the peptide from the EBV DNA polymerase gene demonstrated a second structural mechanism for TCR crossreactivity. The HLA-DR2 haplotype that confers susceptibility to MS encodes two DRβ chain genes (DRB1*1501 and DRB5*0101), both of which can pair with the non-polymorphic DRα chain to form functional DR heterodimers (Sone et al., 1985). Both DR molecules are expressed by antigen presenting cells in subjects with the HLA-DR2 haplotype. The Hy.2E11 clone recognized the MBP peptide bound to DRA, DRB1*1501 molecules, but surprisingly the EBV peptide bound to DRA, DRB5*0101 molecules. Comparison of the two crystal structures demonstrated a striking degree of similarity, in particular for the peptide positions previously shown to be required for TCR recognition (Lang et al., 2002; Wucherpfennig and Strominger, 1995). TCR crossreactivity can therefore involve the recognition of different peptides bound to the same MHC molecule, or recognition of different peptides on other self-MHC molecules.

8. TCR crossreactivity and immunopathology

Several different experimental autoimmune diseases have been induced by immunization with microbial peptides, indicating that crossreactive T cell populations can be pathogenic. EAE has been induced in different strains of mice and in Lewis rats with mimicry peptides of MBP and myelin oligodendrocyte glycoprotein (Ufret-Vincenty et al., 1998; Grogan et al., 1999; Gautam et al., 1998; Mokhtarian et al., 1999; Lenz et al., 2001). However, the physiologically more relevant question is whether autoimmune disease can also result from *infection* with pathogens that carry such T cell epitopes. To address this question, Olson et al. (2001) generated recombinant Theiler's viruses in which the candidate sequences were placed into the leader segment of the virus. The first recombinant virus carried the sequence of the PLP (139–151) peptide that is immunodominant in SJL mice. Infection with this virus resulted in the rapid development of central nervous system (CNS) inflammation and vigorous CD4 T cell responses to the PLP peptide. It

is important to note that the wild-type Theiler's virus also induced CNS pathology, but disease onset was significantly later (day 30, rather than day 10), permitting the two disease states to be distinguished. Also, tolerance induction with the PLP (139–151) peptide prevented induction of the early disease process with the PLP (139–151) expressing virus, but not the late disease caused by wild-type Theiler's virus (Olson et al., 2002). Importantly, CNS autoimmunity could not only be induced by a virus that carried the self peptide, but also by a recombinant virus that expressed a peptide from *Hemophilus influenzae* shown to stimulate PLP (139–151) specific T cells (Olson et al., 2001).

The relationship between autoimmune disease and viral infection has also been examined with natural pathogens, in particular using the Herpes simplex keratitis (HSK) model (Panoutsakopoulou et al., 2001). Infection of the eye with Herpes simplex virus (HSV-1, KOS strain) triggers a T cell-mediated autoimmune process that persists after the virus has been cleared. In order to rigorously test the role of molecular mimicry in this disease process, a single amino acid substitution was made in the crossreactive T cell epitope of the viral UL-6 protein. The mimicry T cell epitope was found to be important for disease induction in C.AL-20 mice since 1000-fold larger quantities of the mutant virus were required than of wild-type HSV-1, even though both viruses replicated at the same rate. However, in mice that expressed the Cl-6 TCR and therefore harbored large numbers of autoreactive T cells, infection with HSV-1 was not required since scratching of the cornea or local application of LPS were sufficient for the induction of disease. The crossreactive T cell epitope was thus required for the expansion of autoreactive T cells, but activation of the innate immune system was sufficient when large numbers of such T cells were already present. This model therefore clarified the potential contributions of TCR crossreactivity and 'bystander' activation in the induction of autoimmune diseases.

The diverse nature of the viral/bacterial peptides that stimulate autoreactive T cell clones suggests that different infectious agents could initiate autoimmunity by molecular mimicry. However, it is important to keep in mind that a number of other mechanisms could also result in the activation of autoreactive T cells. The diverse nature of the mimicry peptides and the ubiquitous presence of some of these pathogens may make it difficult to establish a direct epidemiological link between infectious agents and the occurrence of certain autoimmune diseases. In particular, the temporal relationship between an infection and development of an autoimmune process may in many cases not be clear because of the time that frequently elapses until clinical symptoms become obvious and a diagnosis is made. Such epidemiological relationships may be more readily established for autoimmune disorders with a rapid disease onset since early diagnosis can greatly increase the likelihood of establishing a link with a preceding infection. Recent data have demonstrated a relationship between an inflammatory, demyelinating disease of the peripheral nervous system (Guillain–Barré syndrome) and preceding infections (Rees et al., 1995). Patients with this disease acutely develop severe symptoms and rapid diagnosis permitted isolation of *Campylobacter jejuni* from approximately one-third of new cases, compared to 2% of household controls. A better understanding of the epidemiology of infectious agents and autoimmunity could thus help to advance our understanding of the molecular mechanisms that trigger autoimmune diseases.

9. TCR crossreactivity as a general property of T cell recognition

A large number of studies have now demonstrated TCR crossreactivity for a variety of human and murine T cells (Bhardwaj et al., 1993; Hemmer et al., 1997; Grogan et al., 1999; Evavold et al., 1995; Hagerty and Allen, 1995; Loftus et al., 1996; Misko et al., 1999; Brehm et al., 2002). An interesting example is the melanoma/melanocyte-derived peptide MART-1 (res. 27–35) since it demonstrates how crossreactivity can shape the T cell repertoire. In normal human donors with the HLA-A2 haplotype, T cells specific for this melanocyte peptide were detected at a surprisingly high frequency, and such T cells could be visualized directly ex vivo with HLA-A2/MART-1 tetramers (frequency of ∼0.1%). This suggested that these T cells crossreacted with microbial peptides and a motif search similar to the one that we had performed for human MBP specific T cell clones yielded 12 peptides that were able to sensitize target cells for lysis. One of these peptides was derived from the glycoprotein C of Herpes simplex virus (HSV) and anti-MART effectors lysed cells infected with a recombinant vaccinia virus encoding HSV-1 glycoprotein C (Loftus et al., 1996; Dutoit et al., 2002).

TCR crossreactivity can also have a profound effect on protective immune responses to viral pathogens in vivo, as shown in a murine model where CD8 T cells crossreacted with peptides from two different viruses—lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PV) (Brehm et al., 2002). LCMV and PV are members of the *Arenaviridae* family, but the two viruses are only distantly related as shown by sequence comparison. Prior infection with either LCMV or PV provided partial protection against the heterologous virus and LCMV-immune mice showed a 97% reduction in viral titer compared to naïve mice when challenged with PV. CD8 T cells from mice infected with either virus crossreacted with a nucleoprotein-derived peptide (NP 205–212) from the other virus; these two nucleoprotein peptides shared six of eight residues and differed at positions 5 (Tyr versus Phe) and 8 (Leu versus Met). In LCMV infected mice the NP 205–212 epitope was subdominant and 3.6% of CD8 T cells responded to this epitope on day 8 following infection. However, CD8 T cells specific

for this NP 205–212 peptide became the predominant CD8 T cell population (30% of all CD8 T cells) when mice that had previously encountered PV were infected with LCMV. These experiments demonstrated that TCR crossreactivity influences the hierarchy of CD8 T cell responses and shapes the pool of memory T cells.

TCR crossreactivity is also a critical aspect of T cell development in the thymus and weaker TCR signals are required for positive selection in the thymus compared to activation of mature T cells. Positive selection in the thymus is peptide-dependent and is affected both by the density of a particular MHC/peptide complex and the affinity of the TCR for this complex. In thymic organ cultures, positive selection was observed with peptides that represented weak agonists or antagonists for the corresponding mature T cells, or with low densities of the agonist peptide (Jameson et al., 1994; Sebzda et al., 1994; Alam et al., 1996). The creation of transgenic mice that expressed a single MHC/peptide ligand in the thymus provided a striking demonstration of crossreactive TCR recognition in thymic development (Ignatowicz et al., 1996). In this experiment, a peptide was covalently linked to the N-terminus of the MHC class II β chain so that all MHC class II molecules were occupied with this peptide. The total numbers of CD4 T cells in these mice were ∼20% compared to wild-type mice and these CD4 T cells expressed a wide variety of different Vβ segments, indicating that a relatively diverse T cell repertoire could develop in the presence of a single MHC class II/peptide ligand. T cell hybridomas isolated from these mice reacted with peptides that had no primary sequence identity with the selecting peptide (Ignatowicz et al., 1997). These experiments demonstrated that T cells could be activated by peptides that were unrelated in sequence to their selecting peptide.

The examples described above indicate that TCR crossreactivity is common and represents an important aspect of TCR recognition. The balance between specificity and crossreactivity is likely to represent a compromise that permits a sufficient number of T cells to recognize a pathogen novel to the individual's immune system. The potentially negative impact of TCR crossreactivity may be in part balanced by *in vivo* selection of T cells with high avidity TCR for the relevant MHC/peptide complexes. It has been postulated that a single TCR can recognize 10^6 different peptide ligands, and this estimate is based on the observation that a subset of T cell clones can be activated by complex peptide mixtures in which only one peptide position is specified (Mason, 1998). While the number of peptide variants that can be recognized may be very large, the number of natural ligands from microbial and self-antigens is likely to be considerably smaller. Nevertheless, a number of different peptides can act as agonists for a given T cell and a considerably larger number of peptide ligands may induce weak signals, such as those that promote positive selection in the thymus and survival of naïve T cells in the periphery. TCR specificity and crossreactivity thus represent important aspects of T cell biology.

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