Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8896–8900, September 1995
Immunology

Structure of human T-cell receptors specific for an immunodominant myelin basic protein peptide: Positioning of T-cell receptors on HLA-DR2/peptide complexes

KAI W. WUCHERPFENNIG†, DAVID A. HAFLER‡, AND JACK L. STROMINGER†

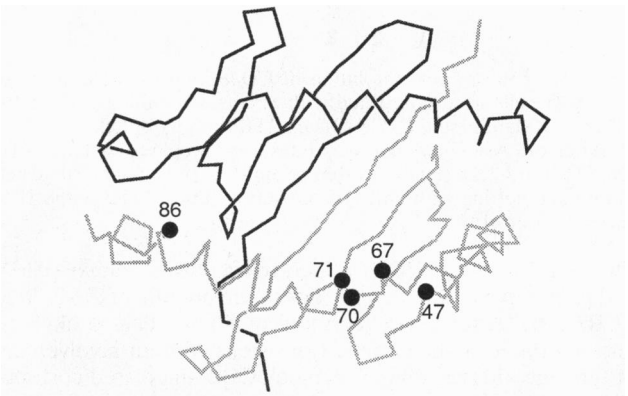
†Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138; and ‡Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Contributed by Jack L. Strominger, March 3, 1995

ABSTRACT **T-cell receptors (TCRs) recognize peptide bound within the relatively conserved structural framework of major histocompatibility complex (MHC) class I or class II molecules but can discriminate between closely related MHC molecules. The structural basis for the specificity of ternary complex formation by the TCR and MHC/peptide complexes was examined for myelin basic protein (MBP)-specific T-cell clones restricted by different DR2 subtypes. Conserved features of this system allowed a model for positioning of the TCR on DR2/peptide complexes to be developed: (*i*) The DR2 subtypes that presented the immunodominant MBP peptide differed only at a few polymorphic positions of the DRβ chain. (*ii*) TCR recognition of a polymorphic residue on the helical portion of the DRβ chain (position DRβ67) was important in determining the MHC restriction. (*iii*) The TCR variable region (V) α3.1 gene segment was used by all of the T-cell clones. TCR Vβ usage was more diverse but correlated with the MHC restriction—i.e., with the polymorphic DRβ chains. (*iv*) Two clones with conserved TCR α chains but different TCR β chains had a different MHC restriction but a similar peptide specificity. The difference in MHC restriction between these T-cell clones appeared due to recognition of a cluster of polymorphic DRβ-chain residues (DRβ67–71). MBP-(85–99)-specific TCRs therefore appeared to be positioned on the DR2/peptide complex such that the TCR β chain contacted the polymorphic DRβ-chain helix while the conserved TCR α chain contacted the nonpolymorphic DRα chain.**

Recognition of antigenic peptides by T-cell receptors (TCRs) is an essential step in the initiation of T-cell-mediated autoimmunity (for review, see ref. 1). TCRs see peptides as complexes with polymorphic self-proteins, the major histocompatibility complex (MHC) class I and class II molecules. Interaction with the structural framework of MHC molecules and with bound peptides may result in a particular orientation of TCRs on MHC/peptide complexes (2, 3). Detailed structural characterization of this recognition unit is important for understanding the structural basis of TCR recognition and for designing therapeutic approaches for T-cell-mediated autoimmune diseases.

Previous studies demonstrated that residues 84–102 of human myelin basic protein (MBP) are immunodominant for human T cells. This peptide as well as the minimum T-cell epitope MBP-(85–99) is presented by HLA-DR2 (DRA, DRB1*1501), which is associated with susceptibility to multiple sclerosis. These peptides bind with high affinity to HLA-DR2 using two hydrophobic residues (Val-89 and Phe-92) as primary anchors (4–8). Structural features critical for ternary complex formation among DR2, MBP-(85–99), and TCRs were characterized in this study. Results indicated that recognition of this peptide favors a particular positioning of the TCR on the DR2/MBP-(85–99) complex.



	DRβ47	DRβ67	DRβ70	DRβ71	DRβ86
DRB1*1501	Phe	Ile	Gln	Ala	Val
DRB1*1502	Phe	Ile	Gln	Ala	Gly
DRB1*1601	Tyr	Phe	Asp	Arg	Gly
DRB1*1602	Tyr	Leu	Asp	Arg	Gly

FIG. 1. Polymorphic residues in the β1 domain of DR2 subtypes. The position of polymorphic residues that differ among DR2 subtypes is shown on the ribbon diagram of HLA-DR1 structure (10). Except for DRβ47, these residues are located in the helical portion of the DRβ1 domain.

MATERIALS AND METHODS

Cloning of MBP-specific T cells, analysis of peptide specificity, and TCR sequence analysis were done as described (7, 8).

RESULTS

Cloning of T Cells Specific for the Immunodominant MBP-(85–99) Peptide. T-cell lines specific for human MBP were generated from blood mononuclear cells of two multiple sclerosis patients with the HLA-DR2 haplotype (8). In both patients, the majority of T-cell lines were MBP-(84–102) specific and HLA-DR restricted. MBP-(84–102)-specific T-cell lines were cloned by limiting dilution. All DR-restricted T-cell clones also recognized the MBP-(85–99) peptide presented by homozygous B-cell lines that express HLA-DR2 (7). T-cell clones from multiple sclerosis patient Ob (DR2/DR4 by serological typing, DRB1*1501 by molecular subtyping of DR2) were DRB1*1501 restricted. T-cell clones from patient

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; V, J, and C, variable, joining, and constant region, respectively; N, short portion of V region encoded by short nucleotide insertions at recombinational junctions.

Table 1. Presentation of the immunodominant MBP-(85–99) peptide by DR2 subtypes

	T-cell proliferation, [^{3}H]thymidine cpm					
	Pair I			Pair II		
	Ob.1A12	Ob.1C3	Ob.3D1	Hy.2E11	Hy.1G11	Hy.2B6
DRB1*1501 (MGAR)	323	714	368	506	758	522
+ MBP-(85–99)	**61,427**	**73,359**	**79,755**	**25,548**	877	617
DRB1*1502 (9011)	492	554	509	5,593	1,319	524
+ MBP-(85–99)	**110,325**	**86,483**	**116,343**	**32,397**	1,526	844
DRB1*1601 (9009)	140	529	481	214	651	571
+ MBP-(85–99)	251	1,059	1,413	211	774	879
DRB1*1602 (9016)	450	620	494	340	638	617
+ MBP-(85–99)	300	824	**113,892**	**7,191**	**36,246**	**16,363**

Presentation of the immunodominant MBP-(85–99) peptide by DR2 subtypes was examined in a T-cell proliferation assay using peptide-pulsed homozygous B-cell lines as antigen-presenting cells. Clones Ob.1A12 and Ob.1C3 (pair I) have the same MHC restriction (DRB1*1501 and 1502) but a different peptide fine specificity; clones Hy.2E11 and Hy.1G11 (pair II) have a different MHC restriction but a similar peptide fine specificity. None of the T-cell clones recognize MBP-(85–99) in the context of DRB1*1601, indicating that DRβ67 on the DRβ chain helix (a TCR contact residue) is critical in defining the MHC restriction. Boldface letters indicate a positive proliferative response.

Hy (DR2/DR7 by serological typing, DRB1*1602 by molecular subtyping of DR2) were DRB1*1602 restricted (Table 1).

DR2-Restricted TCRs Discriminate Between Polymorphic Residues in the DRβ67–71 Cluster of the DRβ-Chain Helix. B-cell lines homozygous for different DR2 subtypes (DRB1*1501, -1502, -1601, and -1602) were used as antigen-presenting cells (Table 1). These DR2 antigens are structurally related: DRB1*1501 and DRB1*1502 differ only at position 86 (Val → Gly); DRB1*1601 and DRB1*1602 differ only at position 67 (Phe → Leu) (Fig. 1) (9). The six DR2-restricted T-cell clones fell into three groups when examined for recognition of the MBP-(85–99) peptide in the context of different DR2 subtypes. Two clones from patient Ob recognized the peptide in the context of DRB1*1501 and DRB1*1502, whereas two other clones from patient Hy recognized it only on DRB1*1602 (Table 1). Recognition of DR2/peptide complexes by two clones (one from Ob, the other from Hy) was more permissive, as they recognized the MBP-(85–99) peptide on three of the four DR2 subtypes (Table 1).

Comparison of peptide presentation by DRB1*1601- and DRB1*1602-encoded proteins was particularly informative because these allotypes differed only at position 67 in the DRβ1 domain (Phe → Leu). This residue is a likely TCR contact residue because it is located at the highest point of the curved DRβ-chain helix (Fig. 1) (10, 11). Two T-cell clones (Hy.1G11 and Hy.2B6) recognized MBP-(85–99) when presented by DRB1*1602 (leucine at DRβ67) but not when presented by DRB1*1601 (phenylalanine at DRβ67) (Table 1). Two other clones (Hy.2E11 and Ob.3D1) recognized the peptide in the context of all subtypes, except DRB1*1601 (Table 1). Thus DRβ67 was a critical residue for TCR recognition of DR2/peptide complexes. A Leu/Ile → Phe substitution of DRβ67 must have interfered with TCR recognition, as the peptide binds with high affinity to DRB1*1601-encoded molecules (12).

DR2-Restricted T Cells Specific for MBP-(85–99) All Use the Variable Region (V) α3.1 Gene Segment and Various Vβ Segments. All of the six DR2-restricted clones used the Vα3.1 gene segment; however, none of the TCR α-chain sequences were identical due to differences in the CD3 regions. The DQ1-restricted clone (Hy.1B11) had a Vα8.1 rearrangement (Table 2) (7). Vα3.1 use was seen only among DR2-restricted clones, as MBP-(84–102)-specific T-cell clones from subjects with other HLA-DR antigens used different TCR Vα and Vβ gene segments (8).

Two sets of clones had strikingly similar TCR α-chain sequences and differed from each other only by a single amino acid substitution in the CDR3 region. The DRB1*1501-restricted clones Ob.1A12 and Ob.1C3 (pair I) differed only by Thr → Ala in this region. Clones Hy.2E11 and Hy.1G11 (pair II) had a conservative substitution at the same position (Ser → Thr) (Table 2).

TCR Vβ usage was more heterogeneous among these MBP-(85–99)-specific clones, but correlated with the MHC restriction (Table 3). The two DRB1*1501-restricted clones (Ob.1A12, Ob.1C3) used Vβ2.1, whereas DRB1*1602-restricted clones (Hy.1G11, Hy.2B6) used Vβ17.1; both sets had a rearrangement of the Vβ gene segment to Jβ2.1. The TCR β-chain sequences of clones Ob.1A12 and Ob.1C3 (pair I) were identical, except for two adjacent positions in the Dβ–Nβ region (TSGAN vs. TSGSL). Interestingly, the junctional sequences of two clones with different Vβ but identical Jβ use (Ob.1C3, Vβ2.1–Jβ2.1 and Hy.1G11, Vβ17.1–Jβ2.1) were also quite similar (RDLTSGSL and R--TSGSY) (Table 3). Still different Vβ and Jβ segments were used by the two clones with dual restriction to DRB1*1501 and DRB1*1602, although all six clones used the same Vα segment.

Table 2. TCR α-chain sequences of MBP-(85–99)-specific T-cell clones

Clone	Vα	Nα/Jα	Cα	Gene segment	MHC restriction
[Ob.1A12	YFCATD	TTSGTYKYIFGTGTRLKVLA	NIQN	Vα3.1	DRB1*1501
[Ob.1C3	YFCATD	ATSGTYKYIFGTGTRLKVLA	NIQN	Vα3.1	DRB1*1501
Ob.3D1	YFCATD	GNGNQFYFGTGTSLTVIP	NIQN	Vα3.1	DRB1*1501,DRB1*1602
[Hy.2E11	YFCATD	SGGSYIPTFGRGTSLIVHP	YIQN	Vα3.1	DRB1*1501,DRB1*1602
[Hy.1G11	YFCATD	TGGSYIPTFGRGTSLIVHP	YIQN	Vα3.1	DRB1*1602
Hy.2B6	YFCATD	AGGQNFVFGPGTRLSVLP	YIQN	Vα3.1	DRB1*1602
	YFCA	EMRPHNNNDMRFGAGTRLTVKP	NIQN	Vα15.1	
Hy.1B11	YFCA	ASSFGNEKLTFGTGTRLTIIP	NIQN	Vα8.1	DQA1*0102 DQB1*0502 DQA1*0101 DQB1*0501

Nomenclature of TCR Vα gene segments is according to Kimura *et al.* (13). N, short portion of V region encoded by short nucleotide insertions at recombinational junctions; J, joining region; C, constant region.

Table 3. TCR β-chain sequences of MBP-(85–99)-specific T-cell clones

Clone	Vβ	N	Dβ	N	Jβ	Cβ	Gene segments	MHC restriction
Ob.1A12	YICSA	RDL	TSG	**AN**	NEQFFGPGTRLTVL	EDLK	Vβ2.1–Jβ2.1	DRB1*1501
Ob.1C3	YICSA	RDL	TSG	**SL**	NEQFFGPGTRLTVL	EDLK	Vβ2.1–Jβ2.1	DRB1*1501
Ob.3D1	YLCASS	IRHRT			NTEAFFGQGTRLTVV	EDLN	Vβw21–Jβ1.1	DRB1*1501,DRB1*1602
Hy.2E11	YLCSA	WPS	GQG	T	YGYTFGSGTRLTVV	EDLN	Vβ4.3–Jβ1.2	DRB1*1501,DRB1*1602
Hy.1G11	YLCAS	R	TSG		SYNEQFFGPGTRLTVL	EDLK	Vβ17.1–Jβ2.1	DRB1*1602
Hy.2B6	YLCASS	TDWS			SYNEQFFGPGTRLTVL	EDLK	Vβ17.1–Jβ2.1	DRB1*1602
Hy.1B11	YLCA	TSALG			DTQYFGPGTRLTVL	EDLK	Vβ6.1–Jβ2.3	DQA1*0102 DQB1*0502 DQA1*0101 DQB1*0501

Nomenclature of TCR Vβ gene segments is according to Kimura *et al.* (13); nomenclature of Vβw21 is according to Ferradini *et al.* (14). Boldface type represents different amino acids in clones Ob.1A12 and Ob.1C3 in both Table 2 and Table 3.

Phe-91 in the Center of the MBP-(85–99) Peptide Is a TCR Contact Residue for All DR2-Restricted T-Cell Clones. In a previous study peptide residues critical for binding to DRB1*1501 molecules were defined (7). Hydrophobic amino acids (Val-89 and Phe-92) in the MBP-(88–95) segment were found to be critical for peptide binding; adjacent residues (His-90, Phe-91, Lys-93) appeared important for interaction with the TCR, as alanine substitutions affected T-cell recognition but not DR binding. Alanine substitutions outside of the MBP-(88–95) core segment had little effect on DR binding or T-cell recognition. The fine specificity of the TCRs was probed using a panel of analog peptides with conservative and nonconservative substitutions (Table 4, Figs. 2 and 3).

A Phe-91 → Ala substitution abolished T-cell recognition of the peptide by all DR2-restricted T-cell clones (Fig. 2 *Upper*, Table 4) but did not affect DR binding (7). The specific recognition of Phe-91 by the T-cell clones was further probed by using analog peptides with substitutions by other aromatic amino acids (tyrosine, tryptophan) and by aliphatic amino acids (valine, leucine, and isoleucine). Three DRB1*1501-restricted clones from patient Ob were exquisitely sensitive to substitution of this position. Two other clones (Hy.2E11 and Hy.1G11, pair II) recognized peptides with aliphatic substitutions (Fig. 2).

There were striking differences between the T-cell clones in the number of residues sensitive to mutation. The most extreme cases were clone Ob.3D1, for which substitution of four residues (His-90, Phe-91, Lys-93, and Ile-95) abolished TCR recognition, and clones Hy.2E11 and Hy.1G11, for which only some analogs of Phe-91 and Lys-93 were not stimulatory (Table 4). Two to four residues in the core of the MBP-(85–99) peptide therefore appeared to be important TCR contact residues for different clones. Secondary contacts in the N- and C-terminal flanking segments were, however, important, as 14- or 15-amino acid peptides were the most potent stimulators (Table 5) (7).

CDR3 Regions of TCR α and β Chains Determine the Fine Specificity of DRB1*1501-Restricted T-Cell Clones. Two T-cell clones (pair I, clones Ob.1A12 and Ob.1C3) used the same Vα3.1–Jα and Vβ2.1–Jβ2.1 rearrangements and were identical in their CDR3 sequences except for one position in the TCR α chain and two positions in the β chain (Tables 2 and 3, Fig. 3). These clones had the same MHC restriction (DRB1*1501 and DRB1*1502) and required the same epitope [MBP-(85–98)] for optimal T-cell stimulation (Tables 1 and 5). The two clones only differed in their specificity for analog peptides of Lys-93 (Tables 4 and 5, Fig. 3). An analog peptide with a Lys-93 → Gln substitution was a potent activator of clone Ob.1A12 but failed to activate clone Ob.1C3 (Fig. 3). These data indicated that the CDR3 regions contribute to the specificity of peptide recognition by these TCRs.

The TCR β Chain Contacts a Cluster of Polymorphic Residues on the DRβ-Chain Helix. T-cell clones Hy.2E11 and Hy.1G11 had identical TCR α-chain sequences, except for a conservative substitution (Ser → Thr) in the Nα region; the Vβ–Jβ rearrangements differed, however (Vβ4.3–Jβ1.2 and Vβ17.1–Jβ2.1, respectively) (Table 3). These TCRs were remarkably similar in their fine specificity for MBP-(85–99) analog peptides (Table 4) but had different MHC restrictions. Clone Hy.2E11 recognized the peptide presented by three DR2 subtypes (DRB1*1501, -1502, and -1602); however, clone Hy.1G11 recognized it only on DRB1*1602 (Tables 1 and 5). Transfection experiments indicated that the Ser → Thr substitution in the TCR α chain was not responsible for the difference in MHC restriction between these clones. Thus, cotransfection of the TCR β chain from clone Hy.1G11 with the TCR α chain either from clone Hy.2E11 or from Hy.1G11 yielded transfectants with the same MHC restriction (DRB1*1602) (Fig. 4).

Table 4. Specificity of MBP-(85–99)-reactive T-cell clones for peptides with single amino acid substitutions of putative TCR contact residues

	Pair I			Pair II	
	Ob.1A12	Ob.1C3	Ob.3D1	Hy.2E11	Hy.1G11
MBP-(85–99)	++++	++++	++++	++++	++++
Substitution of His-90					
Phenylalanine	+++	++	++	+++	++++
Tyrosine	−	−	−	+++	++++
Alanine	+	−	−	++++	++++
Aspartate	+	−	−	+	+++
Substitution of Phe-91					
Tyrosine	−	−	+	+++	++
Tryptophan	−	−	−	+	+
Alanine	−	−	−	−	−
Valine	+	−	−	++++	++
Leucine	−	−	−	++++	++++
Isoleucine	−	−	−	++++	++++
Substitution of Lys-93					
Arginine	++++	+++	−	+++	+
Glutamine	++++	−	−	−	−
Asparagine	+	−	−	−	+
Alanine	++++	++	−	+	+
Aspartate	−	−	−	++	+
Substitution of Asn-94					
Alanine	++++	+++	+	++++	+++
Aspartate	++	+	+++	++++	++
Substitution of Ile-95					
Alanine	++++	+++	++	++++	++++
Phenylalanine	++++	+++	−	++++	++
Tyrosine	++++	+++	−	+++	++
Tryptophan	++++	++++	+++	++++	++
Aspartate	+++	+	++	++++	+

Reactivity of T-cell clones was tested in a T-cell proliferation assay. The T-cell response was graded as follows: +, ++, +++, and ++++, which correspond to a T-cell response at peptide concentrations of 50 μM, 5 μM, 500 nM, or 50 nM, respectively. All peptides had single amino acid substitutions of the MBP-(85–99) sequence (ENPVVHFFKNIVTPR); only peptide Arg-93 was longer (DENPVVHFFRNIVTPRTPPY).

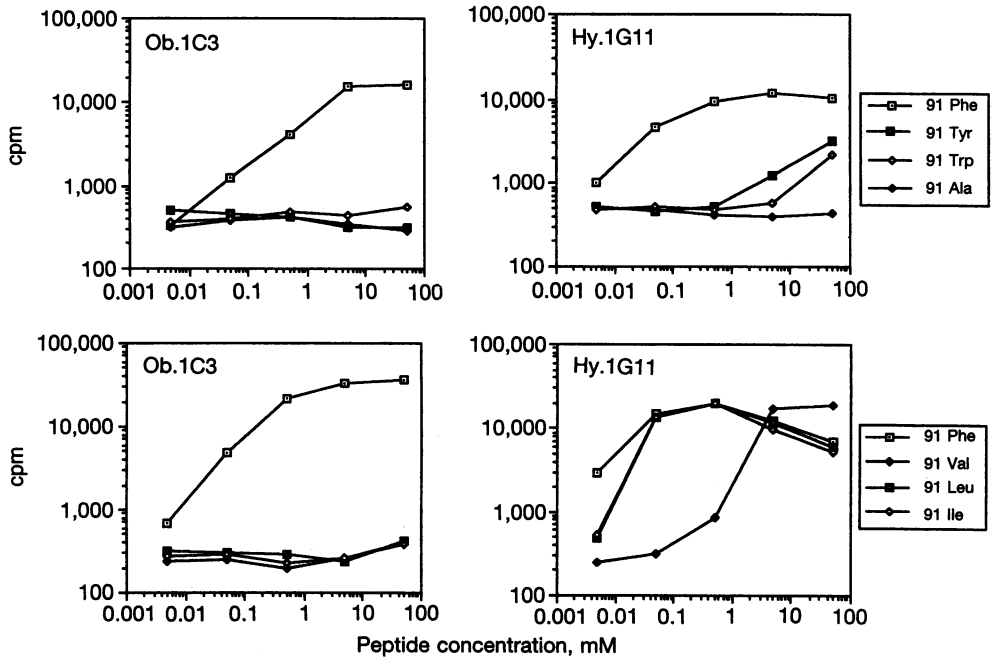


FIG. 2. Phe-91 in the center of the immunodominant MBP-(85–99) peptide is a critical TCR contact residue for all DR2-restricted T-cell clones. T-cell clones were tested in a T-cell proliferation assay for reactivity to analog peptides of MBP-(85–99) (sequence: ENPVVHFFKNIVTPR, residue 91 underlined) in which Phe-91 was substituted by other aromatic amino acids or alanine (*Upper*) or by aliphatic amino acids (*Lower*). B-cell lines MGAR (DRB1*1501 used as antigen-presenting cell for clone Ob.1C3) and 9016 (DRB1*1602 used as antigen-presenting cell for clone Hy.1G11) were treated with peptides at concentrations from 5 nM to 50 μM and cocultured with T-cell clones. T-cell proliferation was measured by [^{3}H]thymidine incorporation (cpm, *y* axis). A Phe-91 → Ala substitution abolished T-cell recognition by all clones but did not affect DR2 binding.

These two TCRs have a strikingly similar fine specificity for the MBP-(85–99) peptide and probably orient themselves with a similar topology on the DR2/peptide complex. Therefore, the difference in MHC restriction was presumably due to direct interaction of the TCR β chain with the polymorphic DRβ-chain cluster (DRβ67–71) and particularly with DRβ67 (Fig. 1).

DISCUSSION

Conserved structural features of this recognition unit facilitated a detailed analysis of the interaction between the DR/peptide complex and the TCR: (*i*) All TCRs used the Vα3.1 gene segment. In addition, two sets of clones that differed either in their peptide fine specificity (pair I) or in their MHC restriction (pair II) had conserved TCR α-chain sequences. (*ii*) Peptide residue Phe-91 was an important TCR contact residue for all MBP-(85–99)-specific TCRs. (*iii*) The DRβ chains of the DR2 subtypes (DRB1*1501, -1502, -1601, and -1602) differed only at a few positions, most notably in the DRβ67–71 cluster (9).

Two T-cell clones (Hy.2E11 and Hy.1G11, pair II) with conserved TCR α-chain but different β-chain sequences had a similar peptide fine-specificity, indicating that a different Vβ–Jβ combination can give a similar three-dimensional structure at the TCR–peptide interface. The clones differed, however, in their MHC restriction because one clone recognized the peptide on three of four DR2 subtypes (DRB1*1501, -1502, and -1602), whereas the other could recognize it only on DRB1*1602. DRβ67 was identified as a critical residue for both clones, as the peptide was not recognized in the context of the DRB1*1601 molecule.

These data suggest a model where the conserved TCR Vα chain is positioned over the nonpolymorphic DRα chain, while the variable TCR β chains determine the MHC restriction by differentiating between polymorphic residues in the DRβ67–71 cluster (Table 5, Fig. 3). A similar observation has been made for two murine T-cell hybridomas specific for moth cytochrome *c* that had a single or a dual MHC restriction (I-E^k or I-E^k/I-E^b). Transfection of the TCR β chain into the hybridoma with the single MHC restriction led to acquisition of I-E^b restriction, indicating that the TCR β chain was responsible for recognizing the I-E β chain (15).

Two possible positions of the TCR αβ heterodimer on DR2/MBP peptide complexes may account for the effect of the TCR β chain on MHC restriction observed in this study: In the first model the CDR3 loop of TCR β contacts the C terminus of the peptide, whereas the CDR1 and CDR2 loops of TCR β interact with the nonpolymorphic helical segment of the DRα chain. This arrangement is similar to the model

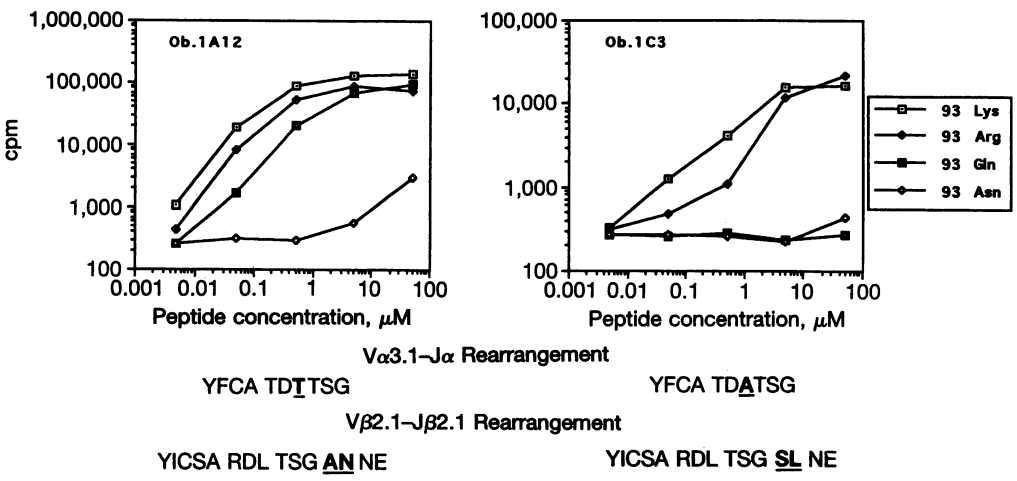


FIG. 3. The CDR3 regions of the TCR determine the fine specificity for the MBP-(85–99) peptide. Differences in peptide fine specificity were observed for two DRB1*1501-restricted T-cell clones that used the same Vα–Jα and Vβ–Jβ gene segments but differed in the CDR3 sequence of the TCR α and β chains. Clones differed in their specificities to analog peptides substituted at Lys-93. In contrast, T-cell responses were similar to peptides substituted at TCR contact residues His-90 and Phe-91. *y* axes represent cpm of incorporated [^{3}H]thymidine as measurements of T-cell proliferation.

Table 5. Relationship among TCR structure, MHC restriction, and peptide specificity

	Pair I			Pair II		
	Ob.1A12	Ob.1C3	Ob.3D1	Hy.2E11	Hy.1G11	Hy.2B6
TCR Vα	Vα3.1	Vα3.1	Vα3.1	Vα3.1	Vα3.1	Vα3.1, Vα15.1
Nα–Jα junction	TDTTSGTY	TDATSGTY	TDGNGNQF	TDSGGSY	TDTGGSY	TDAGGQNF
TCR Vβ–Jβ	Vβ2.1–Jβ2.1	Vβ2.1–Jβ2.1	Vβw21–Jβ1.1	Vβ4.3–Jβ1.2	Vβ17.1–Jβ2.1	Vβ17.1–Jβ2.1
Nβ–Dβ junction	RDLTSGAN	RDLTSGSL	IRHRT	WPSGQGT	RTSG	TDWS
MHC restriction	DRB1*1501	DRB1*1501	DRB1*1501 DRB1*1602	DRB1*1501 DRB1*1602	DRB1*1602	DRB1*1602
Optimal peptide	MBP-(85–98)	MBP-(85–98)	MBP-(87–98)	MBP-(87–99)	MBP-(87–98)	MBP-(87–98)
Putative TCR contacts of MBP-(90–95) core (HFFKNI)						
Major TCR contacts	HF----	HF----	HF-K--	-F-K--	-F-K--	HF-KNI
Minor TCR contacts†	------	---K--	----NI	------	-----I	------

The MBP-(85–99) peptide is recognized by human T-cell clones when presented by DRB1*1501 molecules (clones Ob.1A12 and Ob.1C3), DRB1*1602 molecules (Hy.1G11, Hy.2B6), or by both (Ob.3D1, Hy.2E11). DRB1*1501-restricted clones use Vβ2.1; DRB1*1602-restricted clones use Vβ17.1; all clones use Vα3.1. T-cell clones Hy.2E11 and Hy.1G11 have conserved TCR α-chain but different β-chain sequences. The difference in MHC restriction between these clones is probably due to recognition of the polymorphic DRβ67–71 cluster by the TCR β chains. Clones Ob.1A12 and Ob.1C3 use the same Vα–Vβ pair and differ only by three amino acids in the CDR3 regions (one difference in TCR α chain, two in TCR β chain). These clones have the same MHC restriction but differ in the recognition of analog peptides substituted at position Lys-93.

†Residues 6 and 7 of the peptide core may be positioned so that they can function both in MHC binding and as TCR contact residues (see ref. 11).

proposed for the moth cytochrome c/I-E^k system (16). This model would imply that the CDR3 region of TCR β is also responsible for the different MHC restrictions by contacting polymorphic residue(s) on the helical segment of the DR β chain. In the second model the TCR is rotated by 180° so that the CDR3 loop of TCR β is positioned over the N terminus of the peptide. This arrangement would place CDR1 and CDR2 of TCR β on the polymorphic DRβ-chain helix and allow the CDR2 loop of TCR β to contact the polymorphic DRβ67-71 cluster. The latter orientation was also recently proposed for a murine conalbumin-specific TCR restricted by I-A^k (17) and is more compatible with the present data.

Thus, two different topologies have been suggested for murine T-cell clones specific for moth cytochrome *c*/I-E^k and conalbumin/I-A^k, respectively (16, 17). Positioning of the TCR on the MHC class II peptide complex could depend on the peptide recognized and/or the presenting class II molecule (I-A versus I-E in mice and DR, DQ, or DP in humans). In any case, our data suggest that TCRs specific for the same DR/peptide complex are positioned similarly and that conserved TCR contact residues of the bound peptide and polymorphic MHC residues are critical in defining the interaction with the TCR.

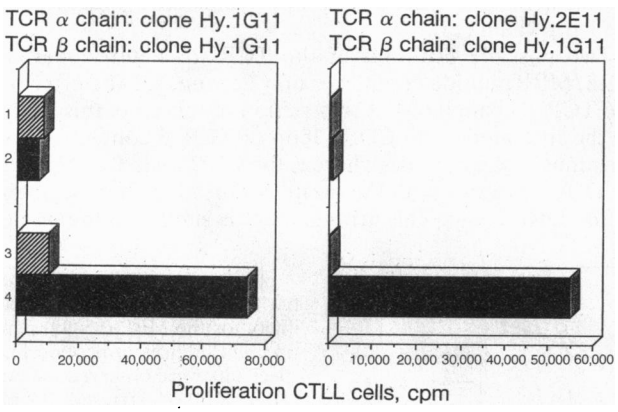


FIG. 4. TCR α chains are not responsible for the difference in MHC restriction between T-cell clones Hy.1G11 and Hy.2E11. The TCR β chain of clone Hy.1G11 (DRB1*1602 restriction) was cotransfected with the TCR α chain of clone Hy.1G11 or clone Hy.2E11 (DRB1*1501/1602 restriction). TCR constructs were made by fusing the extracellular domains of TCR α and β chains to the TCR ζ chain and by cotransfecting BW cells, a murine thymoma cell line. Interleukin 2 release from BW cells upon recognition of the appropriate MHC/peptide complex was monitored by measuring proliferation of an interleukin 2-dependent cell line (CTLL). Bars: 1, DRB1*1501 and no peptide; 2, DRB1*1501 + MBP-(85–99); 3, DRB1*1602 and no peptide; 4, DRB1*1602 + MBP-(85–99).

We thank C. Witek and B. Rybalov for expert technical assistance, Dr. E. Milford for providing B-cell lines, and Dr. L. Berg for critical reading of the manuscript. This work was supported by an award from the National Multiple Sclerosis Society and by grants from the National Institutes of Health (CA47554 to J.L.S. and NS24247 to D.A.H.). K.W.W. is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society.

1. Zamvil, S. S. & Steinman, L. (1990) *Ann. Rev. Immunol.* **8,** 579–621.
2. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 512–518.
3. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334,** 395–402.
4. Ota, K., Matsui, M., Milford, E. L., Mackin, G. A., Weiner, H. L. & Hafler, D. A. (1990) *Nature (London)* **346,** 183–187.
5. Pette, M., Fujita, K., Wilkinson, D., Altmann, D. M., Trowsdale, J., Giegerich, G., Hinkkanen, A., Epplen, J. T., Kappos, L. & Wekerle, H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7968–7972.
6. Martin, R., Jaraquemada, D., Flerlage, M., Richert, J., Whitaker, J., Long, E. O., McFarlin, D. E. & McFarland, H. F. (1990) *J. Immunol.* **145,** 540–548.
7. Wucherpfennig, K. W., Sette, A., Southwood, S., Oseroff, C., Matsui, M., Strominger, J. L. & Hafler, D. A. (1994) *J. Exp. Med.* **179,** 279–290.
8. Wucherpfennig, K. W., Zhang, J., Witek, C., Matsui, M., Modabber, Y., Ota, K. & Hafler, D. A. (1994) *J. Immunol.* **152,** 5581–5592.
9. Marsh, S. G. E. & Bodmer, J. G. (1992) *Hum. Immunol.* **35,** 1–17.
10. Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. & Wiley, D. C. (1993) *Nature (London)* **364,** 33–39.
11. Stern, L. J., Brown, J. H., Jardetzky, T. S., Urban, R., Strominger, J. L. & Wiley, D. C. (1994) *Nature (London)* **368,** 215–221.
12. Valli, A., Sette, A., Kappos, L., Oseroff, C., Sidney, J., Miescher, G., Hochberger, M., Albert, E. D. & Adorini, L. (1993) *J. Clin. Invest.* **91,** 616–628.
13. Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R.-P. & Mak, T. W. (1987) *Eur. J. Immunol.* **17,** 375–383.
14. Ferradini, L., Roman-Roman, S., Azocar, J., Michalaki, H., Triebel, F. & Hercend, T. (1991) *Eur. J. Immunol.* **21,** 935–942.
15. Saito, T. & Germain, R. N. (1987) *Nature (London)* **329,** 256–259.
16. Jorgensen, J. L., Esser, U., de St. Groth, B. F., Reay, P. A. & Davis, M. M. (1992) *Nature (London)* **355,** 224–230.
17. Hong, S.-C., Chelouche, A., Lin, R.-h., Shaywitz, D., Braunstein, N. S., Glimcher, L. & Janeway, C. A. (1992) *Cell* **69,** 999–1009.