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Molecular Mimicry in T Cell-Mediated Autoimmunity: Viral Peptides Activate Human T Cell Clones Specific for Myelin Basic Protein

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Summary

Structural similarity between viral T cell epitopes and self-peptides could lead to the induction of an autoaggressive T cell response. Based on the structural requirements for both MHC class II binding and TCR recognition of an immunodominant myelin basic protein (MBP) peptide, criteria for a data base search were developed in which the degeneracy of amino acid side chains required for MHC class II binding and the conservation of those required for T cell activation were considered. A panel of 129 peptides that matched the molecular mimicry motif was tested on seven MBP-specific T cell clones from multiple sclerosis patients. Seven viral and one bacterial peptide efficiently activated three of these clones. Only one peptide could have been identified as a molecular mimic by sequence alignment. The observation that a single T cell receptor can recognize quite distinct but structurally related peptides from multiple pathogens has important implications for understanding the pathogenesis of autoimmunity.

Introduction

Activation of autoreactive T cells is a critical event in the induction of autoimmunity. In animal models of T cell–mediated autoimmunity, disease can be transferred only with activated but not with resting T cells specific for a central nervous system (CNS)-specific autoantigen (reviewed by Zamvil and Steinman, 1990). Resting autoreactive T cells are part of the normal immune repertoire and do not induce disease as the blood–brain barrier limits access to the CNS to activated T cells (Schluesener and Wekerle, 1985; Burns et al., 1983; Ota et al., 1990; Martin et al., 1990; Pette et al., 1990). This protective mechanism makes circulating self-reactive T cells "ignorant" of the complex set of tissue-specific self-antigens hidden behind the blood–brain barrier. Invasion of the CNS requires autoreactive T cells to be activated in the peripheral immune system. This activation occurs in the absence of their nominal self-antigen, which is sequestered in the CNS (Wekerle et al., 1986; Hickey et al., 1991). Two mechanisms could account for the activation and clonal expansion of autoreactive T cells in the periphery: activation by bacterial or viral superantigens that trigger T cells bearing particular T cell receptor Vβ segments (Brocke et al., 1993; Cole and Griffiths, 1993; Conrad et al., 1994), or activation by viral or bacterial peptides that have sufficient sequence similarity with an immunodominant self-peptide (molecular mimicry) (Oldstone, 1990).

A large body of clinical and epidemiological evidence indicates that infections are important in the induction of autoimmunity. Particular viral infections frequently precede autoimmune myocarditis and type I diabetes (IDDM) (Rose et al., 1986; Ray et al., 1980). Also, an inflammatory CNS disease can follow infection with a number of common viral pathogens, such as measles and rubella. The absence of virus in the CNS and reactivity to myelin basic protein (MBP) in these patients suggest an autoimmune mechanism (Johnson et al., 1984). Environmental agents also influence the risk of developing multiple sclerosis (MS) as demonstrated by migration studies. Individuals that migrate after age 15 carry the risk for developing MS associated with their geographic origin, while individuals who migrate earlier in life acquire the risk of the geographical region to which they migrated (Kurtzke, 1985). These studies are consistent with the hypothesis that a group of pathogens that are relatively ubiquitous in a certain geographic region influence the risk of developing MS.

Recent immunological studies suggest that MBP may be one of the important target antigens in the immunopathogenesis of MS. Several studies have demonstrated that MBP-specific T cells are clonally expanded in MS patients and in an in vivo activated state (Allegretta et al., 1990; Wucherpfennig et al., 1994b; Zhang et al., 1994). Reactivity with the immunodominant MBP(84–102) peptide is found predominantly in subjects carrying HLA-DR2, a genetic marker for susceptibility to MS. The MBP(84–102) epitope can also be presented by other major histocompatibility complex (MHC) class II antigens, including HLA-DQ1 (Ota et al., 1990; Martin et al., 1990; Pette et al., 1990; Wucherpfennig et al., 1994a). In vivo, the T cell response to this peptide appears to be dominated by a few expanded clones. The mechanism(s) leading to clonal expansion of MBP-reactive T cells remains to be identified, but could involve recognition of viral peptides with sufficient structural similarity to the immunodominant MBP peptide. Viruses that cause latent and/or persistent infections (such as the herpes simplex and Epstein–Barr viruses) would be particularly interesting candidates, as they may permit chronic antigenic stimulation of these autoreactive T cell clones. The initiation of autoimmunity by such a mechanism could then lead to sensitization to other CNS self-antigens by determinant spreading (Lehmann et al., 1992; Kaufman et al., 1993; Tisch et al., 1993).

Structural characterization of the immunodominant MBP(85–99) peptide identified residues critical for MHC class II binding and for T cell receptor (TCR) recognition (Wucherpfennig et al., 1994a). The MHC class II and TCR contact residues of the immunodominant MBP(85–99) peptide were then subjected to mutational analysis in order to define the set of amino acids permitted at each critical position. These structural criteria, together with the knowledge that amino acid side chains required for binding to MHC molecules are degenerate, were used to search a protein sequence data base. Selected viral and bacterial peptides were tested for their ability to activate

human MBP(85–99)-specific T cell clones that had been established from blood T cells of MS patients. Of 129 peptides synthesized, seven viral peptides and one bacterial peptide that met the criteria were found to efficiently stimulate MBP-specific T cell clones. These viral and bacterial peptides therefore act as molecular mimics of the immunodominant MBP(85–99) peptide. Molecular mimicry of this immunodominant self-peptide by viruses therefore presents a possible mechanism for the induction of autoimmunity in MS.

Results

Structural Characterization of the Immunodominant T Cell Epitope of Human MBP

Susceptibility to MS is associated with HLA-DR2 (DRA, DRB1*1501, the most common subtype of DR2) (Spielman and Nathenson, 1982; Olerup et al., 1989). This MHC class II molecule may play a critical role in the immunopathogenesis of MS by presenting immunodominant self-peptides to autoreactive T cells. Following injection of MBP in experimental animals, T cells specific for immunodominant peptides of MBP mediate an inflammatory response in the CNS that can be accompanied by marked demyelination (reviewed by Zamvil and Steinman, 1990). In previous studies, two regions of human MBP were found to be immunodominant (residues 84–102 and 143–168) (Ota et al., 1990; Pette et al., 1990; Martin et al., 1990; Wucherpfennig et al., 1994a). Reactivity to the MBP(84–102) peptide was predominantly seen in subjects carrying HLA-DR2. Using L cell transfectants as antigen-presenting cells, HLA-DR2b (DRA, DRB1*1501) was found to serve as the restriction element for these MBP(84–102)-specific T cell clones. The MBP(84–102) peptide bound with high affinity to the HLA-DR2b molecule with two hydrophobic residues serving as the primary anchors (Val-89 and Phe-92 in the MBP(85–99) peptide) (Figure 1) (Wucherpfennig et al., 1994a; Vogt et al., 1994). At the first anchor position, Val-89 could be substituted by other aliphatic amino acids (leucine and isoleucine), as well as by methionine and phenylalanine; alanine was tolerated at this position but reduced the affinity of the peptide for HLA-DR2b. At the second anchor position, all aliphatic and aromatic residues were permitted; again alanine was tolerated but resulted in a loss of binding affinity.

A mutational analysis of putative TCR contact points demonstrated that Phe-91 and Lys-93 were the primary TCR contacts for the MBP(85–99)-specific clones; other residues such as Val-88 and His-90 were important for some clones but not for others. Substitution of Phe-91 by alanine abolished TCR recognition for all clones; some clones tolerated conservative substitutions (e.g., tyrosine or aliphatic amino acids) while other clones did not. Substitution of Lys-93 by arginine was tolerated by most T cell clones, but more drastic changes frequently resulted in a partial or complete loss of T cell reactivity. This analysis demonstrated that His-90, Phe-91, and Lys-93 were the primary TCR contact residues, while Val-89 and Phe-92 were the primary MHC contact residues (Figure 1).

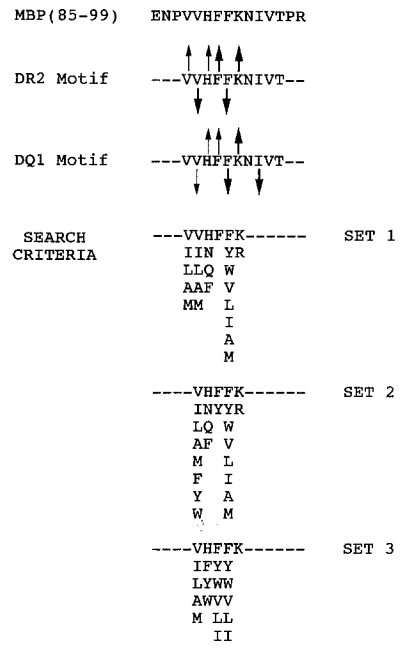

Figure 1. Peptide Motifs Required for MHC Class II Binding and TCR Recognition of the Immunodominant MBP(85–99) Peptide

The MBP(88–97) segment is critical for MHC class II binding and TCR recognition by HLA-DR2- and HLA-DQ1-restricted clones. Peptide residues His-90, Phe-91, and Lys 93 appear to be critical TCR contacts for both sets of clones; hydrophobic amino acids are important for anchoring the peptide to the MHC class II molecules. Based on the recognition motifs and a detailed mutational analysis, molecular mimicry motifs were defined for searching a protein data base for viral and bacterial sequences that fit these criteria. The different sets represent search criteria for different sets of clones with different preferences at TCR contact points (Val-88, His-90, Phe-91, and Lys-93). Hydrophobic amino acids (aliphatic and/or aromatic) were specified for the two major MHC anchor residues (Val-89 and Phe-92). The following number of peptides were synthesized for each set: set 1, 24 peptides; set 2, 46 peptides; set 3, 59 peptides. Random peptide sequences would be expected to match the motifs at the following frequencies: set 1, 1:40,000; set 2, 1:27,750; set 3, 1:3692 sequences.

Can Structural Motifs Be Used to Identify Viral Peptides That Activate MBP-Specific T Cells?

Based on this structural characterization of the immunodominant MBP(85–99) peptide, a set of structural criteria (i.e., a molecular mimicry motif) was developed to search a protein data base for viral and bacterial peptides that matched these requirements. These search criteria focused on the core region of the peptide, residues 88–93, which contained the MHC and TCR contacts common to all clones. In the first set, aliphatic amino acids were allowed at the first MHC anchor residue (Val-89); aliphatic and aromatic residues were permitted at the second MHC anchor (Phe-92). For the TCR contacts, Phe-91 was absolutely conserved, Lys-93 could only be substituted by arginine, while His-90 and Val-88 could be substituted by several structurally related amino acids (Figure 1).

The second set of criteria omitted Val-88 as a TCR contact residue (used only by some clones) and also permitted aromatic amino acids at the first MHC anchor (Val-89).

This was done since the MBP(85–99) peptide is presented by different HLA-DR2 subtypes: presentation by DRB1*1501 requires an aliphatic amino acid or phenylalanine at this position, while aliphatic and all aromatic residues at this position can serve as anchor for DRB1*1602 (K. W. W. and J. L. S., unpublished data). This difference relates to the size of the primary pocket binding this hydrophobic residue and is determined by the Val/Gly dimorphism at DRβ86 (Val in *1501 and Gly in *1602) (Busch et al., 1991; Brown et al., 1993).

The third set represented a modification of the TCR contact residues preferred by a subgroup of MBP(85–99)-specific clones. For these clones, Lys-93 was absolutely conserved while Phe-91 could be substituted by some aromatic or aliphatic amino acids (Figure 1).

These search criteria also matched well with the structural requirements for a HLA-DQ1-restricted clone specific for the MBP(85–99) peptide. This clone required the same minimal peptide segment as DR2-restricted clones (residues 87–97). As in the DR2-restricted clones, His-90, Phe-91, and Lys-93 appeared to be the primary TCR contact residues (unpublished data). Based on pool sequencing of naturally processed peptides bound to HLA-DQ1, three hydrophobic positions (at position 1, 4, and 7 relative to the first anchor; Val-89, Phe-92, and Ile 95 in the MBP(85–99) peptide) are thought to contribute to binding (K. Falk and O. Rötschke, submitted). Substitution of these hydrophobic positions by aspartic acid greatly diminished the stimulatory capacity of the peptide, while substitutions by other hydrophobic amino acids were tolerated. These data suggest that the MBP(85–99) peptide is bound in a similar fashion to HLA-DR2b and HLA-DQ1 and that the same peptide residues are critical for interaction with the TCR.

These complex criteria were used to search protein data bases (PIR and SwissProt) using the Genetics Computer Group software (program: findpatterns). More than 600 sequences of viral and bacterial origin were identified that matched these criteria. From this pool, sequences were selected based on the following criteria: one, viruses known to cause human pathology; two, viruses prevalent in the Northern Hemisphere where MS occurs most frequently; three, selected bacterial sequences associated with inflammatory CNS disease (such as Borrelia burgdorferi) and with invasive infections (such as Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa). Not included were most viruses that cause infections in tropical countries, sequences derived from vaccinia virus as well as a large number of sequences from Escherichia coli, which is part of the normal intestinal flora. When multiple antigenic variants were present, one or several sequences that best fit the search criteria were chosen. The selected peptides were synthesized by Pin-Technology on a 1 mg scale (Chiron Mimotopes, San Diego); 70 peptides were made for criteria sets 1 and 2 (Figure 1) and 59 peptides for set 3.

Activation of Human MBP(85–99)-Specific T Cell Clones by Viral Mimicry Peptides

Peptides were tested for their ability to activate human MBP(85–99)-specific T cell clones that had been previously established from blood T cells of two patients with relapsing–remitting MS (Wucherpfennig et al., 1994a, 1994b). As previously, homozygous B cell lines that expressed DR2 (DRB1*1501 or DRB1*1602) or DQ1 were used as antigen-presenting cells in these T cell proliferation experiments (Wucherpfennig et al., 1994a). Seven clones were tested with the 70 viral/bacterial peptides from sets 1 and 2. As expected, all clones were activated by the MBP(85–99) peptide that served as the positive control.

Interestingly, three of the seven clones tested were also efficiently activated by several viral/bacterial peptides (Tables 1 and 2, data for five clones are shown). The first clone (Hy.1B11) recognized the MBP(85–99) peptide on HLA-DQ1; clones Hy.2E11 and Hy.1G11 were HLA-DR2 restricted (Wucherpfennig et al., 1994a). Among the first 70 peptides tested, three mimicry peptides stimulated the DQ1-restricted clone; two mimicry peptides from Epstein–Barr virus (EBV) and influenza type A virus stimulated two of the DR2-restricted T cell clones (Table 1). Among the second group of peptides (set 3, 59 peptides), one mimicry peptide from human papillomavirus was identified for the DQ1-restricted clone, while a reovirus peptide and a herpes simplex virus peptide were identified for the DR2-restricted clones (Hy.2E11 and Hy.1G11, respectively) (Table 2; data not shown). This second group of peptides has not yet been tested with the other DR2-restricted clones.

Taken together, the DQ1-restricted T cell clone recognized five structurally related peptides: the immunodominant MBP(85–99) peptide, three viral peptides (from herpes simplex, adenovirus type 12, and human papillomavirus) and a bacterial peptide (Pseudomonas aeruginosa). Two of the DR2-restricted clones were activated by four peptides. Both clones recognized the MBP(85–99) peptide as well as mimicry peptides from EBV and influenza virus. In addition, each clone recognized a viral peptide from reovirus (T cell clone Hy.2E11) and herpes simplex virus (clone Hy.1G11) (Table 2).

Efficient T Cell Stimulation by Mimicry Peptides

If viral/bacterial mimicry peptides are indeed important for the initiation of autoimmunity, they have to be capable of potent T cell stimulation that results in marked clonal expansion of autoaggressive T cell clones. The stimulatory capacity of the mimicry peptides was therefore compared with the MBP(85–99) peptide in a titration experiment (Figure 2). The peptides were found to be efficient stimulators of these MBP-specific T cell clones; in particular, the EBV peptide (DR2-restricted clones) and the adenovirus peptide (DQ1-restricted clone) were similar to the MBP(85–99) peptide in their stimulatory capacity. These results demonstrate that T cell mimicry is not the result of a minor degree of "cross-reactivity" but rather the result of structural similarity sufficient for potent T cell activation.

The efficient stimulation of MBP(85–99)-specific T cell clones by these peptides also argues against the presence of a small contaminating T cell population that responds to these peptides. To ensure that the peptides were indeed seen by a single T cell clone, both T cell clones were

Table 1. Screening of Five MBP (85–99)-Specific T Cell Clones for Reactivity to a Panel of Viral/Bacterial Mimicry Peptides

Peptide	Sequence	Source	Hy.1B11	Ob.1A12	Ob.1C3	Hy.2E11	Hy.1G11
None			6,090	420	452	1,471	200
2	ENPVVHFFKNIVTPR	**MBP(85–99)**	**80,102**	**32,631**	**21,171**	**70,350**	**8,859**
3	GMSLIHFLKGCIISY	Human papillomavirus	4,751	821	769	897	234
4	SGFALHFFRLLPTAS	Epstein–Barr virus	3,850	412	391	772	160
5	FRQLVHFVRDFAQLL	**Herpes simplex virus**	**20,164**	426	402	981	157
6	YQTIIHFARTLNRMY	Parainfluenza virus type 2	4,689	393	443	531	178
7	WRGIVHFLRYQGQEF	Human papillomavirus	4,344	529	436	621	197
8	SCTAAHFIKRFIKDG	Rickettsia prowazekii	5,443	547	438	2,502	219
9	TQRLAHFYRRWTGAK	Yersinia pseudotuberculosis	4,902	414	442	924	143
10	IVVILFFFKIPQRLR	Cytomegalovirus	3,712	444	490	963	175
11	YVVLVQFVKHVALFS	Cytomegalovirus	3,158	441	460	815	201
12	KSLVLNFAKNEELNN	Staphylococcus aureus	3,086	407	449	919	204
13	NSNAINFLKTWAKN	Borellia burgdorferi	3,684	444	442	605	199
14	TRPAAQFVKEAKGFT	Klebsiella pneumoniae	2,912	419	454	797	221
15	YEAMAQFFRGELRAR	Herpes simplex	4,626	430	400	1,058	193
16	SPGLVQFARATDTYF	Adenovirus type 40 and 41	4,987	474	509	1,235	213
17	ACIVLFFARRAFNKK	Human cytomegalovirus	7,969	376	412	959	171
18	ILGLLNFARNFIPNF	Human spuma retrovirus	5,259	415	430	936	144
19	VVALVNFLRHLTQKP	Human cytomegalovirus	6,103	401	408	926	154
20	PVHLLNFARLDLIKQ	Varizella–Zoster virus	5,620	340	437	858	163
21	FRDLLNFIRQRLCCE	Human cytomegalovirus	2,619	426	510	967	144
22	DLRVLNFIRGTKVIP	Influenza A virus	3,517	486	451	741	191
23	TVDVANFLRAYSWSD	Marburg virus	3,660	434	427	1,045	175
24	LQKALNFVRMGDRFI	Staphylococcus aureus	4,868	354	344	977	213
25	TLLLIFFYRFMRPLI	Staphylococcus aureus	3,884	420	455	834	200
26	HELLANFLRQQGGVR	Pseudomonas aeruginosa	3,738	388	442	987	195
27	SDDFIHFFKAKSYDD	Dhori virus	3,373	431	459	879	651
28	LVDEAHFIKKEAFNT	Human cytomegalovirus	3,553	425	429	2,306	208
29	TGGVYHFVKKHVHES	**Epstein-Barr virus**	3,667	503	435	**96,613**	**8,915**
30	EACNAHFWRDLQGEA	Herpes simplex virus	3,517	494	446	1,214	224
31	IGSQVHFYRDLSSIN	Human papillomavirus	2,562	398	456	1,172	207
32	EQVLFHFARKNGVMR	Human papillomavirus	5,127	510	421	1,115	189
33	PLGRIHFFRRGFWTL	Human cytomegalovirus	6,459	231	417	973	122
34	ISIFLHFVRIPTHRH	LCMV	8,921	342	341	944	162
35	NGQYIHFYREPTDIK	LCMV	4,408	371	401	948	209
36	SGCYVHFFREPTDLK	Lassa virus	4,687	359	388	1,094	175
37	QESYAHFIRDSVGLP	Adenovirus type 7	4,307	415	399	957	245
38	SKYLYHYLRTLALGT	HTLV-I	4,404	326	468	1,049	159
39	NFEDWHYAKFGFTPL	Human corona virus	4,809	434	367	944	184
40	LAYSLNFLKVIQQIL	Measles virus	5,383	443	393	936	223
41	DFEVVTFLKDVLPEF	**Adenovirus type 12**	**57,504**	393	445	1,148	194

(continued)

recloned by limiting dilution. The subclones had the same specificity for MBP(85–99) and for the viral peptides as the parental T cell clones. Two subclones were obtained for Hy.1B11 (DQ1 restriction); these reacted with the MBP(85–99) peptide as well as with the herpesvirus, adenovirus, and Pseudomonas peptides (the papillomavirus peptide from the second synthesis was not tested on the subclones). One subclone was obtained for Hy.2E11 (DR2 restriction); this clone reacted with MBP(85–99) as well as with the EBV and the influenza peptides as did the parental clone (the reovirus peptide from the second synthesis was not tested). In addition, previous polymerase chain reaction (PCR) analysis of these T cell clones using a panel of TCR Vα and Vβ family-specific primers had indicated that they represented clonal populations (Wucherpfennig et al., 1994b). Surface staining of clone Hy.1G11 with a monoclonal antibody specific for the Vβ17.1 segment (MAb C1, Friedman et al., 1991) showed that all cells expressed the Vβ17.1 segment, proving that it represented a clonal population.

Mimicry Peptides Have Conserved Features but Are Quite Distinct from the MBP Peptide

Comparison of peptide sequences that stimulate the same TCR revealed several interesting points. First, only one peptide (from human papillomavirus L2 protein) had striking sequence similarity with the MBP(85–99) peptide, in that all amino acids in the MBP(89–95) segment except position 94 (asparagine to aspartic acid) were identical (Table 2). For all other sequences, simple alignment would not have predicted them to be efficient stimulators of MBP(85–99)-specific T cell clones. Second, at positions not specified by the search criteria, the selection for particular amino acids was apparent (Table 2). For example, for the DQ1-restricted clone, aspartic acid was selected at position 94 (probably a TCR contact) in all four mimicry peptides. This position is occupied by asparagine in the MBP peptide (similar size, but no negative charge). Substitution of Asn-94 for aspartic acid in the MBP peptide markedly increased its stimulatory capacity for the DQ1-restricted clone but reduced it for the DR2-restricted clone. Selection also oc-

Table 1. (*continued*)

Peptide	Sequence	Source	Hy.1B11	Ob.1A12	Ob.1C3	Hy.2E11	Hy.1G11
42	RTLVLAFVKTCAVLA	Adenovirus type 2	3,756	405	400	962	156
43	TLMVIPFVKLDYADT	Coxsackie A9 virus	3,411	427	451	744	188
44	VEGVATFLKNPFGAF	Human cytomegalovirus	3,137	426	458	786	148
45	VGGVVSFLKNPFGGG	Human herpes virus 6	3,710	338	469	774	193
46	DSHLICFYKRGEGLS	Human cytomegalovirus	3,936	371	416	921	203
47	VLNLLEFLKDWSGHL	Epstein–Barr virus	3,913	371	410	1,055	201
48	INTVLCFVKSGILLY	Hepatitis A virus	4,472	374	430	1,088	197
49	GFIAALFYKHGFNSS	Hepatitis C virus	4,180	397	418	1,332	168
50	GFLAALFYKHKFNAS	Hepatitis C virus	3,581	360	468	987	202
51	YRNLVWFVKKGNSYP	Influenza A virus	3,628	371	399	567	183
52	YRNLVWFIKKNTRYP	**Influenza type A virus**	3,911	368	420	**45,094**	**5,885**
53	DLRVLSFIKGTKVVP	Influenza A virus	4,315	406	414	438	214
54	SGRLIDFLKDVIESM	Influenza A virus	3,982	380	488	720	205
55	GEILIDFFKKGNLSA	Influenza C virus	4,908	355	425	884	161
56	VLALITFFKFTALAP	Japanese encephalitis virus	11,189	329	415	1,128	159
57	ILQVLSFFKGTIING	Newcastle disease virus	3,895	389	392	1,121	195
58	CAAMVRFYKRGQMRE	Human papillomavirus	3,428	365	410	587	161
59	NSNAAAFLKSNSQAK	Human papillomavirus	3,698	433	476	665	189
60	TLDILVFLKTFGGLL	Parainfluenza virus type 3	4,138	399	499	708	206
61	DLPMVTFLKDELRKK	Rhinovirus type 89	4,375	365	426	453	211
62	PKAAALFAKTYNLVP	Sindbis virus	4,156	404	443	571	200
63	DTKLAGFLKHYNSVW	Rotavirus	4,185	398	376	775	169
64	FVIVLPFIKAQNYGI	Rotavirus	4,350	399	468	1,015	212
65	YARVIDFAKFREIAD	Campylobacter jejuni	2,769	379	447	1,034	181
66	EQFKAHYFRNVTKGE	Hemophilus influenza	2,961	312	392	828	132
67	PRAAAAFVKFNCAAL	Klebsiella	5,096	355	364	631	134
68	ERFLARFVKDYGRPA	Pseudomonas aeruginosa	4,894	350	374	899	143
69	APGAILFAKAKHEVG	Pseudomonas aeruginosa	3,965	390	305	983	163
70	DRLLMLFAKDVVSRN	**Pseudomonas aeruginosa**	**32,872**	311	373	887	169
71	NQEAAGFIKHFEQLL	Staphylococcus aureus	3,830	345	413	956	202
72	EEKLLAFAKADKTYS	Streptococcus pneumoniae	4,916	325	445	1,061	191

The MBP(85–99) peptide (peptide 2) was used as a positive control; mimicry peptides 3–26 were from peptide set 1, mimicry peptide 27–72 from peptide set 2. T cell clone Hy.1B11 is HLA-DQ1 restricted, clones Ob.1A12, Ob.1C3, Hy.2E11, and Hy.1G11 are HLA-DR2 restricted. Irradiated homozygous B cell lines were used as antigen-presenting cells: 9001 (DQ1) for clone Hy.1B11; MGAR (DR2 [DRB1*1501]) for clones Ob.1A12, Ob.1C3, Hy6.2E11, and Hy.1G11. B cells were pulsed with peptides for 2 hr at a concentration of approximately 60 μg/ml (clones Hy.1B11, Ob.1A12, and Hy.1G11) and at approximately 12.5 μg/ml for clones Ob.1C3 and Hy.2E11. After the 2 hr pulse, T cells were added (50 × 10^3/well; final peptide concentrations: 12.5 μg/ml and 3.1 μg/ml, respectively). Numbers represent incorporation of [^{3}H]thymidine that was determined after a 3 day culture period. Two other T cell clones (Hy.2B6 and Ob.3D1) were also tested and responded only to the MBP(85–99) peptide (data not shown).

curred at the neighboring MHC contact (Ile-95) for which isoleucine, valine, or phenylalanine were selected (all hydrophobic). Third, different selection events occurred for the DQ1- and the DR2-restricted clones: at position 94 (selection of aspartic acid [negative charge] for DQ1 peptides), a positive charge (lysine) was selected in two of the three mimicry peptides presented by DR2. Fourth, in the flanking segments (residues 85–87 and 97–99), no apparent selection took place as amino acids with different size and charge were allowed.

The majority of the viruses are common human pathogens: influenza type A frequently causes respiratory tract infections; human papillomavirus infects epithelial tissues and has been linked to cervical carcinomas; EBV causes an acute viral syndrome (infectious mononucleosis) in young adults. Human herpesvirus I (herpes simplex), EBV, and human papillomavirus cause latent/persistent infections; the respective reservoirs are neurons (herpes simplex), B cells (EBV), and epithelial cells (papillomavirus). Virus expression can be reactivated by UV exposure and stress (herpes simplex) and by B cell activation (EBV) (Schwarz et al., 1985; Epstein and Achong, 1977; Spruance, 1985; Tovey et al., 1978). For the induction and maintenance of an autoimmune response, these persistent viral infections are of particular interest as they could explain the chronicity of the clinical disease and the clonal expansion and persistence of MBP-specific T cells. Reactivation of viral expression may also be involved in triggering clinical relapses. By this mechanism, viral mimicry peptides could activate resting MBP-specific T cells in periphery and allow them to invade the CNS (Figure 4).

Presentation of a Naturally Processed Viral Mimicry Peptide

Are these mimicry epitopes actually presented to autoreactive T cells during a viral infection? The mimicry peptide from the EBV DNA polymerase allowed this question to be addressed. In EBV-transformed B cells (which were used as antigen-presenting cells in the T cell assays), the lytic viral cycle is repressed. The DNA polymerase gene

Table 2. Sequence Alignment of Viral/Bacterial Mimicry Peptides That Stimulate MBP-Specific T Cell Clones That Are DQ1 or DR2 Restricted

	85 90 94 99
Peptides Recognized by Clone Hy.1B11 (DO1 Restricted)	
MBP(85–99)	ENPVVHFFKNIVTPR
Herpes simplex, UL15 protein	FRQLVHFVRDFAQLL
Adenovirus type 12, ORF	DFEVVTFLKDVLPEF
Pseudomonas, phosphomannomutase	DRLLMLFAKDVVSRN
Human papillomavirus type 7, L2 protein	IGGRVHFFKDISPIA
Peptides Recognized by Clone Hy.2E11 (DR2 Restricted)	
MBP(85–99)	ENPVVHFFKNIVTPR
EBV, DNA polymerase	TGGVYHFVKKHVHES
Influenza type A, hemagglutinin	YRNLVWFIKKNTRYP
Reovirus type 3, sigma 2 protein	MARAAFLFKTVGFGG
Peptides Recognized by Clone Hy.1G11 (DR2 Restricted)	
MBP(85–99)	ENPVVHFFKNIVTPR
EBV, DNA polymerase	TGGVYHFVKKHVHES
Influenza type A, hemagglutinin	YRNLVWFIKKNTRYP
Herpes simplex, DNA polymerase	GGRRLFFVKAHVRES

Only the human papillomavirus peptide has obvious sequence similarity with the MBP(85–99) peptide (residues identical in the 89–95 segment are underlined). All mimicry peptides that stimulate the DQ1-restricted clones have aspartic acid (D) at position 94 (a putative TCR contact); hydrophobic residues were selected at position 95 (a putative MHC contact). In contrast, two of three mimicry peptides for the DR2-restricted clones have a positive charge (lysine) at position 94.

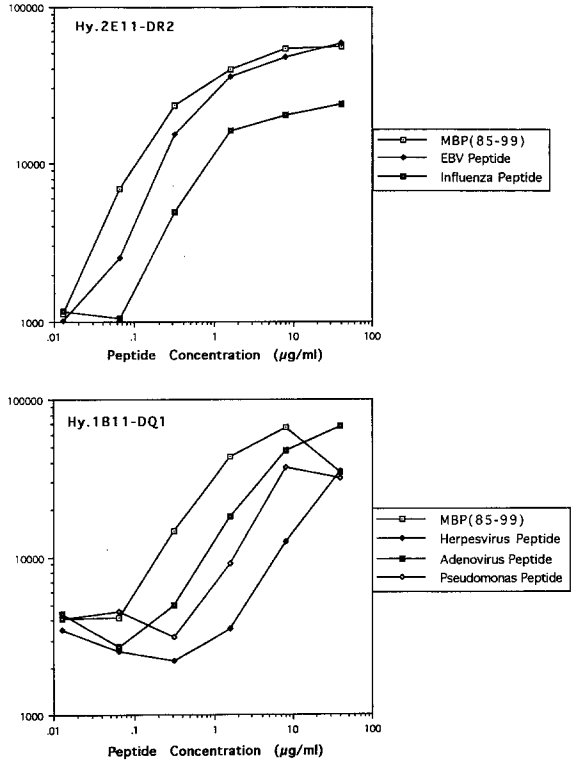

Figure 2. Efficient Stimulation of MBP(85–99)-Specific Clones by Mimicry Peptides

Peptides were compared with the MBP(85–99) peptide in a titration experiment. The top panel shows the DR2-restricted clone (Hy.2E11) with MGAR (DRB1*1501) as antigen-presenting cells, while the bottom panel shows the DQ1-restricted clone (Hy.1B11) with 9001 (DQ1) as antigen-presenting cells. B cells were pulsed with peptides for 2 hr, washed, and cocultured with T cells for 3 days. Numbers represent cpm of incorporated [^{3}H]thymidine as a measure of T cell proliferation.

is not transcribed in this latent state; however, B cell activation results in activation of the lytic cycle and in the expression of the DNA polymerase gene (Datta et al., 1980). To examine MHC class II–restricted presentation of the EBV DNA polymerase, an HLA-DR2$^+$ EBV-transformed B cell line (MGAR) and an MHC-mismatched control (9001, HLA-DR1) were pretreated for 36 hr with phorbol ester, which was removed by extensive washing prior to coculture of antigen-presenting cells with T cells. T cell clones Hy.2E11 and Hy.1G11, which recognize the EBV DNA polymerase peptide presented by HLA-DR2, were activated by a HLA-DR2$^+$ EBV-transformed B cell line pretreated with phorbol ester. This effect was specific because MHC-mismatched B cells did not activate the clones; also, a control clone (Ob.1A12) that recognized MBP(85–99) but not the EBV peptide was not activated (Figure 3). In a separate experiment, T cell activation was blocked by a MAb specific for HLA-DR (MAb L243) but not by a MAb specific for HLA-DQ (G2a.5). These results demonstrate that the MBP-specific T cell clones recognize not only the viral peptide but also antigen-presenting cells infected with the virus. In vivo, this recognition event could lead to chronic antigenic stimulation of MBP-specific T cells as B cell activation results in the reexpression of EBV genes, including the DNA polymerase gene.

Viral Variants Differ in Their Capacity to Activate MBP-Specific T Cell Clones

Two peptides from different strains of influenza type A matched the initial search criteria; only one of them was stimulatory for clone Hy.2E11. These two viral sequences were derived from the hemagglutinin subtype H13 (there are 13 serologically defined hemagglutinin subtypes of influenza type A) (Chambers et al., 1989). These hemagglutinin peptides differed at four positions: the first substitution was at position 92 (isoleucine for valine), which anchors the peptide to DR2; the other substitutions were at positions 95–97 (Table 3). Single amino acid substitutions demonstrated that the change at position 97 (arginine to serine) was responsible for the loss of reactivity (Table 3). This was surprising since threonine is present in the MBP peptide (which is structurally similar to serine) and since peptide position 97 was not subject to strong selection among the mimicry peptides (the stimulatory peptides have threonine, histidine, arginine, or phenylalanine at this position). Therefore, it is likely that this substitution affects the overall peptide conformation as described for MHC class I–bound peptides (Madden et al., 1993). These effects on the overall peptide conformation are probably responsible for the fact that the majority of viral peptides do not stimulate MBP(85–99)-specific clones.

The observation that certain viral strains are capable of stimulating MBP-specific T cells while other strains are

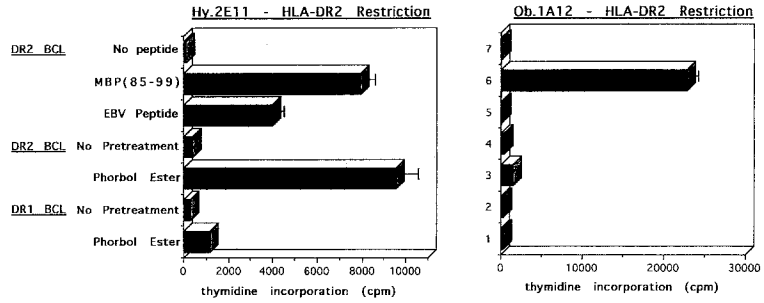

Figure 3. Recognition of the Naturally Processed EBV DNA Polymerase Peptide by MBP-Specific T Cells Following Reactivation of EBV Transcription in B Cells

The EBV lytic cycle is suppressed in EBV-transformed B cells; however, B cell activation results in reactivation of the lytic cycle and in transcription of the DNA polymerase gene. A HLA-DR2 B cell line (MGAR) and a MHC-mismatched control (9001, HLA-DR1) were pretreated with phorbol ester (PMA, 25 ng/ml for 36 hr), washed, and cocultured with MBP(85–99)-specific T cell clones. T cell clone Hy.2E11 recognized both the MBP(85–99) and the EBV DNA polymerase peptide; clone Ob.1A12 that recognized only MBP(85–99) in the context of HLA-DR2 served as a control. Clone Hy.2E11 was activated by phorbol ester–pretreated B cells that expressed HLA-DR2 but not by MHC-mismatched B cells. In a separate experiment, this effect could be blocked by a MAb specific for HLA-DR (L243) but not by a MAb specific for HLA-DQ (G2a.5). T cell clone Hy.1G11 that also recognized MBP(85–99) and the EBV DNA polymerase peptide were also activated by phorbol ester–pretreated, HLA-DR2$^+$ B cells (data not shown). T cell proliferation assays were performed as described in Table 4.

not may be important in defining the epidemiology of the disease. "Epidemic" outbreaks of MS have been noted (for example on the Faroe islands [Kurtzke and Hyllestad, 1979]) that may be related to particular viral strains that possess strongly cross-reactive T cell epitopes. These results also indicate that epidemiological studies will have to take viral subtypes and viral antigenic variation into account. Influenza viruses are among the interesting candidates as presentation of the influenza hemagglutinin (from which the mimicry peptide is derived) by MHC class II molecules is well documented (Brown et al., 1991).

MBP(85–99) and Its Viral Mimicry Peptides Are Efficiently Presented by the Disease-Associated DR2 Molecule

The presentation of the viral mimicry peptides by different DR2 subtypes was compared to determine whether they are efficiently presented by the disease-associated molecule (DRB1*1501, the most common DR2 subtype). The MBP peptide was presented by three of the four DR2 subtypes; the peptide was not presented by DRB1*1601, which differs from DRB1*1602 by a single amino acid substitution (at position DRβ67, a possible TCR contact). The two viral peptides were presented much better by the DR15 molecules (DRB1*1501 and 1502, which differ only at position DRβ86) than by DRB1*1602. This was particularly evident for the influenza peptide, which only activated the T cell clone when presented by the DRB1*1501/1502 molecules but not by DRB1*1602 (Table 4). These results indicate that the viral mimicry peptides identified here are more effectively presented by the MS-associated DR2 molecules (DRB1*1501/1502).

Discussion

This study establishes that some TCRs actually recognize not a single peptide but rather a limited repertoire of structurally related peptides derived from different antigens. This was true for three of the seven MBP(85–99)-specific T cell clones studied. One of these clones recognized five different peptides; the other clone recognized four peptides from different antigens. This recognition did not merely represent a minor degree of cross-reactivity, since these peptides efficiently activated the MBP(85–99)-specific clones. The other T cell clones were probably negative because the search criteria had focused only on the core region of the peptide (residues 88–93) that contained the most important MHC and TCR contact points common to all clones. Several clones for which no mimicry peptides were identified do, however, need a longer peptide segment than the positive clones (residues 85–97 versus residues 87–97). It is therefore likely that mimicry peptides could also be identified for these clones if all positions of the peptide were subjected to a detailed mutational analysis. As only a small fraction of the peptides that matched the criteria were synthesized, it is likely that many more peptides capable of activating MBP(85–99)-specific T cells could be identified.

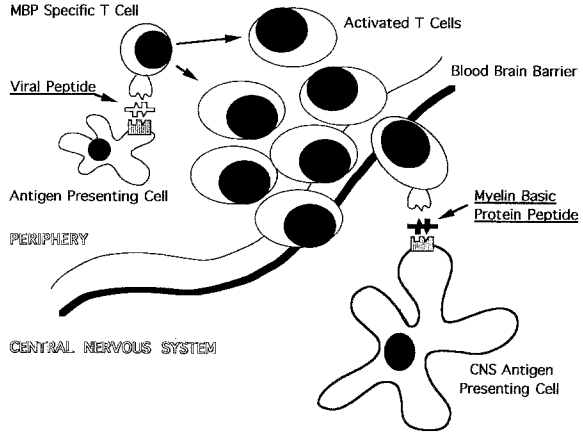

Figure 4. Proposed Mechanism for Activation of MBP-Specific T Cells in the Peripheral Immune System by Molecular Mimicry

Since only activated T cells can cross the blood–brain barrier, activation of T cells specific for CNS-specific antigens occurs in the peripheral immune system in the absence of the self-antigen. Viral peptides with sufficient structural similarity to the immunodominant MBP peptide activate these autoreactive T cells allowing them to undergo clonal expansion and CNS infiltration. Recognition of the MBP peptide in the CNS initiates the autoimmune destruction of myelin in the white matter (modified after Wucherpfennig et al., 1991).

Table 3. Antigenic Variation of Viral Epitopes Determines Capacity for T Cell Stimulation by Molecular Mimicry

MBP(85–99)	ENPVVHFFKNIVTPR
Influenza peptide 52 (Stimulatory)	YRNLVWFIKKNTRYP
Influenza peptide 51 (Nonstimulatory)	YRNLVWFVKKGNSYP
Point mutation position 92 (Ile to Val)	YRNLVWFVKKNTRYP
Point mutation position 95 (Asn to Gly)	YRNLVWFIKKGTRYP
Point mutation position 96 (Thr to Asn)	YRNLVWFIKKNNRYP
Point mutation position 97 (Arg to Ser)	YRNLVWFIKKNTSYP
	Hy.2E11
No Peptide	1,025
MBP(85–99)	**40,532**
Influenza peptide 52	**20,116**
Influenza peptide 51	805
Influenza peptide 52 (92 Val)	**16,070**
Influenza peptide 52 (95 Gly)	**13,998**
Influenza peptide 52 (96 Asn)	**14,604**
Influenza peptide 52 (97 Ser)	1,299

Two influenza type A hemagglutinin peptide sequences obtained from different viral isolates matched the search criteria; one of these sequences stimulated the DR2-restricted clone, the other peptide did not. Using single amino acid substitutions of the four positions different between these two peptides, the defective stimulation of peptide 51 was mapped to position 97 (Arg to Ser). These results indicate that antigenic variation of viruses can have a profound influence on molecular T cell mimicry. Numbers represent cpm of incorporated [^{3}H]thymidine as a measure ot T cell proliferation (mean of triplicates).

Table 4. Presentation of Viral Peptides by Subtypes of the Disease-Associated HLA-DR2 Molecule

Peptide	DRB*1501	DRB*1502	DRB*1601	DRB*1602
No peptide	310	1,064	360	423
MBP(85–99)	11,487	10,189	601	14,557
EBV peptide	12,005	11,277	521	3,389
Influenza peptide	6,266	10,079	456	412

B cell lines homozygous for different DR2 subtypes (DRB1*1501, 1502, 1601, and 1602) were compared for their ability to present viral mimicry peptides. B cell lines were pulsed with peptides at a concentration of 60 μg/ml for 2 hr, followed by washing to remove free peptide. Irradiated B cells (25×10^3) were cocultured with 50×10^3 T cells for 3 days. T cell proliferation was quantitated by [^{3}H]thymidine incorporation. Numbers represent cpm of incorporated [^{3}H]thymidine as a measure of T cell proliferation (mean of triplicates).

It was, however, surprising that the majority of peptides did not result in T cell activation. Most likely, amino acid substitutions at single positions have only limited predictive value as the simultaneous substitution of multiple neighboring positions may profoundly affect the peptide conformation. This has been elegantly demonstrated for MHC class I–bound peptides by comparing the crystal structures of HLA-A2 complexed with five different peptides (Madden et al., 1993). The effect of multiple simultaneous substitutions on peptide conformation makes peptides structurally more unique than appreciated by simple sequence comparison. The power and the novelty of the approach chosen in the present study is, however, demonstrated by the fact that the stimulatory mimicry peptides identified only have limited primary sequence similarity and would not have been predicted (with the exception of the papilloma virus peptide) based on simple alignments between MBP(85–99) and viral antigens. A better understanding of the structural consequences of multiple simultaneous substitutions on peptide conformation will certainly enhance the predictive power of this approach.

How specific is T cell recognition of MHC–peptide complexes? The molecular mimicry hypothesis postulates that there is significant cross-reactivity between viral T cell epitopes and human self-peptides. At first sight, TCR recognition appears to be exquisitely specific, since even minor substitutions in a T cell epitope can diminish or abrogate T cell activation (Reay et al., 1994). Several lines of evidence do, however, indicate that there is a significant degree of cross-reactivity. In shaping the TCR repertoire, two conflicting requirements have to be met. The first is comprehensive coverage of pathogen-derived epitopes by production of a large panel of specificities that can deal with the rapidly changing antigenic composition of pathogens. The second is elimination of TCRs that react with self-antigens that could initiate autoimmune responses. However, tolerance achieved by clonal deletion cannot be complete due to the complexity of self-antigens expressed in some organs, in particular the brain. The T cell receptor repertoire generated represents a compromise between these conflicting needs and allows a certain degree of self-reactivity in return for reasonably comprehensive coverage of foreign antigens.

Cross-reactivity is part of the positive selection process in the thymus that selects T cells with low affinity for self-MHC molecules (plus peptide) and deletes T cells with higher affinity for their ligand (Kappler et al., 1987; Kisielow et al., 1988; Sebzda et al., 1994). The relatively high frequency of alloreactive T cells also indicates that T cell recognition is not absolutely specific. Cytotoxic T cells specific for an EBV peptide bound to HLA-B8 were found to cross-react with HLA-B44 (Burrows et al., 1994). This alloreactivity is probably the result of molecular mimicry between the HLA-B8-bound EBV peptide and a HLA-B44-bound self-peptide that remains to be identified.

Molecular mimicry may also be an important mechanism in experimental autoimmune diseases. Immunization of Lewis rats with complete Freund's adjuvant induces a strong T cell response to a mycobacterial heat shock protein 65 (hsp65) peptide. Hsp65-reactive T cell clones mediate arthritis by cross-reacting with a joint proteoglycan (van Eden et al., 1985, 1988). While the cross-reacting joint peptide remains to be biochemically defined, molecular mimicry between hsp65 and a joint proteoglycan appears to be the underlying pathogenetic mechanism. Significant sequence similarity had also been noted between a T cell epitope of rabbit MBP and hepatitis B virus. Administration of this hepatitis B virus peptide was found to induce histological signs of CNS inflammation and T cell reactivity to MBP in a polyclonal population (Fujinami and Oldstone, 1985).

The diverse nature of the molecular mimicry peptides and the ubiquitous presence of some of these pathogens may make it difficult to establish a direct epidemiological

link between these viral infections and the occurrence of MS. In addition, the temporal relationship between a viral infection and the initiation of MS is not clear in most cases, as the clinical diagnosis is frequently made at a time when magnetic resonance scans demonstrate a relatively large number of old lesions (Stadt et al., 1990). It may, however, be possible to directly demonstrate the causal relationship in patients with postinfectious encephalomyelitis by establishing both virus-specific and MBP-specific T cell clones. The importance of molecular mimicry could also be demonstrated in MS patients with a recent viral infection (high IgM antibody titers to a particular virus) and magnetic resonance scans indicative of a recent onset of disease.

The diverse nature of the viral peptides that stimulate MBP(85–99)-specific T cell clones makes it unlikely that a single virus is responsible for initiating autoimmunity in MS. The observation that several pathogens carry mimicry epitopes of MBP(85–99) could explain why it has been so difficult to link the immunopathogenesis of MS to a single viral agent. Rather, it appears that a group of common viral pathogens, in particular the herpes virus family (EBV, herpes simplex, and cytomegalovirus), influenza viruses, and papillomaviruses could be involved in initiating the autoimmune process. This notion is supported by clinical and epidemiological data that suggest the involvement of several pathogens; the observation that oligoclonal immunoglobulins in the cerebrospinal fluid of MS patients are specific for several different viruses also points in this direction (Kurtzke, 1985; Baig et al., 1989). Childhood immunization against viral pathogens that carry mimicry T cell epitopes may reduce the risk of developing MS later in life. Genetic modifications of viral vaccines that eliminate proven mimicry epitopes could make viral vaccines safer and reduce the frequency of postvaccinal encephalomyelitis.

Experimental Procedures

Cell Lines

Homozygous EBV-transformed B cell lines used were the following: MGAR (DRB1*1501), 9011 (DRB1*1502), 9009 (DRB1*1601), 9016 (DRB1*1602), and 9001 (DQ1 [DQA1*0101, DQB1*0501]).

Peptide Synthesis

Mimicry peptides were synthesized on a 1 mg scale using the Multipin Peptide Synthesis System (Chiron Mimotopes). Peptides were synthesized on pins with a cyclic dipeptide (diketopiperazine, DKP) group attached to the C-terminus of the peptide that allowed cleavage in aqueous solution at a neutral or slightly basic pH. Peptide synthesis was monitored by including a standard peptide sequence as a control, which was subjected to HPLC and mass spectroscopy analysis. The immunodominant MBP(85–99) peptide was also included as a positive control for the T cell experiments.

Pin peptides were lyophilized and resuspended at a concentration of 2 mg/ml in 40% acetonitrile, 100 mM HEPES (pH 7.4). These conditions allowed the majority of peptides to be completely solubilized. Preliminary experiments had indicated that adding acetonitrile to a final concentration of 2% or less had no detrimental effect on the degree of T cell stimulation observed in the proliferation assay.

Viral and bacterial peptides that stimulated MBP(85–99)-specific clones were resynthesized by conventional methods (free C-terminus instead of the DKP group); the identity of peptides was further confirmed by mass spectroscopy (using a API III Sciex Ionspray Spectrometer) and by amino acid analysis.

Cloning of MBP(84–102)-Specific T Cells

Previously established MBP-specific T cell clones specific for the immunodominant MBP(85–99) peptide were used in this study (Wucherpfennig et al., 1994a, 1994b). These clones had been generated from blood mononuclear cells of two patients with relapsing–remitting MS carrying HLA-DR2 (DR2 subtypes: DRB1*1501 for patient Ob, DRB1*1602 for patient Hy). In both patients, the T cell response was focused on the immunodominant MBP(84–102) peptide. T cell lines specific for MBP had been generated from blood mononuclear cells by stimulation with MBP (100 μg/ml) in RPMI 1640 supplemented with 10% human serum, 2 mM L-glutamine, 10 mM HEPES, 100 U/100 μg/ml penicillin/streptomycin in 96-well plates at 2×10^5 cells/well (Ofa et al., 1990). On day 3, IL-2 was added to 5% (Human T-Stim, Becton Dickinson, Bedford, MA). On day 14, an aliquot of each cell line was assayed for reactivity to human MBP followed by proliferation assays with a panel of 13 synthetic peptides encompassing the human MBP sequence. Following a third round of stimulation (generally two stimulations with MBP and one stimulation with peptide), T cell lines were cloned by limiting dilution using allogeneic feeder cells and phytohemagglutinin (PHA) (1 μg/ml) (Murex Diagnostics, Dartford, England) for stimulation. Allogeneic MNC were irradiated with 5000 rad and cocultured in 96-well plates (10^5 cells/well) with T cells. IL-2 was added on day 3, and cells were fed every 3–4 days with media containing 5% IL-2-containing supernatant. On day 12–14, growth-positive wells were expanded by restimulation with PHA, IL-2, and allogeneic feeder cells.

Recloning of MBP-specific clones was also done by limiting dilution using allogeneic feeder cells, PHA, and recombinant human IL-2 (Boehringer Mannheim). Clones were maintained by weekly stimulation with irradiated allogeneic feeder cells, PHA, and rIL-2. Alternatively, clones were expanded by stimulation with MBP(85–99) peptide-pulsed B cells and rIL-2.

T Cell Proliferation Assays

T cell proliferation assays were done using EBV-transformed homozygous B cell lines as antigen-presenting cells. B cells were irradiated (5000 rad) and pulsed with peptide for 2 hr prior to addition of T cells. For screening of pin-peptides, 25×10^3 B cells were added per well of a 96-well microtiter plate in a 50 μl volume (in triplicates). Peptide was added to a concentration of approximately 60 μg/ml. Following a 2 hr incubation at 37°C/5% CO_2, 50×10^3 T cells were added to a total culture volume of 200 μl/well (final peptide concentration: approximately 12.5 μg/ml). After 3 days, T cell proliferation was determined with a [^{3}H]thymidine pulse (1 μCi/well) and liquid scintillation counting.

For MHC restriction experiments, irradiated B cells were pulsed with peptide for 2 hr at 37°C, followed by extensive washing. Peptide-pulsed B cells (25×10^3) were cocultured with 50×10^3 T cells for 3 days; T cell proliferation was determined with a [^{3}H]thymidine pulse (1 μCi/well) and liquid scintillation counting.

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