A Myelin Basic Protein Peptide Is Recognized by Cytotoxic T Cells in the Context of Four HLA-DR Types Associated with Multiple Sclerosis

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Summary

We have examined previously the peptide specificity of the T cell response to myelin basic protein (MBP) in patients with multiple sclerosis (MS) and healthy controls, and demonstrated that an epitope spanning amino acids 87–106 was frequently recognized. Because this region is encephalitogenic in some experimental animals, it has been postulated that the response to the epitope may have relevance to MS. In this study, the fine specificity of this response is studied using four well-characterized, monospecific T cell lines from three MS patients and an identical twin of a patient. Each of the lines recognized a peptide with the same core sequence, amino acids 89–99, although the responses were affected to various degrees by truncations at the COOH- or NH_2 terminal ends of the 87–106 epitope. Importantly, the epitope was recognized in conjunction with four different HLA-DR molecules. Also, the T cell receptor β chain usage was heterogeneous, and each line expressed a different VDJ sequence. The four HLA-DR molecules restricting the response to this epitope have been shown to be overrepresented in MS populations in various geographic areas, suggesting that the response to this region of the MBP molecule may be relevant to the pathogenesis of MS. These findings may have important implications in designing therapeutic strategies for the disease.

Although the cause of multiple sclerosis (MS)[1] is not known, a T cell–mediated autoimmune process has been postulated. Myelin basic protein (MBP) is a potential target antigen because it induces experimental allergic encephalomyelitis (EAE) in susceptible animals. Encephalitogenic epitopes of MBP differ among susceptible strains and correlate with the MHC class II genotype (1). The TCRs expressed by encephalitogenic T cells from PL/J and B10.PL mice and Lewis rats use the same TCR Vβ chain, Vβ8.2, and have similarities in their VDJ regions, as reviewed in reference 2. Thus, the pathogenesis of EAE is related to the capacity of T cells with the appropriate TCRs to recognize epitopes of MBP presented in conjunction with class II MHC molecules. These requirements have provided rationales for therapeutic strategies that prevent or treat EAE (3–7).

The analysis of the T cell response to MBP in patients with MS and in healthy controls has shown that several regions of the molecule are frequently recognized (8–10). One region, an epitope spanning amino acids (aa) 87–106, was recognized by >50% of T cell lines (TCL) derived from both MS patients and controls (9). Our findings indicated that several HLA-DR molecules could serve as restriction elements for MBP or fragments of the molecule. In contrast, other investigators have reported that an epitope spanning aa 84–102 is predominantly recognized by TCL from MS patients and largely restricted by HLA-DR2 (11). To examine the fine specificity of the T cell response to the 87–106 region of MBP, we have used TCL that were generated by repeated stimulation with MBP until they were specific for a single region

[1] *Abbreviations used in this paper:* aa, amino acid; EAE, experimental allergic encephalomyelitis; HTC, homozygous typing cells; ICAM-1, intracellular adhesion molecule 1; MBP, myelin basic protein; MS, multiple sclerosis; TCL, T cell line.

of the molecule. Four lines specific for peptide 87–106 were identified and selected to examine the HLA restriction, the peptide specificity, and TCR β chain usage.

Materials and Methods

T Cell Lines and Cytotoxic Assays. PBL from three patients with chronic progressive MS: MS1 (DR2,4; DQ1,3), MS18 (DR15,-; DQw6,-; Dw2), and MS20 (DR4,13; DQw6,w7), and the healthy identical twin of MS20 (C8) were isolated from a leukapheresis. MBP-specific long-term TCL were established by weekly stimulation with MBP (prepared as described; 10). Each line was continuously kept in culture between 8 and 16 mo. Cytotoxic activity was tested in ^{51}Cr release assays. Briefly, 5×10^5 cells of HLA-typed EBV-transformed B cell lines, homozygous typing cells (HTC; 10th International Histocompatibility Workshop), or fibroblast transfectants (see below) were incubated with 200 μg/ml MBP or 100 μg/ml peptides (solid phase method; the aa sequence of all peptides is based on the porcine MBP; reference 1) for 1 h before 200 μCi ^{51}Cr-sodiumchromate (New England Nuclear, Boston, MA) was added for another 90 min. 100 μl of target cells (5×10^4/ml) was seeded in triplicates into 96-well microtiter plates (Costar, Cambridge, MA) prefilled with 100 μl of effector cells (TCL) according to desired E/T ratios. Supernatants were harvested after 4 h (transfectants, 5–6 h), and percent specific lysis was calculated as described (9). The HLA class II types of HTC used as targets for the experiments shown in Fig. 1 and 2 are: 9033 (DR4; DQw8; Dw4; DP4); 9028 (DR4; DQw8; Dw14; DP4); 9053 (DR13; DQw6; Dw19; DP4), and 9081 (DR15, DQw6; Dw2; DP4). Antibody blocking, proliferative assays, and phenotyping were performed as described (9).

Transfectants. Mouse L cells transfected with full-length cDNA clones for DRα and DR2 Dw2a or DR2 Dw2b will be described elsewhere (Jaraquemada et al., manuscript in preparation). 5B.6 and 3B.4 are clones of DAP.3 cells expressing DR2 Dw2a and DR2 Dw2b. The vector used for these transfections contained the *Escherichia coli* gpt gene, and cells were grown in medium supplemented with 6 μg/ml mycophenolic acid (Gibco Laboratories, Grand Islands, NY), 250 μg/ml xanthine (Calbiochem-Behring Corp., La Jolla, CA), and 15 μg/ml hypoxanthine (Sigma Chemical Co., St. Louis, MO). 3B.4 and 5B.6 were retransfected with the gene for intracellular adhesion molecule 1 (ICAM-1) inserted in the vector CDM8 under the control of a cytomegalovirus promoter (12) together with the plasmid pSV2-neo for selection in 0.5 mg/ml G418 (active ingredient; Gibco Laboratories). Human M1 fibroblasts were transfected with the full-length cDNA clone for DRα inserted into the RSV.5 (neo) vector (Long, E. O., manuscript in preparation) together with a full-length cDNA for the DRB1 0401 (Dw4) gene inserted into the RSV.3 vector as described (13). Transfected cells were selected in 0.5 mg/ml G418 (active ingredient), and cells expressing HLA-DR were isolated by cell sorting.

Analysis of TCR β Chain Usage. Total cellular RNA was prepared from 10^7 T line cells after stimulation with MBP and 6 d in IL-2-containing medium. TCR β chain genes were amplified using the PCR and directly sequenced (Howell, M.D., manuscript in preparation). Briefly, RNAs were reverse transcribed and cDNAs amplified using a human TCR Cβ primer (5′-GCG GCT GCT CAG GCA GTA-3′) and a consensus Vβ primer (5′-T G/T T A/C/T C/T T GGT A C/T A/C A/G A/T C A-3′), which was designed to amplify β chain rearrangements containing virtually any of the known human TCR β chain genes. Amplification with these primers produces an ~500-bp double-stranded product, which contains one-half of the V region gene and extends through the VDJ junction approximately one-third of the way into the C region. This provides enough information to identify the Vβ genes present in the rearrangements isolated. Amplified DNAs were gel purified, base denatured, and directly sequenced with T7 polymerase (Sequenase; United States Biochemicals Corp., Cleveland, OH) using a Cβ sequencing primer (5′-CGA CCT CGG GTG GGA ACA-3′). In each of the samples, a predominant rearrangement was present and sequenceable using this strategy. The MS20 sample was also cloned into plasmid and six independent isolates sequenced, each of which had a sequence identical to that obtained by directly sequencing the PCR product.

Results and Discussion

The fine specificity of the four lines was characterized using truncated peptides within the 87-106 sequence (Fig. 1). Peptides representing either the nine NH_2-terminal or 10 COOH-terminal aa of this region were not recognized. Aa 90 and 96 at the NH_2- and COOH-terminal end, respectively, are required for recognition. However, peptide 90–96 is not recognized by the lines indicating that this core sequence needs extension in either the NH_2-terminal or the COOH-terminal direction. Peptide 91–102, which lacks the phenylalanine in position 90 and is extended by the tripoline sequence at the COOH terminus, is only recognized in the context of DR15 Dw2 by TCL MS18, indicating either minor differences in peptide binding to this HLA-DR molecule or influences on TCR recognition of the peptide/MHC complex, or both. Similarly, for TCL C8, the NH_2-terminal histidin at position 89 is required for recognition. Support for the potential importance of the 87–106 region comes from the examination of the amphipathicity of the MBP molecule, which has been analyzed in blocks of 11 aa representing three helical turns (13a). In the region of aa 87–106, the highest amphipathic indices (2.52–3.38) were obtained at midpoint aa at positions 88–91. An earlier study testing PBL for primary lymphoproliferative responses to human MBP and a number of MBP peptides obtained positive responses to MBP peptide 83–96 with PBL from MS patients, but not from controls (14). In addition, the peptide 87–106 includes regions encephalitogenic in experimental animals. The epitope 91–101 represents one minimal sequence encephalitogenic in SJ/L mice (1, 2). The region 87–99 of human MBP (aa 88–100 according to the nomenclature in this study) has also been found to be encephalitogenic in the Lewis rat (15). The encephalitogenic epitope in SJ/L mice and the region of human MBP encephalitogenic in the Lewis rat are all included in the peptide 87–106. Minor differences in the location of the core sequence may be due to differences in the MHC class II elements in various species.

We next examined the restriction elements used by the four TCL. All of the MBP-specific TCL were restricted by HLA-DR based on antibody blocking (data not shown). Detailed examination of restriction using HTC showed that each of the lines was restricted by a different DR molecule (Fig. 2). The restriction of MS1 and MS18 was confirmed using fibroblasts cotransfected with cDNAs for DRα and DRβ chains

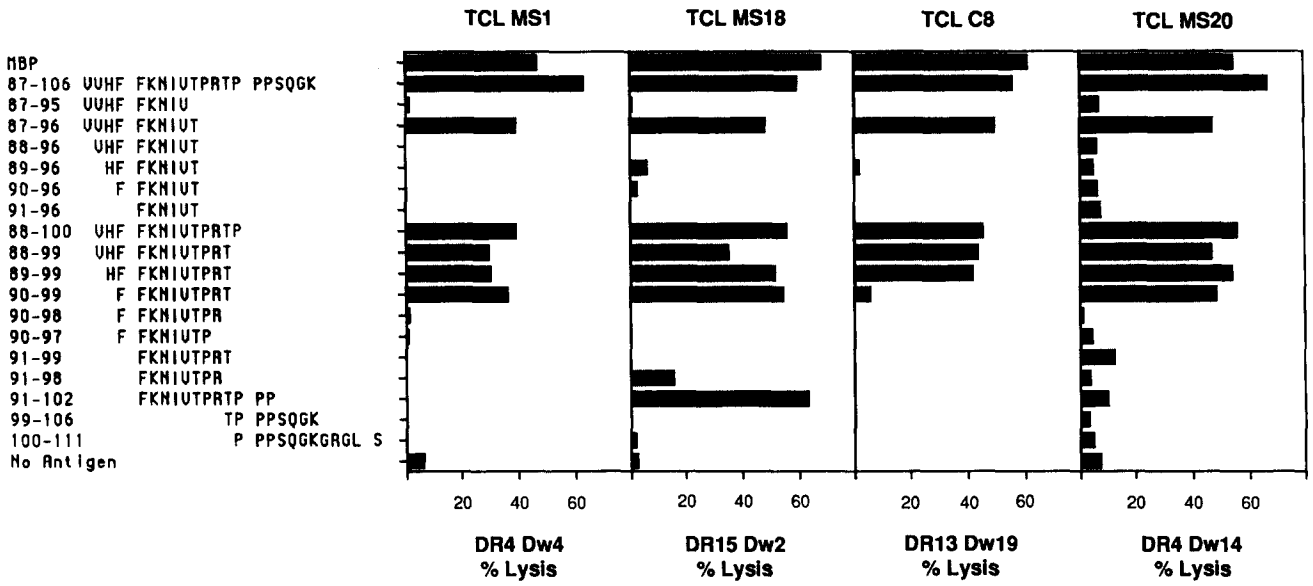

Figure 1. Fine specificity of MBP-specific cytotoxic TCL MS1, MS18, MS20, and C8. Targets pulsed with MBP peptide 87–106 are lysed by each TCL. Patients 87–96 and 89–99 represent the minimal sequences recognized. Minor differences exist for the recognition pattern of MS18 and C8 and are discussed in the text.

derived from the DR4 Dw4 (M1 DR4) and the DR15 Dw2 (DR2a; transfectant 5B.6 ICAM1) haplotypes, respectively (Fig. 3). The inability of TCL MS20 to lyse L cells transfected with DRα and DRβ from the DR4 Dw14 (L300.7) haplotype (kindly provided by Dr. R. Karr, University of Iowa, Iowa City) is probably due to the absence of human adhesion molecules on murine L cells. MS20 did proliferate when peptide 87–106 was presented by L300.7 (data not shown). L cells transfected with α and β chains from the DR13 Dw19 haplotype were not available, however, the restriction of TCL C8 by HLA-DR had been established earlier by antibody blocking (9). Similar observations have been made with a malaria circumsporozoite antigen that can be presented to T cells in the context of multiple HLA class II antigens (16). The present report is the first known description of such a permissive restriction for a human autoantigen.

The restriction elements used by the TCL derived from MS patients, DR15 (a split of DR2) and DR4, have been shown to be overrepresented in caucasian and Arab populations of MS patients (17). DR13 (a split of DR6), the restriction element of TCL C8, is associated with MS in Japanese and Mexican MS patients, populations that show a low incidence of MS (18, 19).

TCL MS20 and C8 are derived from a pair of monozygotic HLA-DR4, DR13-positive twins discordant for MS. Both lines recognize the 87–106 peptide, but use different restriction molecules, demonstrating that the peptide can be presented by both DR molecules. Since the expression of the two DR molecules should be similar in both twins, it is likely that the difference in the dominant reactivity is due to TCR usage. To address this question, the TCR β chains used by the four lines were studied by PCR amplification and direct

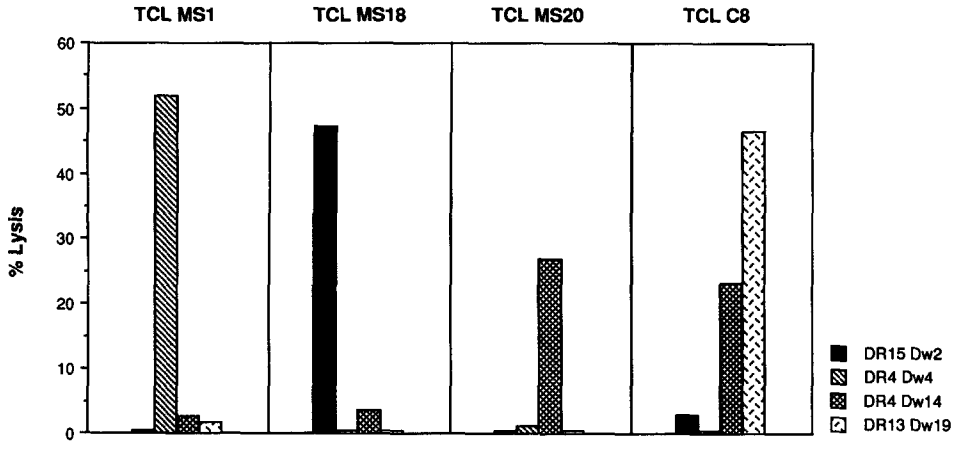

Figure 2. HLA restriction of TCL MS1, MS18, MS20, and C8 using HTC pulsed with MBP peptide 87–106. MS1 is restricted by DR4 Dw4, MS18 by DR15 Dw2, MS20 by DR4 Dw14, and C8 by DR13 Dw19. TCL C8, to a lesser extent, also recognizes the peptide in association with DR4 Dw14, the restriction element used by TCL MS20 derived from the affected twin.

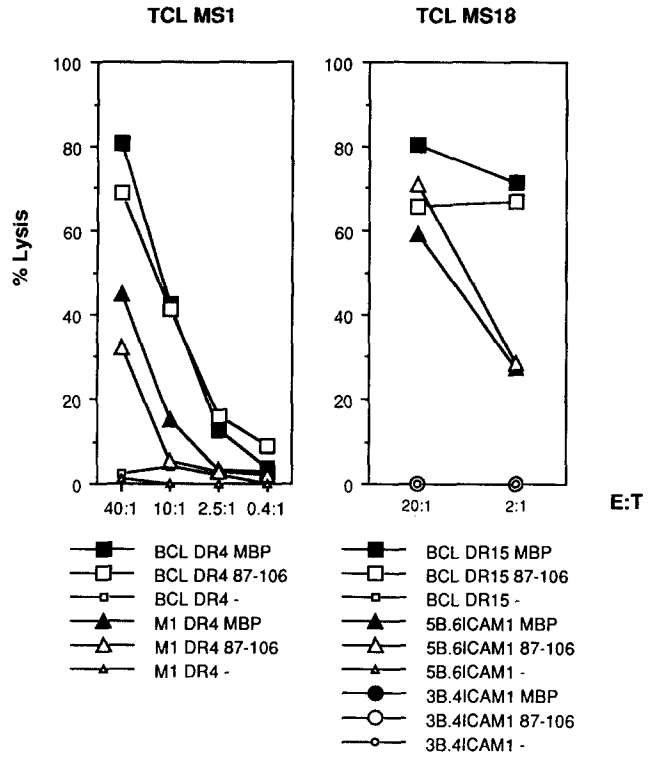

Figure 3. TCL MS1 recognizes M1 fibroblasts transfected with DRα and DRB1 derived from the DR4 Dw4 haplotype (M1 DR4) or DR4 Dw4-positive HTC pulsed with MBP or peptide 87–106. Untransfected M1 cells or M1 cells transfected with other DR antigens were not lysed (data not shown). TCL MS18 recognizes MBP or peptide 87–106 when presented on HTC in association with DR15 Dw2. L cell transfectants expressing ICAM-1 and DR15 Dw2a (5B.6 ICAM-1) and pulsed with MBP or peptide 87–106 are also lysed, but not those transfectants expressing DR15 Dw2b (3B.4 ICAM-1).

sequencing (Fig. 4). Three different Vβ families were used with the affected and the unaffected twins, both expressing Vβ8. Each line used a distinct Jβ gene. The overall sequence of the VDJ regions differed among the four lines. This is in contrast with a recent report documenting a restricted usage of Vβ17 in the response to a similar MBP peptide (84–102) in MS patients (20). It is also in contrast with the common usage of Vβ8.2 found in encephalitogenic T cells from PL/J and B10.PL mice and the Lewis rat (reviewed in reference 2). The different Vβ usage (Vβ17) in SJ/L mice (2) indicates that TCR α chain usage or factors other than TCR are important for susceptibility. Support for this notion comes from a recent report demonstrating a limited heterogeneity of rearranged TCR Vα transcripts in the brains of MS patients (21). In addition, although a similar VDJ motif is found in B10.PL mice and the Lewis rat (2, 6), differences have been reported in T cells from PL/J mice with the same MBP specificity (2). By analogy, a considerable heterogeneity in TCR usage in the outbred human population is not surprising. The differences in the Vβ usage in the human lines that recognize the same peptide are consistent with the hypothesis that parts of the TCR V region presumably bind to the third hypervariable region (HVR) of the DRβ chain (22). Each of the TCL uses a different restriction element and each of the DRβ chains shows differences in the third HVR (23). These findings suggest that individual responses to this region of the MBP molecule will show differences with respect to HLA class II restriction, peptide fine specificities, and TCR usage.

It is postulated that recognition of an immunogenic region of MBP (aa 87–106), which is encephalitogenic in some experimental animals, may be related to the pathogenesis of MS. Support for this hypothesis comes from the observation that this peptide can be presented by multiple HLA-DR molecules each previously associated with MS. Since the peptide is recognized by T cells from healthy individuals, it is likely that the cellular response to MBP and to the 87–106 peptide specifically is not sufficient for disease. The fact that only one twin in this study is affected indicates that either somatic events such as the generation of individual TCR or environmental factors are responsible for the expression of disease. In addition to the possible relationship between TCR usage and disease other genetic or environmental factors may affect T cell entry into the central nervous system or HLA class II expression on brain cells. Crossreactive epitopes between infectious agents and autoantigens also need to be considered. Although these findings require confirmation in larger numbers of patients, they may have therapeutic implications. Treatment of experimental animals with antibody to the V regions of the TCR (7) or immunization with peptide homologous to V or J regions of the TCR (5, 6) have been shown to modify EAE. Our results are not consistent with

	Vß	Dß	Jß	
MS1	Y L C A S tat ctc tgt gcc agc	R P G P R agg ccg gga ccg aga	D T Q Y gat acg cag tat	Vß6.6-Jß2.3
MS18	Y L C A S S tat ctc tgt gcc agc agc	L R G A L ttg agg ggg gcg cta	N I Q Y aac att cag tat	Vß5.2-Jß2.4
MS20	Y F C A S S tac ttc tgt gcc agc agc	R K D S P S cgt aaa gac agt ccc agt	S P L H tca ccc ctc cac	Vß8-Jß1.6
C8	Y F C A S tac ttc tgt gcc agc	T W T N acc tgg acg aat	N E K L aat gaa aaa ctg	Vß8-Jß1.4

Figure 4. Nucleotide sequences and predicted aa sequences of TCR β chain genes from MBP-specific TCL MS1, MS18, MS20, and C8.

a recent report of common Vβ usage in MBP-specific TCL from MS patients (20), and suggest that therapies targeting TCR regions may be more complicated than anticipated from experimental systems. Encephalitogenic peptides, mutated so as to allow binding to MHC, but to eliminate T cell recognition, also have been used to block induction of EAE (3, 4). Although the 87–106 peptide could be used in a similar approach, the blockade of DR molecules, especially with peptides capable of binding to multiple HLA-DR molecules, has a potential for marked immuno-suppression. Nevertheless, clinical trials using the above therapies may be important in substantiating the role of MBP in the pathogenesis of MS.

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