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

By Roland Martin,* Mark D. Howell,† Dolores Jaraquemada,§ Marjorie Flerlage,* John Richert,¶ Steven Brostoff,† Eric O. Long,§ Dale E. McFarlin,* and Henry F. McFarland*

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 NH2 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) 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.

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.

19 The Journal of Experimental Medicine • Volume 173 • January 1991 19-24
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 by cell sorting. Amplified DNAs were gel purified, base denatured, and directly sequenced with T7 polymerase (Sequenase; United States Biochemicals Corp., Cleveland, OH) using a CB (C.CG) 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 specificity of the four lines was characterized using truncated peptides within the 87-106 sequence (Fig. 1). Peptides representing either the nine NH2-terminal or 10 COOH-terminal aa of this region were not recognized. Aa 90 and 96 at the NH2- 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 NH2-terminal or the COOH-terminal direction. Peptide 91–102, which lacks the phenylalanine in position 90 and is extended by the triptoline 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 NH2-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 91–101 which 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.

![Figure 1](image1.png)

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 2](image2.png)
Figure 3. TCL MS1 recognizes M1 fibroblasts transfected with DRα and DRβ1 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).

Figure 4. Nucleotide sequences and predicted a sequences of TCR \( \beta \) chain genes from MBP-specific TCL MS1, MS18, MS20, and C8.

Multiple Sclerosis Cell Lines Recognize Myelin Basic Protein Peptide
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.

We thank Dr. W. E. Biddison, Neuroimmunology Branch, NIH, for comments; Dr. R. Karr, University of Iowa, Iowa City, for providing the transfectant L300.7; Dr. W. L. Maloy, Biological Resources Branch, NIAID, NIH, for MBP peptides; Dr. J. A. Berzofsky, Metabolism Branch, NCI, NIH, for analyzing human MBP for amphipathic sites; Dr. J. Whitaker, University of Birmingham at Alabama for cathepsin D–derived peptides; Laura Quigley and Roger Stone for preparing human MBP; and Jocely Diveley and Katherine Lundeen for technical assistance with TCR sequence analysis.

Roland Martin is a research fellow of the Deutsche Forschungsgemeinschaft.

Address correspondence to R. Martin, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, Building 10, Room 5B-16, National Institutes of Health, Bethesda, MD 20892.

Received for publication 11 September 1990.

References

1. Fritz, R., and D.E. McFarlin. 1989. Encephalitogenic epitopes of myelin basic protein. Chem. Immunol. 46:101.
2. Zamvil, S.S., and L. Steinman. 1990. The T lymphocyte in experimental allergic encephalomyelitis. Annu. Rev. Immunol. 8:579.
3. Wraith, D.C., D.E. Smilke, D.J. Mitchell, L. Steinman, and H.O. McDevitt. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. Cell. 59:247.
4. Urban, J.L., S.J. Horvath, and L. Hood. 1989. Autoimmune T cells: immune recognition of normal and variant peptide epitopes and peptide-based therapy. Cell. 59:257.
5. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T cell receptor V-region peptide protects against autoimmune encephalomyelitis. Nature (Lond.). 341:541.
6. Howell, M.D., S.T. Winters, T. Olee, H.C. Powell, D.J. Carlo, and S.W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. Science (Wash. DC). 246:668.
7. Zaller, D.M., G. Osman, O. Kanagawa, and L. Hood. 1990. Prevention and treatment of murine experimental allergic encephalomyelitis with T cell receptor Vβ-specific antibodies. J. Exp. Med. 171:1943.
8. Chou, Y.K., M. Vainiene, R. Whitham, D. Bourdette, C.H.-J. Chou, G. Hashim, H. Offner, and A.A. Vandenbark. 1989. Response of human T lymphocyte lines to myelin basic protein: association of dominant epitopes with HLA class II restriction molecules. J. Neurosci. Res. 23:207.
9. Martin, R., D. Jaraquemada, M. Flerlage, J. Richert, J. Whitaker, E.O. Long, D.E. McFarlin, and H.F. McFarland. 1990. Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. J. Immunol. 145:540.
10. Richert, J., E.D. Robinson, G.E. Deibler, R.E. Martenson, L.J. Dragovic, and M.W. Kies. 1989. Evidence for multiple human T cell recognition sites on myelin basic protein. J. Immunol. 143:3512.
11. Ota, K., M. Matsui, E.L. Milford, G.A. Mackin, H.L. Weiner, and D.A. Hafer. 1990. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature (Lond.). 346:183.
12. Simmons, D., M.W. Makgoba, and B. Seed. 1988. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature (Lond.). 331:624.
13. Jacobson, S., R.P. Sekaly, C. Jacobson, H.F. McFarland, and E.O. Long. 1989. HLA class II restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. J. Virol. 65:1756.
14. Baxevanis, C.N., G.J. Reclos, C. Servis, E. Anastasopoulos, P. Arsenis, A. Katsyiannis, N. Matikas, J.D. Lambris, and M. Papamichail. 1989. Peptides of myelin basic protein stimulate T lymphocytes from patients with multiple sclerosis. J. Neuroimmunol. 22:23.
15. Vandenbark, A.A., G.A. Hashim, B. Celnik, A. Galang, X. Li, E. Heber-Katz, and H. Offner. 1989. Determinants of human myelin basic protein that induce encephalitogenic T cells in Lewis rats. J. Immunol. 143:3512.
16. Sinigaglia, F., M. Gutttinger, J. Kilgus, D.M. Doran, H. Mattle, H. Etlinger, A. Trzeciak, D. Gillessen, and J.R.L. Pink. 1988. A malaria T cell epitope recognized in association with most mouse and human HMC class II molecules. Nature (Lond.). 336:778.
17. Tiwari, J.L., and P.I. Terasaki. 1985. HLA and Disease As-
18. Naito, S., Y. Kuroiwa, T. Itoyama, T. Tsubaki, A. Horikawa, T. Sasazuki, S. Noguchi, S. Ohtsuki, H. Tokuomi, T. Miyatake, N. Takahata, S. Kawanami, and A.J. McMichael. 1978. HLA and Japanese MS. *Tissue Antigens.* 12:19.

19. Gorodezky, C., R. Najera, B.E. Rangel, L.E. Castro, J. Flores, G. Velazquez, J. Granados, and J. Sotelo. 1986. Immunogenetic profile of multiple sclerosis in Mexicans. *Hum. Immunol.* 16:364.

20. Wucherpfennig, K.W., K. Ota, N. Endo, J.G. Seidman, A. Rosenzweig, H.L. Weiner, and D.A. Hafler. 1990. Shared human T cell receptor Vβ usage to immunodominant regions of myelin basic protein. *Science (Wash. DC)*. 248:1016.

21. Oksenberg, J.R., S. Stuart, A.B. Begovich, R.B. Bell, H.A. Erlich, L. Steinman, and C.A. Bernard. 1990. Limited heterogeneity of rearranged T-cell receptor Vα transcripts in brains of multiple sclerosis patients. *Nature (Lond.)*. 345:344.

22. Reinsmoen, N.L., and F.H. Bach. 1990. Structural model of T-cell recognition of HLA class II-associated alloepitopes. *Hum. Immunol.* 27:51.

23. Brown, J.H., T. Jardetzky, M.A. Saper, B. Samraoui, P.J. Bjorkman, and D.C. Wiley. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature (Lond.)*. 332:845.