Brief Definitive Report

Experimental Autoimmune Peripheral Neuritis Induced in BALB/c Mice by Myelin Basic Protein-specific T Cell Clones

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Summary

In vivo adoptive transfer of $CD4^+$ T helper cell type 1 clones reactive with autologous myelin basic protein (MBP) may initiate an inflammatory demyelinating disease of the central nervous system called experimental autoimmune encephalomyelitis. Although MBP is also a component of peripheral nervous system (PNS) myelin, previous studies have failed to demonstrate inflammation in the PNS induced by MBP-reactive T cells. Here, we report on two MBP-specific T cell clones that preferentially initiate inflammatory and demyelinating peripheral neuritis when adoptively transferred to syngeneic recipients. The MBP epitope recognized by these clones spans the junction of exons 6 and 7 and, therefore, is present in the 21- and 18.5-kD but not the 14- and 17-kD MBP isoforms, in which exon 5 is spliced to exon 7. The data suggest that MBP may be processed and presented differently in the central nervous system and PNS, and they provide evidence for MBP as a potential target for autoimmune reactions in the PNS.

Widespread multifocal inflammation and demyelination of the peripheral nervous system (PNS) characterize both chronic and acute inflammatory demyelinating polyradiculoneuropathies. Indications that these diseases are of an autoimmune nature were supported by animal studies of experimental autoimmune neuritis (EAN), which can be experimentally induced by immunization with peripheral nerve tissue in adjuvant (1). Investigations of the neuritogenic components of peripheral nerve demonstrated that EAN can be induced by immunization of rats with either the P_0 or the P_2 proteins of peripheral nerve myelin (2, 3). EAN can be adoptively transferred to native recipients with $CD4^+$ cells specific for either of these two myelin proteins (4, 5).

In addition to the P_0 and P_2 proteins, peripheral nerve myelin contains a third major protein, designated P_1. The P_0 and P_2 proteins are unique to peripheral nerve myelin and constitute ~45–65% and 2–15% of PNS myelin proteins, respectively (6, 7). In contrast, the P_1 protein is identical in amino acid sequence to central nervous system (CNS)-derived myelin basic protein (MBP) (8, 9). Despite the presence of MBP in the PNS as well as in the CNS, MBP-recognizing T cell clones that passively transfer experimental autoimmune encephalomyelitis in mice have not been shown to induce peripheral nerve inflammation and demyelination (10, 11).

The relative percentages of MBP in CNS and PNS myelin differ dramatically. While MBP accounts for 30–40% of myelin proteins in the CNS, it accounts for only 2–16% of peripheral nerve myelin proteins, with variations depending upon the precise location within the PNS (7). Precise quantitative and qualitative comparisons of PNS and CNS MBP are further complicated by more recent findings of several MBP isoforms (12–15). In the fully developed mouse CNS, four isoforms of MBP, with molecular masses of 21.5, 18.5, 17.2, and 14 kD, predominate. While all major MBP isoforms present in the CNS are also present in the PNS, as judged by PCR amplified cDNA synthesized from sciatic nerve RNA, their relative quantities in CNS various PNS are not known (16).

MBP does, however, appear to play a role in certain instances of PNS disease. Inflammatory polyneuropathy has been shown, in some patients, to correlate with antibodies to MBP (17). Pender et al. have shown demyelination of spinal roots after adoptive transfer of MBP-sensitized lymphocytes to Lewis rat recipients (18), although no involvement of dorsal root ganglia, spinal nerves, and peripheral nerves was observed.

We previously characterized encephalitogenic MBP-specific T cell clones from BALB/c mice (19, 20). 12 out of 14 I-A^d–restricted clones recognizing an epitope in the 59-76 peptide of MBP were capable of causing severe autoimmune encephalomyelitis upon adoptive transfer into BALB/c recipients (19). Histologically, severe inflammation and demyelination in the CNS were observed; no evidence of PNS involvement was found. In contrast, two I-E^d–restricted T cell clones recognizing an epitope present in the COOH-terminal

J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/08/0587/06 $2.00
Volume 182 August 1995 587–592

region of MBP comprising residues 151–168 were shown to cause only moderate clinical and histological signs of disease (20). Here we report that, although the latter two T cell clones induce moderate inflammation in the CNS, they induce significant PNS disease. The MBP epitope recognized by these clones spans an alternative MBP exon splice site.

Materials and Methods

Mice. Female BALB/c mice were purchased from Harlan Bioproducts for Science, Inc. (Indianapolis, IN) and used between the ages of 6–10 wk.

Antigens. Mouse MBP peptides 59-76 (HTRTTHYGSLPQKSQHGR), 151-168 (IFKLGGRDSRSGSPMARR), 155-168, and 111-114/155-168 (RFSWGGRDSRSGSPMARR) were synthesized by Dr. Charles Dahl (Harvard Medical School, Boston, MA).

T Cell Clones. The derivation and specificity of MBP-reactive BALB/c clones has been previously described (19, 20). Clone A2 was derived from a BALB/c mouse immunized with MBP peptide 151-168 and selected in vitro with homologous peptide.

Assays of Antigen-specific Proliferative Responses and IL-2. For antigen-specific proliferation, triplicate cultures containing 5×10^4 cloned T cells were incubated with 5×10^5 irradiated (3,000 R) spleen cells in the presence or absence of Ag as detailed elsewhere (19, 20). Similarly, production of supernatants and IL-2 assays were performed as described previously (19). The means of triplicate cultures are presented. Standard deviations were <20%.

In Vivo Adoptive Transfer of Disease. T cell clones were activated in vitro for 3 h with irradiated syngeneic spleen cells, 10 μg/ml MBP peptide, and IL-2. Cells were harvested, washed, and injected intravenously into lightly irradiated (390 R) BALB/c recipients. Immediately after cell injections, and again after 3 d, recipients were injected intravenously with 200 ng purified pertussis toxin (List Biological Laboratories, Inc., Campbell, CA).

Clinical and Histopathological Evaluation. Mice were monitored daily for clinical signs of disease and scored as follows: 0, no clinical signs; 1, complete tail limpness, incontinence and ruffling of fur; 2, limp tail and moderate hind limb weakness; 3, complete hind limb paralysis; 4, complete hind limb paralysis and some forelimb weakness; and 5, moribund state.

For histopathological analysis, tissues from affected mice were fixed in Bouin's solution followed by 10% formalin before embedding in paraffin. Multiple cross sections of brain and spine, with spinal cord in situ, as well as sections of whole legs, were stained with hematoxylin and eosin. Replicate sections were stained with luxol fast blue and cresyl violet, and with Bodian stain for axons. The assignment of a severity score is on the basis of a semiquantitative evaluation of the extent of mononuclear cell infiltration and of demyelination.

Results and Discussion

BALB/c-derived T cell clones reactive with two distinct MBP epitopes were studied for their ability to induce inflammatory and demyelinating disease in syngeneic recipients. All clones used in these studies are Th1s, secreting IL-2 and IFN-γ but not IL-4 after activation (19 and data not shown). 3 d after in vitro activation with syngeneic spleen cells and appropriate peptide antigens, 10^7 MBP-reactive T cell clones were injected intravenously into lightly irradiated (390-R) BALB/c recipients (Table 1). The recipients developed signs of disease 7–14 d after transfer. Recipients of clones 1E2 and 2C2, specific for the COOH-terminal MBP peptide 151-168 in association with I-E^d, showed ruffling of fur, significant and progressive weight loss, complete tail paralysis, and hind limb ataxia. Paralysis of a hind leg was observed in only 1 out of 11 1E2 recipients, but not among 13 2C2 recipients. These clinical signs appear modest compared with the paraplegia and tetraplegia consistently observed upon transfer of BALB/c-derived T cell clones recognizing the MBP 59-76 peptide in association with I-A^d, such as clones 8-4.G6, 8-7.D8, and 8-3.E8.

Histological studies of CNS from recipients of MBP 151-168–specific clones 1E2 or 2C2 revealed only very mild CNS disease: Very few inflammatory foci were observed, and there was no evidence of demyelination (20 and Table 1). By contrast, recipients of clones recognizing the 59-76 peptide of MBP had numerous inflammatory foci accompanied by severe demyelination in CNS tissue (19 and Table 1). However, upon examination of PNS tissue, evidence of significant inflam-

Table 1. *CNS versus PNS Disease Induction by Adoptively Transferred BALB/c Anti-MBP T Cell Clones*

Clone	MBP peptide	Recipient (No. mice)	Clinical disease	Histological findings			
				CNS		PNS	
				Inflammation	Demyelination	Inflammation	Demyelination
1E2	151-168	BALB/c (6)	2.0	+	No	+ + + +	Yes
2C2	151-168	BALB/c (3)	2.0	+	No	+ + + +	Yes
8-4.G6	59-76	BALB/c (4)	5.0	+ + + +	Yes	–	No
8-7.D8	59-76	BALB/c (3)	5.0	+ + + +	Yes	–	No
8-3.E8	59-76	BALB/c (3)	5.0	+ + + +	Yes	–	No

10^7 of the indicated T cell clones were injected intravenously into BALB/c recipients 3 d after activation in vitro with the indicated peptides and syngeneic BALB/c spleen cells. Mice were killed when moribund or by day 21, and tissue sections were evaluated as described.

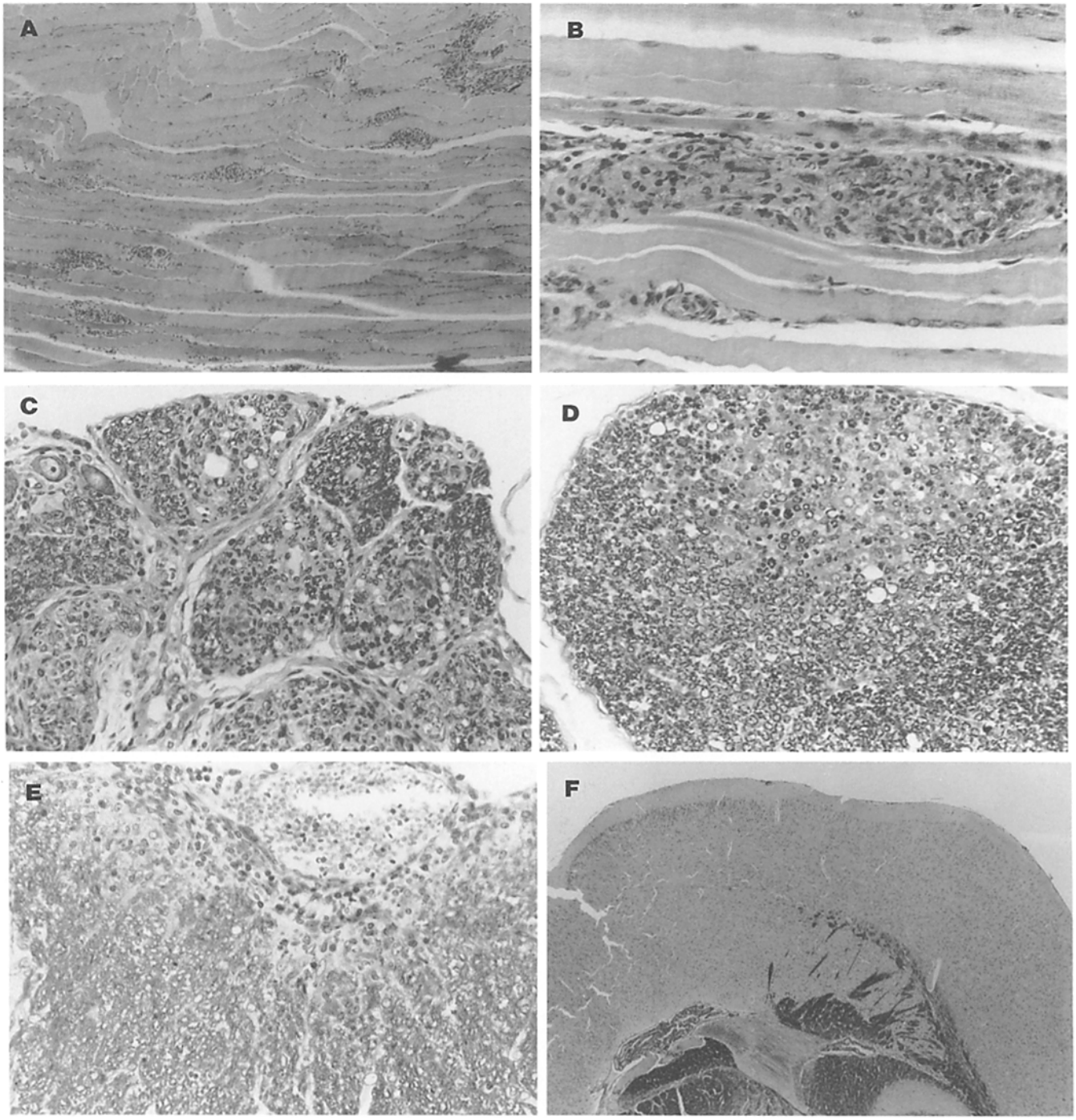

Figure 1. Histological sections from recipients of PNS disease–inducing clones. (*A*) Longitudinal section through quadriceps muscle from a BALB/c recipient of clone 2C2 (day 26). Many nerves are severely inflamed. Luxol fast blue–cresyl violet (LFB) ×93. (*B*) Higher power view of a small nerve from *A*. A few small foci stain with LFB, but most of the nerve has been replaced by inflammatory cells. ×373. (*C*) Cross section of cauda equina from the same mouse shown in *A* and *B*. Here, some roots are partially preserved, but most have undergone partial demyelination. LFB ×373. (*D*) Cross section of a spinal root from a recipient of clone 1E2 22 d earlier. One portion of the root is demyelinated, while much of the root is quite normal. LFB ×373. (*E*) Midline dorsal column of lumbar spinal cord from mouse shown in *A*–*C*. The meninges around a vein are inflamed, as is the immediately underlying white matter where no myelin remains. LFB ×373. (*F*) Low power view of brain from the mouse in *A*. There is no evidence of inflammation or demyelination. LFB ×37.

mation and demyelination was observed in recipients of clones 1E2 and 2C2 but not in recipients of peptide 59-76–reactive clones.

In all recipients of clones 1E2 and 2C2, the most severely affected parts of the nervous system were peripheral nerves in the hind leg. Most nerves were diffusely and densely in-

Table 2. *COOH-terminal Amino Acid Sequences of Mouse MBP Peptides Used in This Study*

	Exon 6				Exon 7														Splice
Peptide	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	site
151-168	I	F	K	L	G	G	R	D	S	R	S	G	S	P	M	A	R	R	6/7
155-168					G	G	R	D	S	R	S	G	S	P	M	A	R	R	7 only
	Exon 5				Exon 7														
"5/7"*	R	F	S	W	G	G	R	D	S	R	S	G	S	P	M	A	R	R	5/7

* This peptide corresponds to residues 111-114/155-168 of the 18.5-kD isoform of mouse MBP but constitutes the COOH-terminal 18 residues of the 14- and 17.2-kD isoforms.

filtrated by mononuclear inflammatory cells, predominantly macrophages, with fewer lymphocytes (Fig. 1, *A* and *B*). Only a few scattered areas of intact myelin remained (Fig. 1 *B*). Bodian stains of severely inflamed nerves revealed a deficiency of axons, suggesting that they had undergone necrosis. More proximally, some larger nerves and spinal roots were unaffected, while others were partially demyelinated (Fig. 1, *C* and *D*). Examination of many cross sections of spinal cord revealed inflammation and partial demyelination in some spinal roots and foci of inflammatory cell infiltration in meninges. Inflammation and demyelination were present in subpial white matter as well. All such foci were superficial and extended into the spinal cord only a short distance (Fig. 1 *E*). Very rare small foci of inflammation were observed in the brains of a few animals, but demyelination in the brain was never observed (Fig. 1 *F*).

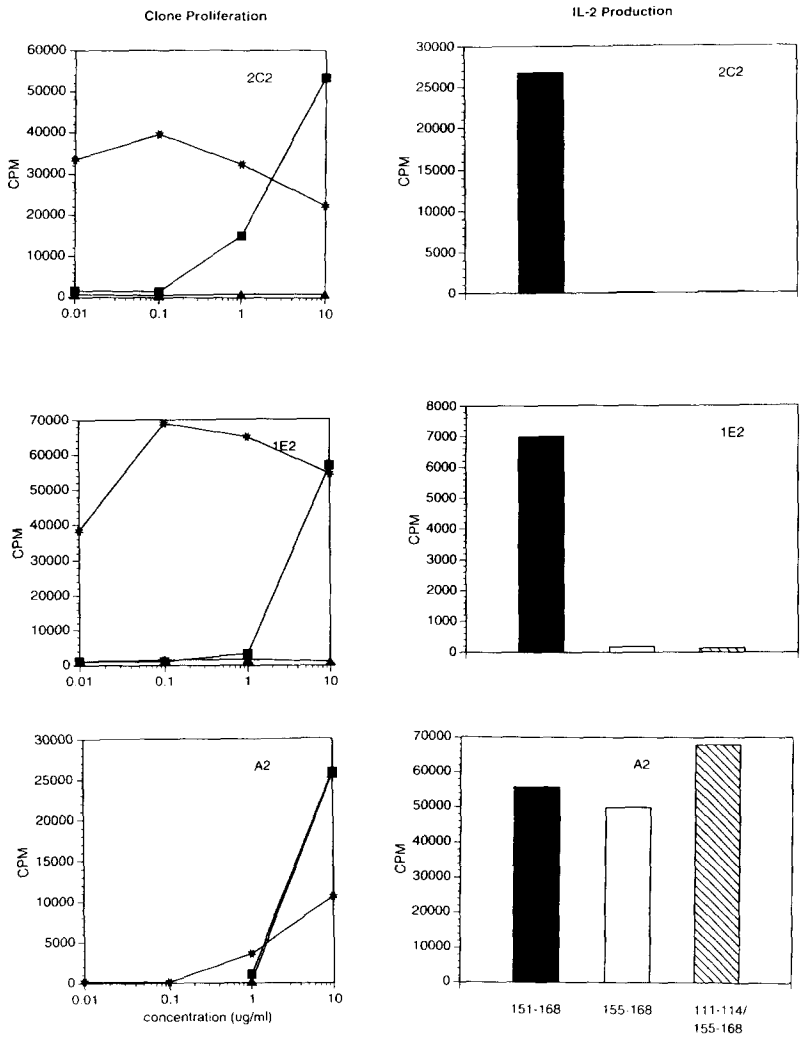

Figure 2. Specificity of peptide-induced proliferation and IL-2 secretion by MBP COOH-terminal specific BALB/c T cell clones. Parallel 96-well plates of clones 2C2, 1E2, or A2 were incubated with the indicated concentrations of MBP peptides 151-168 (—), 155-168 (—■—), or 111-114/155-168 (—▲—) in the presence of 5 × 10^5/well irradiated BALB/c spleen cells. One set of plates was pulsed with [^{3}H]thymidine for 20–44 h of culture, while supernatants of triplicate culture wells were harvested and pooled from the second set; these were then assayed for IL-2 content using HT-2 cells. Proliferation in the absence of added peptides was 563, 1,902, and 145 cpm for clones 2C2, 1E2, and A2, respectively. Proliferation of HT-2 in the absence of added supernatant was 135 cpm. All standard deviations were <20%.

MBP exists predominantly in four different isoforms in the adult nervous system (14, 15). In the 14- and 17.2-kD isoforms, exon 5 is spliced directly to exon 7. The adult brain has over threefold more of the exon 5/7 spliced forms (14 and 17.2 kD) than 6/7 spliced forms (21 and 18.5 kD) (12). We synthesized a number of peptides of the MBP COOH terminus, shown in Table 2, including one corresponding to exon 7 only (155-168), one consisting of 4 residues from exon 6 spliced to exon 7 (151-168, representing the 18 COOH-terminal residues of the 21- and 18.5-kD isoforms), and one consisting of 4 residues from exon 5 spliced to exon 7 ("5/7"), representing the 17.2- and 14-kD isoforms. Epitope mapping using this series of COOH-terminal MBP peptides shows that the MBP epitope recognized by clones 1E2 and 2C2 lies within residues 151-168 and requires the junction of exons 6 and 7. Fig. 2 shows the proliferative responses and the patterns of IL-2 production by clones 1E2 and 2C2 to the series of synthetic COOH-terminal MBP peptides described. These data show that proliferative responses of clones 1E2 and 2C2 to peptide 155-168 (exon 7 only) require 100-fold more peptide as compared with their responses to 151-168. The requirement for exon 6 residues for optimal activation of these clones is confirmed by the data in Fig. 2, showing that, without the exon 6 residues, production of IL-2 by clones 2C2 and 1E2 is not detectable. Furthermore, peptide "5/7" (111-114/155-168), representing the COOH-terminal residues of the 14- and 17.2-kD forms of MBP, was incapable of activating clones 1E2 and 2C2 either to proliferate or to produce IL-2 (Fig. 2). Responses of A2, a clone which recognizes a sequence encoded within exon 7 alone and is unaffected by flanking sequences beyond the splice site, are included. Clone A2 responds equally to peptide 155-168 (exon 7 only) or to 155-168 with residues from either exons 5 or 6 on its NH_2 terminus. Because clone A2 is also I-E^d restricted (data not shown), these data confirm that peptide 5/7 is capable of binding to, and being presenting by, the relevant class II molecule.

Despite the presence of MBP in peripheral nerve myelin (P_1), encephalitogenic MBP-reactive T cell clones have not previously been shown to initiate peripheral nerve disease when adoptively transferred in vivo. Data presented here define a murine model of autoimmune peripheral neuritis, in which PNS MBP is the predominant target autoantigen for MBP-recognizing T cell clones.

There are a number of possible non-mutually exclusive explanations for the ability of clones 1E2 and 2C2 to induce disease primarily in the PNS. Because the epitope for these two clones spans an alternative MBP splice site, they appear to recognize MBP sequences present only in the 18.5- and 21-kD isoforms of MBP. In the CNS, the 17.2- and 14-kD isoforms, which contain the 5/7 splice site, are approximately threefold more abundant (12). Possible quantitative differences in MBP isoform composition in the PNS and CNS could, at least partially, account for the preferential retention and activation of these clones in the PNS.

Another possibility is that differential processing of MBP by tissue-specific resident APCs may yield different epitopes. That different epitopes may be presented is made even more likely by the fact that MBP is endogenously synthesized by PNS Schwann cells, which have been shown to function as APC (21). Moreno et al. reported that qualitatively different epitopes of hen egg lysozyme are generated when comparing exogenously provided to endogenously synthesized hen egg lysozyme (22). Quantitative differences in I-E^d–restricted T cell activation by exogenous, compared with endogenous, β_2-microglobulin peptides have also been reported (23). Intimately linked with "determinant selection" of epitopes is MHC class II expression. Quantitative differences in MHC class II expression on Schwann cells as compared with microglia and astrocytes could affect ligand presentation, particularly in the case of the 1E2 and 2C2 ligand, where the affinity between peptide and class II is relatively low (19). Finally, unique trafficking and/or homing properties of these two clones may account for induction of PNS disease, although preferential retention and focus initiation are probably ultimately determined by optimal ligand (peptide/MHC) presentation.

In summary, this report provides the first demonstration that autoreactive MBP–recognizing T cell clones can preferentially induce inflammation and demyelination in the PNS. The data also highlight the differential recognition of MBP epitopes in the CNS and PNS and suggest that functional availability of antigenic epitopes is a determining factor in expression of autoimmunity.

This work was supported by grants from the National Multiple Sclerosis Society (S. Abromson-Leeman), the Multiple Sclerosis Foundation, and the National Institutes of Health (grant NS31152 to M. E. Dorf).

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Received for publication 20 March 1995 and in revised form 17 April 1995.

References

1. Waksman, B.H., and R.D. Adams. 1955. Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants. *J. Exp. Med.* 102:213–236.

2. Kadluboski, M., and R.A.C. Hughes. 1979. Identification of the neuritogen responsible for EAN. *Nature (Lond.).* 277:140–141.

3. Carlo, D.J., Y.D. Karkhanis, P.J. Bailey, H.M. Wisniewski, and S.W. Brostoff. 1975. Experimental allergic neuritis: evidence for the involvement of the P_0 and P_2 proteins. *Brain Res.* 88:580–584.
4. Linington, C., S. Izumo, M. Suzuki, K. Uyemura, R. Meyermann, and H. Wekerle. 1984. A permanent rat T cell line that mediates experimental allergic neuritis in the Lewis rat *in vivo*. *J. Immunol.* 133:1946–1950.
5. Linington, C., H. Lassmann, K. Ozawa, S. Kosin, and L. Mongan. 1992. Cell adhesion molecules of the immunoglobulin supergene family as tissue-specific autoantigens: induction of experimental allergic neuritis (EAN) by P_0 protein-specific T cell lines. *Eur. J. Immunol.* 22:1813–1817.
6. Greenfield, S., S. Brostoff, E.H. Eylar, and P. Morell. 1973. Protein composition of myelin of the peripheral nervous system. *J. Neurochem.* 20:1207–1216.
7. Whitaker, J.N. 1981. The protein antigens of peripheral nerve myelin. *Ann. Neurol.* 9(Suppl.):54–64.
8. Brostoff, S.W., and E.H. Eylar. 1972. The proposed amino acid sequence of the P1 protein of rabbit sciatic nerve myelin. *Arch. Biochem. Biophys.* 153:590–598.
9. Greenfield, S., S.W. Brostoff, and E.L. Hogan. 1980. Characterization of the basic proteins from rodent peripheral nervous system myelin. *J. Neurochem.* 34:453–455.
10. Raine, C.S., F. Mokhtarian, and D.E. McFarlin. 1984. Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse. Neuropathologic analysis. *Lab Invest.* 51:534–546.
11. Tabira, T., and K. Sakai. 1987. Demyelination induced by T cell lines and clones specific for myelin basic protein in mice. *Lab. Invest.* 56:518–525.
12. Barbarese, E., P.E. Braun, and J.M. Carson. 1978. Accumulation of the four myelin basic proteins in mouse brain during development. *J. Neurochem.* 31:779–782.
13. Yu, Y.-T., and A. Campagnoni. 1982. In vitro synthesis of the four mouse myelin basic proteins: evidence for the lack of a metabolic relationship. *J. Neurochem.* 39:1559–1568.
14. Takahashi, N., A. Roach, D.B. Teplow, S.B. Prusiner, and L. Hood. 1985. Cloning and characterization of the myelin basic protein gene from mouse: one gene can encode both 14 kD and 18.5 kD MBPs by alternate use of exons. *Cell.* 42:139–148.
15. Newman, S., K. Kitamura, and A.T. Campagnoni. 1987. Identification of a cDNA coding for a fifth form of myelin basic protein. *Proc. Natl. Acad. Sci. USA.* 84:886–890.
16. Mathisen, P.M., S. Pease, J. Garvey, L. Hood, and C. Readhead. 1993. Identification of an embryonic isoform of myelin basic protein that is expressed widely in the mouse embryo. *Proc. Natl. Acad. Sci. USA.* 90:10125–10129.
17. Hemachudha, T., D.E. Griffin, J.J. Giffels, R.T. Johnson, A.B. Moser, and P. Phanupak. 1987. Myelin basic protein as an encephalitogen in encephalomyelitis and polyneuritis following rabies vaccination. *N. Engl. J. Med.* 316:369–374.
18. Pender, M.P. 1988. The pathophysiology of myelin basic protein-induced acute experimental allergic encephalomyelitis in the Lewis rat. *J. Neurol. Sci.* 86:277–289.
19. Abromson-Leeman, S., J. Alexander, R. Bronson, J. Carroll, S. Southwood, and M. Dorf. 1995. Experimental autoimmune encephalomyelitis-resistant mice have highly encephalitogenic myelin basic protein (MBP)-specific T cell clones that recognize a MBP peptide with high affinity for MHC Class II. *J. Immunol.* 154:388–398.
20. Abromson-Leeman, S., M. Hayashi, C. Martin, R. Sobel, A. Al-Sabbagh, H. Weiner, and M.E. Dorf. 1993. T cell responses to myelin basic protein in experimental autoimmune encephalomyelitis-resistant BALB/c mice. *J. Neuroimmunol.* 45:89–102.
21. Wekerle, H., M. Schwab, C. Linington, and R. Meyermann. 1986. Antigen presentation in the peripheral nervous system: Schwann cells present endogenous myelin autoantigens to lymphocytes. *Eur. J. Immunol.* 16:1551–1557.
22. Moreno, J., D.A.A. Vignali, F. Nadimi, S. Fuchs, L. Adorini, and G.J. Hammerling. 1991. Processing of an endogenous protein can generate MHC Class II-restricted T cell determinants distinct from those derived from exogenous antigen. *J. Immunol.* 147:3306–3313.
23. Guery, J.-C., A. Sette, E. Appella, and L. Adorini. 1995. Constitutive presentation of dominant epitopes from endogenous naturally processed self-β_2-microglobulin to Class II-restricted T cells leads to self-tolerance. *J. Immunol.* 154:545–554.