T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis

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Chronic relapsing paralysis and demyelination within the central nervous system (CNS), features associated with the human disease multiple sclerosis (MS)[1–4], develop in mice after injection of murine T-cell clones specific for the autoantigen myelin basic protein (MBP)[5,6]. We examined the fine specificity of three independently derived encephalitogenic T-cell clones using synthetic polypeptides derived from portions of the N-terminal sequence of MBP. These clones appear functionally identical; they all respond to an epitope in the N-terminal nine amino acid residues in association with the same class II (I-A) molecules of the major histocompatibility complex (MHC). Both the N-terminal acetyl moiety and the first residue (Ala) are necessary for recognition. Only N-terminal MBP peptides recognized by these clones were found to cause encephalomyelitis (EAE) *in vivo*. These results show that the N-terminal MBP-specific T lymphocytes that mediate autoimmune encephalomyelitis are a small population with a limited repertoire; they all recognise the same combination of MHC and target.

EAE is a model for autoimmune diseases mediated by class II restricted T lymphocytes[5–10]. Immunization with intact MBP or fragments of MBP causes EAE in susceptible mouse strains[10–12]. However, not all such fragments are encephalitogenic. Only the N-terminal 37 amino-acid MBP fragment causes EAE in PL/J (H-2^u) and (PL/J × SJL/J (H-2^s))F_1 ((PLSJ)F_1) mice[11,12]. Encephalitogenic T-cell clones with the L3T4$^+$, Lyt2$^-$ phenotype have been isolated from both these strains following immunization with either intact rat or bovine MBP[5,6]. Clones PJR-25 and F1-12 were isolated from homozygous PL/J and (PLSJ)F_1 mice, respectively, after immunization with intact rat MBP. Encephalitogenic clone PJB-20 was isolated from homozygous PL/J mice after immunization with intact bovine MBP. These clones respond to a determinant shared with mouse (self) MBP within the encephalitogenic N-terminal MBP fragment in association with I-A^u($A_\alpha{}^u A_\beta{}^u$) class II molecules. Class II-restricted T lymphocytes recognize discrete, contiguous sequences within proteins, sometimes fewer than ten residues[13] in length via their antigen-specific cell surface receptors[14,15]. To investigate the pathogenesis of this autoimmune disease further, we examined whether these encephalitogenic T lymphocytes all respond to a common epitope, or have distinct N-terminal MBP specificities.

Separate forms of intact MBP with different N-terminal sequences were tested for their ability to stimulate proliferation of encephalitogenic T-cell clones. Bovine MBP was found to be less stimulatory than mouse or rat MBP at the same concentration (results not shown). Since the N-terminal 37 amino-acids of bovine MBP differ from mouse MBP only at residues 2 and 17 (see Fig. 1)[16], we predicted that the epitope(s) recognized by these T-cell clones would include one of these two residues. The overlapping peptides pR(rat)1-16 and pR5-20 were made and tested for their ability to stimulate proliferation of these clones. The epitope recognized by all three clones is found in the N-terminal 16 residues; peptide pR1-16 is as effective as intact

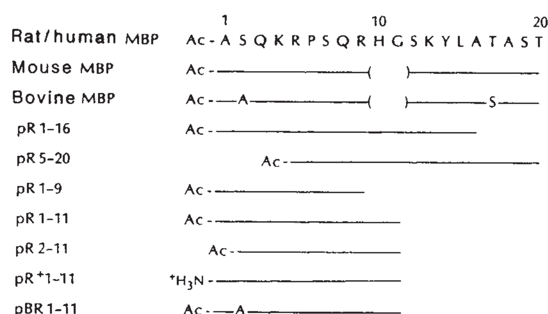

Fig. 1 Amino-acid sequences of MBP from different species, and synthetic MBP peptides. The sequences of intact rat, human, mouse, and bovine MBP have been published previously[16]. Peptides corresponding to the sequences of rat (R) and bovine (B) MBP were synthesized as described previously[29,30] by solid phase techniques on a Beckman Model 990 B peptide synthesizer; using commercially available amino acid polystyrene resins and t-Boc-protected amino acids from Peninsula Laboratories. The protecting groups used were: o-benzyl esters for Thr and Ser; tosyl for His; orthochlorobenzyloxycarbonyl for Lys; and 2,6-dichlorobenzyl for Tyr. Couplings were performed using a 2.5 molar excess of t-Boc amino acid and dichlorohexylcarbodiimide (DCC) over the number of milliequivalents of mino acid on the resin. All couplings were >99% complete, as determined by reaction with ninhydrin. The peptides were deprotected and removed from the resin simultaneously by treatment with anhydrous hydrogen fluoride in the presence of anisole, dimethylsulphide, and indole, then separated from organic side products by extraction with ether and isolated from the resin by extraction with 5% acetic acid and subsequent lyophilization. Purity was determined by high pressure liquid phase column (Merck) and amino acid analysis. The peptides used in these experiments were not further purified as they all contained >90% of the desired peptide.

MBP (Fig. 2*a*) whereas pR5-16 and pR5-20 do not stimulate proliferative responses. These clones also respond to peptides pR1-9 and pR1-11. Since pR1-11 and pR1-16 are equipotent, residues 12-16 do not contribute significantly to the epitope recognized by these T-cell clones.

Acetylation of the first residue (NAc-Ala_1) is essential for recognition. When the acetyl group is removed, revealing a positively charged amino terminus (pR^+1-11, Fig. 1), proliferative activity is eliminated (Fig. 2*b*). The amino-acid Ala_1 is also necessary, since the MBP peptide pR2-11, containing NAc-Ser_2 is not stimulatory even at the highest concentrations tested. Bovine MBP, which has an alanine instead of a serine residue at position 2 (see Fig. 1), is less encephalitogenic than intact rat or mouse MBP, which both have Ser_2, in homozygous PL/J and (PLSJ)F_1 mice[11]. Peptide pBR1-11, which has Ala as the second residue in a rat MBP1-11, sequence (Fig. 1) is less effective than pR1-11 and requires higher concentrations to stimulate proliferation. Thus, these representative T-cell clones isolated after stimulation with rat MBP or bovine MBP share the same N-terminal specificity. We also identified a second T-cell epitope in the encephalitogenic N-terminal fragment of rat MBP. T-cell clones that are restricted to $A\alpha^s A\beta^u$ class II molecules isolated from (PLSJ)F_1 mice recognize rat but not mouse (self) MBP, and are not encephalitogenic. The epitope recognized by these T-cell clones is found in pR9-16 (Fig. 3). Thus there are two distinct epitopes in the N-terminal 16 residues of MBP.

MBP peptide fragments have been shown to cause acute encephalomyelitis in other animal models[17–22]. However, peptides as small as those described in this report have not previously been shown to cause EAE in mice. Peptides pR1-9, pR1-11, and pR1-16, all of which are recognized *in vitro* by encephalitogenic T cell clones, cause EAE in (PLSJ)F_1 mice when given in adjuvant, without carrier protein (Table 1). Histo-

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Table 1 Induction of EAE by synthetic N-terminal MBP peptides

Antigen	Dose (nanomoles)	Incidence	Mean severity	Mean day of onset
pR1-9	100 (110 μg)	10/10	4.2 (±0.63)	11.9 (±0.74)
pR1-9	20	4/5	1.8 (±0.96)	12.5 (±1.7)
pR1-11	100 (128 μg)	10/10	4.4 (±1.3)	12.8 (±1.4)
pR1-11	20	3/5	3.7 (±1.2)	12.3 (±1.2)
pR1-16	100 (186 μg)	3/5	1.7 (±0.57)	14.3 (±1.5)
pR1-16	20	3/5	2.7 (±1.2)	13.7 (±1.2)
pR2-11	200 (244 μg)	0/10	—	—
pR2-11	100	0/5	—	—
pR2-11	20	0/5	—	—
pR5-16	100 (144 μg)	0/5	—	—
pR9-16	100 (97 μg)	0/5	—	—
pR11-27	100 (184 μg)	0/5	—	—
pR20-37	100 (216 μg)	0/5	—	—

MBP peptides were tested for their ability to cause EAE following the protocol described previously[11,12] for induction of EAE in mice with intact MBP. Each peptide was dissolved in phosphate buffered saline (PBS) and emulsified with complete Freund's adjuvant (CFA) in a 1:1 mixture of PBS:CFA containing 4 mg ml^{-1} H37Ra (Difco). Recipient $(PLSJ)F_1$ female mice (Jackson Laboratories, Maine), aged 12–20 weeks, were injected with 0.1 ml emulsion at the base of the tail. On the same day 10^{10} heat killed *Bacillus pertussis* (Michigan Department of Health, Lot 91B) were injected intravenously. Forty-eight hours later 10^{10} *B. pertussis* were injected a second time. Mice were examined daily for signs of EAE by two independent observers. Severity of EAE was graded using a scale previously described[6]: 0, no sign of EAE; 1, decreased tail tone only; 2, mild paraparesis; 3, moderately severe paraparesis; 4, complete paraplegia; 5, moribund. Synthetic peptides pB1-9, pBR1-11, and pR5-20 have also been tested (results not shown). When $(PLSJ)F_1$ mice are injected with 100 μmoles pB1-9 or pBR1-11, peptides that are recognized by encephalitogenic T cell clones, clinical and histological EAE are observed. Peptide pR5-20 is not encephalitogenic.

logical examination of paralysed mice shows perivascular infiltration of mononuclear cells in the CNS, as observed in EAE caused by immunization with whole MBP[10,11]. In contrast, peptides pR2-11, pR5-16, pR9-16, pR11-27 and pR20-37, which do not stimulate encephalitogenic T-cell clones to proliferate *in vitro*, do not cause clinical or histological signs of EAE in $(PLSJ)F_1$ mice (Table 1).

T-cell clones recognize peptide sequences in association with a particular class II MHC product. Encephalitogenic T-cell clones isolated after immunization with intact MBP recognize pR1-11 in association with $I\text{-}A^u$ class II molecules (Fig. 2*b*). It is possible that encephalitogenic $(PLSJ)F_1$ T lymphocytes may recognize this epitope in association with other class II molecules. However, immunization with pR1-11 causes EAE in $H\text{-}2^u$ strains (PLJ 7/10 mice paralysed and B10.PL 10/10 paralysed), but not $H\text{-}2^s$ strains (SJL/J 0/10 paralysed and B10.S 0/10 paralysed), and lymphocytes primed *in vivo* with pR1-11 are inhibited from responding *in vitro* to either pR1-11 or intact MBP by monoclonal antibodies specific for I-A, but not I-E molecules (results not shown). Thus, the encephalitogenic responses to intact MBP and N-terminal MBP peptides in PL/J and $(PLSJ)F_1$ mice are restricted to $I\text{-}A^u$ class II molecules.

We have thus identified the epitope recognized by T-cell clones which mediates autoimmune encephalomyelitis. These independently-derived T-cell clones appear to have functionally identical peptide specificity and class II restriction. Further, the induction of autoimmune encephalomyelitis by *in vivo* administration of N-terminal MBP peptides correlates with T cell recognition *in vitro*; of all N-terminal MBP peptides tested, only those peptides which are recognized by these independently-derived T-cell clones are encephalitogenic. T-cell clones derived after immunization with pBR1-11 or pR1-11 also have the same N-terminal MBP and class II specificity as the clones

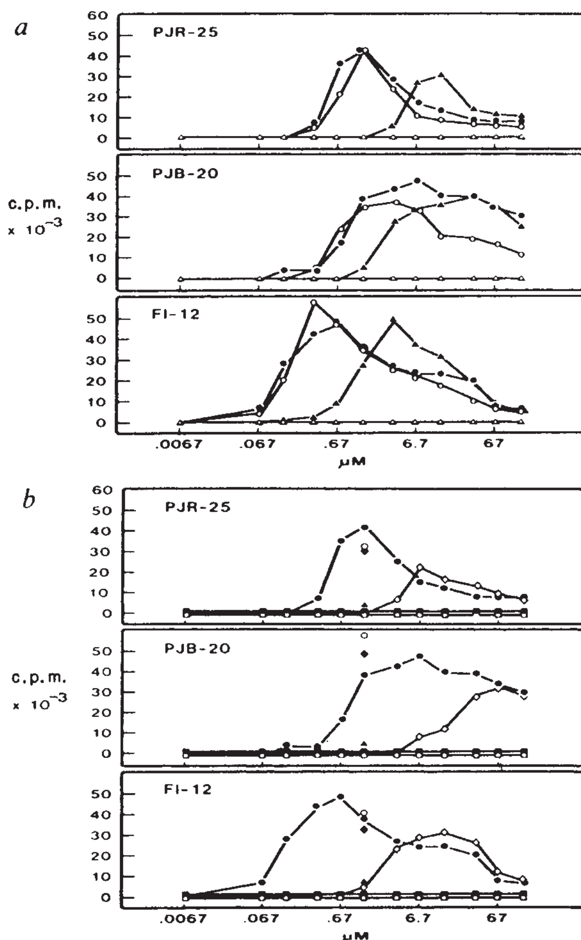

Fig. 2 Recognition of N-terminal MBP peptides by encephalitogenic T cell clones. ***a***, Peptides pR1-16 (○), pR1-11 (●), pR1-9 (▲), and pR5-20 (△) were tested for their ability to stimulate encephalitogenic proliferative T cell clones, isolated as described previously[1,6,31] To assay for proliferative responses, 10^4 T cells were cultured with 5×10^5 X-irradiated (3,000 rad) PL/J splenic antigen presenting cells (APC) in 0.2 ml culture media in 96 well flat-bottomed microtitre plates (Falcon). Culture medium was RPMI 1640 (Gibco; supplemented with 10% fetal calf serum (Hyclone), 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin). Peptides were added to cultures giving the final concentrations indicated. At 48 h each well was pulsed with 1 μCi ^3H-thymidine and cells collected 16 h later. The mean c.p.m. for thymidine incorporation was calculated for triplicate cultures. Standard deviations are within 10% of the mean. The same pattern of response to N-terminal peptides is found when these T cell clones are cultured with $(PLSJ)F_1$ APC. ***b***, Peptides pR1-11 (●), pBR1-11 (◇), $pR^+1\text{-}11$ (□), and pR2-11 (■) were tested as described above. In a separate assay, the proliferative response to pR1-11 (○) was inhibited with 1 μg per well anti I-A monoclonal antibody 10-2.16 (ref. 32) (▲), but not anti I-E monoclonal antibody 14-4-4[33] (◆). When tested at this concentration, 14-4-4 inhibits T cell clones restricted to I-E molecules[6] (results not shown).

described here. Clones derived from mice immunized with pBR1-11 respond in a heteroclitic manner; like PJB-20 they respond better to pR1-11 than to pBR1-11, the original immunogen (results not shown). Thus, *in vitro* and *in vivo* results suggest that the encephalitogenic T-cell response to MBP 1-37 is limited to a discrete population of MBP-reactive T cells.

It has recently become possible to examine the genes that encode antigen-specific T-cell receptors (TCR)[23-25] and investigate the relationship between specificity and TCR gene expression. Ovalbumin-specific T-cell clones that recognize the same epitope and share the same class II restriction have been shown to use the same V_α/J_α and V_β/J_β TCR genes[26]; T-cell clones that share the same fine specificity for cytochrome C use the same V region genes, but can express different J elements[27]. However examination of TCR genes from clones of different specificities suggests that there may not be a simple correlation

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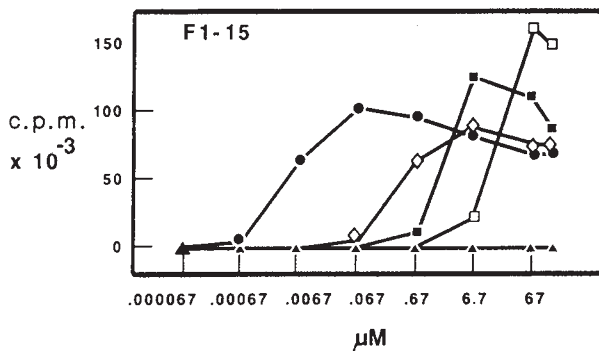

Fig. 3 N-terminal peptides recognized by a representative non-encephalitogenic MBP specific T cell clone. Peptides pR1-16 (●), pR5-16 (◇), pR7-16 (■), pR9-16 (□) and pR10-20 (▲), were tested as described (Fig. 2, legend) F1-15 T cells were cultured with (PLSJ)F_1 splenic APC. These N-terminal MBP-specific non-encephalitogenic T cell clones are restricted to $A\alpha^s A\beta^u$ class II molecules. N-terminal peptides that are recognized by these non-encephalitogenic T cell clones do not cause EAE (see Table 1).

between V_β expression and T-cell specificity[28]. The TCR genes from encephalitogenic T-cell clone PJR-25 are being cloned, and should be useful probes allowing us to examine whether other encephalitogenic clones, which share the same epitope and class II restriction, express the same TCR genes.

We have described here the first investigation of the fine specificity of T cell clones mediating an autoimmune disease. Two distinct T-cell epitopes in MBP 1-16 have been characterized. Examination of the response to MBP peptides, both *in vitro* and *in vivo*, has revealed that only a limited repertoire of N-terminal specific T lymphocytes, expressing a common class II restriction and peptide specificity, mediate induction of EAE. These results may provide insights into the immunogenetic mechanisms of other autoimmune diseases. In cases where only a limited repertoire of T lymphocytes mediate autoimmune pathogenesis, specific forms of therapy might be possible. For example measures which selectively inactivate or eliminate only autoaggressive T cells bearing the N-terminal MBP specificity may be beneficial in the treatment of autoimmune encephalomyelitis.

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1. Wisniewski, H. M. & Keith, A. B. *A. Neurol.* **1**, 144-148 (1977).
2. Raine, C. S., Snyder, D. H., Valsamis, M. P. & Stone, S. H. *Lab. Invest.* **31**, 369-380 (1974).
3. Raine, C. S. in *Multiple Sclerosis—Pathology, Diagnosis and Management* (eds Hallpike, J., Adams, C. W. M. & Tourtellote, W. W.) 413-460 (Chapman & Hall, London, 1983).
4. Lassman, H. K. & Wisniewski, H. M. *Archiv. Neurol.* **36**, 490-497 (1979).
5. Zamvil, S. *et al. Nature* **317**, 355-358 (1985).
6. Zamvil, S. *et al. J. exp. Med.* **162**, 2107-2124 (1985).
7. Petinelli, C. B. & McFarlin, D. E. *J. Immun.* **127**, 1420-1423 (1981).
8. Brostoff, S. W. & Mason, D. W. *J. Immun.* **133**, 1938-1942 (1984).
9. Waldor, *et al. Science* **227**, 415-417 (1985).
10. Petinelli, C. B., Fritz, R. B., Jen-Chou, C. H. & McFarlin, D. E. *J. Immun.* **129**, 1209-1214 (1982).
11. Fritz, R. B., Jen-Chou, C. H. & McFarlin, D. E., *J. Immun.* **130**, 191-194 (1983).
12. Fritz, R. B., Skeen, M. J., Jen-Chou, C. H., Garcia, J. & Egorov, I. K., *J. Immun.* **134**, 2328-2332 (1985).
13. Schwartz, R. H., Fox, B. S., Frago, E., Chen, C. & Singh, B. *J. Immun.* **135**, 2598-2608 (1985).
14. Haskins, K. *et al. J. exp. Med.* **157**, 1149-1169 (1983).
15. Kaye, J., Porcelli, S., Tite, J. Jones, B. & Janeway, C. A. Jr. *J. exp. Med.* **158**, 836-856 (1983).
16. Martenson, R. E. *Prog. clin. biol. Res.* **146**, 511-521 (1984).
17. Eylar, E. H., Caccam, J., Jackson, J. J., Westall, F. C. & Robertson, R. B. *Science* **168**, 1220-1223 (1970).
18. McFarlin, D. E., Blank, S. E., Kibler, R. F., McKneally, S. & Shapira, R. *Science* **179**, 478480 (1972).
19. Teitlebaum, D. C., Webb, C., Arnon, R. & Sela, M. *Cell. Immun.* **29**, 265-271 (1977).
20. Kibler, R. F. *et al. J. exp. Med.* **146**, 1323-1331 (1977).
21. Hashim, G. A., Carvalho, E. F. & Shapiro, R. D. *J. Immun.* **121**, 665-670 (1978).
22. Arnon, R. *Immun. Rev.* **55**, 5-30 (1981).
23. Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
24. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
25. Chien, Y. *et al. Nature* **312**, 31-35 (1984).
26. Yague, J. *et al. Cell* **42**, 81-87 (1985).
27. Fink, P. J., Matis, L. A., McEllingott, D. L., Bookman, M. & Hendrick, S. M. *Nature* **321**, 219-226 (1986).
28. Goverman, J. *et al. Cell* **40**, 859-867 (1985).
29. Erickson, B. W. & Merrifield, R. B. in *The Proteins* Vol. II (ed. Neurath, H.) 255-527 (Academic, New York, 1976).
30. Rothbard, J. B., Fernandez, R. & Schoolnick, G. K. *J. exp. Med.* **160**, 208-221 (1984).
31. Kimoto, M. & Fathman, C. G. *J. exp. Med.* **152**, 759-770 (1980).
32. Oi, V. T., Jones, P. P., Goding, J. W. & Herzenberg, L. A. *Clin. Topics Microbiol. Immun.* **81**, 115-129 (1978).
33. Ozato, K., Mayer, N. & Sachs, D. H. *J. Immun.* **124**, 533-540 (1980).

Interaction of peptide antigens and class II major histocompatibility complex antigens

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T lymphocytes require a foreign antigen to be presented on a cell surface in association with a self-transplantation antigen before they can recognize it effectively. This phenomenon is known as major histocompatibility complex (MHC) restriction[1]. It is not clear how an incalculably large number of foreign proteins form unique complexes with a very limited number of MHC molecules. We studied the recognition properties of T cells specific for a peptide derived from bacteriophage λ cI protein. Analogues of this peptide, as well as peptides derived from other unrelated antigens which can be presented in the context of the same MHC molecule, can competitively inhibit activation of these T cells by the cI peptide. Furthermore, these unrelated antigens can stimulate cI-specific T cells if certain specific amino-acid residues are replaced. Here we suggest a model in which all antigens give rise to peptides that can bind to the same site on the MHC molecule. T-cell recognition of this site (which is presumed to be polymorphic) with or without antigen bound can explain self-selection in the thymus and MHC restriction.

We immunized BALB/c mice with the 102 amino-acid N-terminal domain of bacteriophage λ cI protein (cI) and produced cI-specific, class II-restricted T-cell hybridomas. More than 75% of specific hybridomas could be stimulated by a peptide comprised of residues 12-26 (P12-26) in the context of 1-A^d. Independently isolated T cells could be divided into at least three groups on the basis of their reactivity to truncated analogues of P12-26 (Fig. 1). Following Rock and Benacerraf[2] and others[3], who showed competition for presentation between related antigens, we examined whether inactive truncated analogues could inhibit activation of T cells stimulated with the parent peptide or active truncated analogues. In the case of the hybridoma 7B7.3, P12-24 can inhibit activation by P15-26, but not in the case of hybridoma 9H35 (see Fig. 2*a*). Failure to obtain competition has also been noted in other systems[1,4], suggesting that competition depends on the T cell involved. It may well be that the active peptide and the analogues compete for binding to the same site on I-A (ref. 5) but the T cell enhances the avidity of the active peptide for I-A so that competition cannot be seen. This hypothesis agrees with recent findings from several laboratories[6,7]. To test the hypothesis that other antigens which can be presented in the context of 1-A^d inhibit cI peptide stimulation, we screened 14 overlapping peptides derived from

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