TWO MINOR DETERMINANTS OF MYELIN BASIC PROTEIN INDUCE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN SJL/J MICE

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Experimental allergic encephalomyelitis (EAE)[1] is an autoimmune inflammatory demyelinating disease in the central nervous system (CNS) of animals immunized with myelin basic protein (MBP). The disease is directly mediated by T helper cells that recognize MBP in the context of class II antigens of the MHC (1-3). In nude mice, a single clone of adoptively transferred MBP-reactive T helper cells can cause EAE (4), suggesting that these are the only T cells required for disease induction. As a prototypic model of T helper cell-mediated autoimmune disease, observations in EAE could likely be applicable to other T helper cell-mediated diseases such as murine lupus (5), thyroiditis (6), collagen arthritis (7), and adjuvant arthritis (8), as well as human autoimmune diseases.

The MBP epitope is determined in part by the MHC. Using proteolytic peptide fragments of MBP, SJL/J ($H\text{-}2^s$) and B10.T(6R) ($H\text{-}2^q$) mice were found to develop EAE to the COOH-terminal peptide of MBP, whereas PL/J ($H\text{-}2^u$) and A/J ($H\text{-}2^k$) mice developed EAE to the NH_2-terminal peptide of MBP (9). Recently, by using synthetic peptides that overcome the difficulties of obtaining pure uncontaminated proteolytic peptides, a single T cell encephalitogenic epitope for PL/J mice has been identified. This epitope consists of the first nine NH_2-terminal amino acid residues of MBP which must be acetylated at the α amino group to induce disease (10). Similar fine mapping of the encephalitogenic T cell epitope(s) for SJL/J mice has not been done, in part because of the large size of the COOH-terminal peptic fragment of MBP (residues 89-169 of rat MBP, reference 9).

Mouse MBP consists of four major forms due to differential RNA splicing of exons II and VI (11), resulting in molecular masses of 21, 18.5, 17.5, and 14 kD, in the relative amounts of 1:10:3.5:35. Since EAE can also be induced with the small form of rat MBP (14 kD), which has exons II and VI of the MBP gene deleted (12), the COOH-terminal encephalitogenic determinant for SJL/J mice must be present within a segment of only 42 amino acid residues. Consistent with this notion is the observa-

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[1] *Abbreviations used in this paper:* EAE, experimental allergic encephalomyelitis; LNC, lymph node cell; MBP, myelin basic protein.

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tion that this peptide sequence is identical among the MBPs of several mammalian species, including mouse, rat, bovine, guinea pig, and porcine, all of which can induce EAE in SJL/mice (13, 14).

To identify the SJL/J encephalitogenic T cell epitope(s), overlapping peptides to the COOH-terminal region of the small form of mouse MBP were synthesized. Two overlapping peptides encompassing an 18-amino acid region were found to elicit EAE in SJL/J mice. The finding of a single peptide region of MBP that is responsible for encephalitogenic T cell epitopes in SJL/J mice is analogous to that of the PL/J mice and has implications for the development of specific therapy for T cell-mediated autoimmune diseases.

Materials and Methods

Mice. SJL/J and PL/J mice (6-10 wk old) were purchased from The Jackson Laboratory, Bar Harbor, ME. (SJL/J × PL/J)F_1 mice were bred at the Caltech animal facility.

Peptides. Peptides were synthesized on a Peptide Synthesizer (model 430A; Applied Biosystems Inc., Foster City, CA) by using a stepwise solid-phase chemistry protocol and machine program developed at Caltech (15). The crude peptide product was purified by semipreparative HPLC on a C8, 10 mm I.D. × 10 cm Brownlee column; 60 m linear gradient 100% A to 100% B; A: 0.1% TFA/H_2O; B: 0.1% TFA/60% CH_3CN/40%/H_2O; flow rate 0.7 ml/m.

Myelin Basic Protein. MBP was prepared from frozen brains (Pel-Freeze Biologicals, Rogers, AR) as previously described (16).

Immunization. MBP or peptides were emulsified with an equal volume of CFA supplemented with 4 mg/ml *Mycobacterium tuberculosis* H37Ra (Difco Laboratories Inc., Detroit, MI). Mice were immunized subcutaneously with 0.05 ml at two sites. At 24 and 72 h after immunization, mice were given an intravenous injection of 6 × 10^9 washed, killed *Bordetella pertussis* in saline (gift from Dr. Dale E. McFarlin, National Institutes of Health, Bethesda, MD).

Disease Severity. Clinical EAE was graded on a scale of 1 to 5 using previously established standard criteria (1): grade 1, flaccid tail; grade 2, moderate hind or front leg weakness; grade 3, severe hind or front leg weakness; grade 4, complete paralysis of limb(s); grade 5, death.

T Cell Lines, Clones, and Hybridomas. Draining lymph node cells (LNC) were harvested 10 d after subcutaneous immunization with an emulsion of 200 μg rat MBP or 20 nmol of peptide and an equal volume of CFA. 3 × 10^6 cells and 20 μg of rat MBP in a total volume of 1 ml were incubated for 4 d in Ventrex HL-1 medium (Fisher Scientific Co., Pittsburgh, PA). The cell line was subsequently stimulated every 2 wk using a final concentration of 10^4 line cells/ml, 10% rat Con A-stimulated supernatant, 20 μg/ml rat MBP or 4 μM peptide, and 2 × 10^6 ml SJL/J irradiated (3,300 rad) spleen cells in DME supplemented with 10% FCS, 2 mM glutamine, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM pyruvate (DME growth media). T cell clones were derived by limiting dilutions at 0.4 cells per well and stimulated every 2 wk as described above.

T cell hybrids were produced by 50% PEG 1450 (J. T. Baker Chemical Co., Phillipsburg, NJ) fusion of equal numbers of a HGPRT-deficient BW5147 thymoma cell line and T cell line stimulated for 24 h in 10% rat Con A supernatant. Hybridomas were selected with HAT (100 μM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine), cloned twice at 0.4 cells per well, and tested for antigen reactivity by the IL-2 assay.

Proliferation Assays. Antigen-primed LNC proliferation assays were set up by obtaining 10 d after immunization, draining LNC, and plating them at 4 × 10^5 per 200 μl of Ventrex HL-1 with and without antigen per well in 96-well microtiter plates. 4 d later, 1 μCi [^{3}H]thymidine was added to each well. Cells were harvested 18 h later using a PHD cell harvester (Cambridge Technology, Inc., Cambridge, MA) and counts per minute were determined by a liquid scintillation counter. Samples were run in triplicate and the values are expressed as the cpm antigen minus cpm control.

Line and clone proliferation assays were performed using 10^5 line or clone cells, 4 × 10^5

irradiated SJL/J spleen cells, and antigen in 200 μl Ventrex HL-1 medium per 200 μl in a 96-well microtiter plate. 2 d later 1 μCi [^{3}H]thymidine was added to each well and cells were harvested 18 h later. Values are expressed as a stimulation index: cpm antigen/cpm control.

IL-2-release assay was done using HT-2 cells as described (17). Briefly, 10^5 hybridoma cells, 5×10^5 PL/J irradiated spleen cells, and varying concentrations of antigen per 200 μl of DME growth media were cultured in 96-well plates for 24–48 h. 50 μl of supernatant was then transferred to 5×10^3 HT-2 cells in 50 μl of DME growth media per well. After 24 h, the proliferation was determined by pulsing each well with 1 μCi of [^{3}H]thymidine for 6 h.

Results

Selection of Peptides. Previous studies have established that a COOH-terminal peptic fragment of rat, guinea pig, and bovine MBPs, each encompassing 81 amino acids, contains the encephalitogenic epitope causing EAE in SJL/J mice (9). Since the synthetic peptide must most likely be homologous to mouse MBP in order to be encephalitogenic, and since both small (14 kD) and large (21 and 18.5 kD) mouse MBPs are known to contain the intact determinant (9), we first prepared two peptides selected to encompass either side of deleted exon VI of the murine small MBP gene, pM87–114 and pM155–168 (Fig. 1). In this paper, synthetic murine MBP peptides are designated pM followed by the residue numbers. By this numbering system, exon VI consists of residues 114–154, making pM87–114 and pM155–168 contiguous sequences of the murine small MBP. We included residue 114 on pM87–114, even though residue 114 belongs to exon VI. Since residues 114 and 155 are both glycines, this amino acid is at the carboxy end of pM87–114 in both the small and large forms of MBP (Fig. 1). Subsequently, additional overlapping peptides were synthesized to delineate the location of encephalitogenic epitopes in more detail.

Screening of Peptides, pM87–114 and pM155–168 on an MBP-specific T Cell Line. Peptides were initially screened for proliferation on an SJL/J, MBP-specific cell line, SML-4. Since an encephalitogenic synthetic peptide must stimulate a T cell response in order to cause EAE, it was expected that the potentially large number of peptides needed to be screened for their encephalitogenic potential could be initially screened for their ability to stimulate this T cell line. In addition, this would allow us to find, if present, T cell epitopes that do not cause disease. The T cell line used was derived from an SJL/J mouse primed with whole rat MBP and was restimulated with this same preparation of MBP in vitro. This cell line, designated SML-4, was then used to test both MBPs from various mammalian species and the synthetic peptides. Table I shows that SML-4 reacted with MBP from all the species tested, including rat, rabbit, porcine, guinea pig, and bovine. This broad pattern of reactivity is consis-

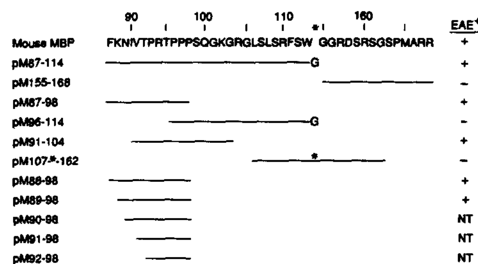

FIGURE 1. Encephalitogenic synthetic peptides of small mouse MBP COOH-terminal fragment. Peptides were synthesized with the ABI 430A Peptide Synthesizer and purified by semipreparative HPLC. Peptides were tested for encephalitogenicity by immunizing SJL/J mice with 20 nM of peptide mixed with CFA supplemented with H37Ra followed by 6×10^9 killed *B. pertussis* i.v. 24 and 72 h later. (*) Location of the deleted exon VI of the MBP gene. +, encephalitogenic; –, nonencephalitogenic.

TABLE I
Response of SML-4 T Cell Line to Xenogeneic MBP and Synthetic Peptides, pM87-114 and pM155-168

MBP	cpm	Synthetic peptides	cpm
Control	1,907	Control	776
Rat	202,988	Rat MBP	45,719
Rabbit	213,579	pM87-114	11,741
Guinea pig	144,620	pM155-168	853
Bovine	190,281		
Porcine	168,779		

10^5 SML-4 cells, 3×10^5 SJL/J irradiated spleen cells, and either 5 μg MBP/ml or 4 μM peptide were incubated in 200 μl of Ventrex HL-1 medium supplemented with 2 mM glutamine per well for 3 d. 18 h before harvesting, 1 μCi of [^{3}H]thymidine was added to each well. Results are the mean value of triplicate experiments. Additional 10-fold higher and lower concentrations of antigens were also tested and only the optimal values are shown on this table.

tent with the notion that SML-4 may be directed against the highly conserved COOH-terminal region of small MBP. When tested against the two synthetic peptides, the SML-4 cell line reacted to pM87-114, but was totally unreactive to pM155-168 (Table I). Thus, pM87-114 contains an MBP T cell determinant for SJL/J mice.

EAE Develops After Immunization with pM87-114 but not pM155-168. Mice were immunized with the two peptides along with *B. pertussis*. Confirming the SML-4 proliferation findings, five of five mice developed EAE after immunization with

TABLE II
Incidence of EAE After Immunization with Synthetic Peptides

Peptides	EAE incidence*	Day of onset‡ (range)	Average maximal grade§
pM87-114	5/5	10.8 (9-14)	3.2
pM155-168	0/5		
pM87-98	3/5	9.7 (9-10)	2.6
pM96-114	0/5		
pM91-104	3/5	10.0 (10)	3.3
	3/5	10.0 (9-12)	2.3
pM107-‖-162	0/5		
pM88-98	2/5	10.3 (10-11)	3.0
	2/5	10.0 (10)	3.0
pM89-98	0/5		
	2/10		0.5

* Values are number of affected individuals per total tested. Each line represents a separate immunization experiment.
‡ Mean value of day of onset.
§ Average maximal grade during first episode of EAE: 0 = normal, 1 = tail paralysis, 2 = hindlimb weakness, 3 = hind and forelimb weakness, 4 = complete paralysis of limb, and 5 = death.
‖ Location of the deleted exon VI of the MBP gene (see Table I).

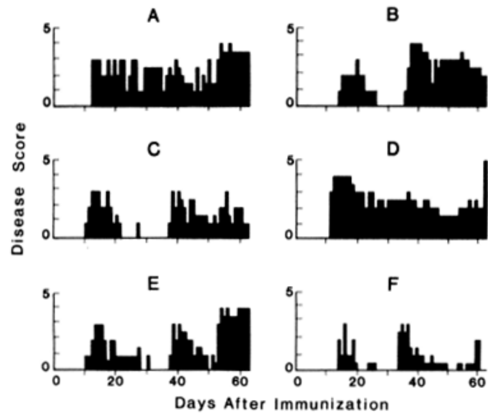

FIGURE 2. Severity of EAE after immunization with synthetic COOH-terminal MBP peptides. SJL/J mice were immunized at the base of the tail with an emulsion containing 20 nM of either pM87-114 (*A–E*) or pM88-98 (*F*) with an equal volume of CFA supplemented with H37Ra. 6 × 10^9 killed *B. pertussis* in 0.25 ml saline were given i.v. 24 and 72 h later. Mice were observed daily for EAE and graded on a five-point scale (see Table I).

pM87-114, but none of five after immunization with pM155-168 (Table II). The course of EAE in pM87-114-immunized mice was relapsing or chronic neurologic disease (Fig. 2, *A–E*). Consistent with previous reports, each relapse led to a greater degree of residual neurologic impairment.

EAE-inducing Epitopes Reside Between Murine MBP Residues 87–104. To further define the encephalitogenic T cell epitope and to determine if other encephalitogenic epitopes are present within the COOH-terminal peptide of mouse MBP, additional smaller and overlapping peptides were synthesized (Fig. 1). The first of these peptides synthesized and tested were pM87-98 and M96-114. When tested against the SML-4 cell line, only pM87-98 along with the parent molecules, pM87-114 and whole MBP were recognized (Fig. 3).

After several in vitro passages using rat MBP, the SML-4 line became unreactive to pM87-98 and all other peptides listed, although it was still highly reactive to rat MBP. Therefore, peptides were screened for their ability to induce EAE by direct immunization with peptide followed by intravenous *B. pertussis*. Table II shows that two overlapping peptides, pM87-98 and pM91-104, induced severe EAE in SJL/J mice. The disease was generally more severe with these peptides than with intact rat MBP. Peptide pM88-98 could also cause EAE, whereas pM89-98 elicited only very mild acute disease in 2 of 10 mice (partial tail paralysis). The disease course

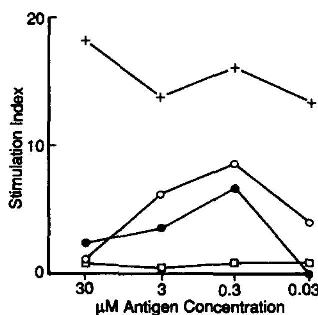

FIGURE 3. Proliferative response of SML-4 T cell line to rat MBP and synthetic COOH-terminal peptides pM87-114, pM87-98, and pM96-114. See Table II for details of proliferation assay. Results are expressed as a stimulation index = cpm antigen/cpm control. Rat MBP (●), pM87-114 (O), pM87-98 (+), and pM96-114 (□).

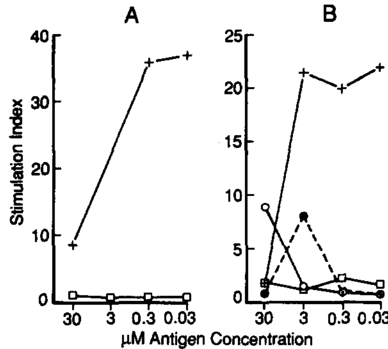

FIGURE 4. Proliferative response of the SML-4 T cell line restimulated for two cycles with pM87-98 and a representative T cell clone, 12#3, cloned from SML-4 using pM87-98 as the antigen. Cells were tested with the two overlapping encephalitogenic peptides, pM87-98 and 91-104. The details of the proliferation assay are in Table II. Results are expressed as a stimulation index = cpm antigen/cpm control. (*A*) SML-4 T cell line and (*B*) SML-4-derived T cell clone 12#3. pM87-98 (+), pM91-104 (□), pM88-98 (●), and pM89-98 (O).

was again similar to that after immunization with native MBP and varied from acute to chronic relapsing disease. An example of a mouse showing a classic relapsing disease after immunization with pM88-98 is shown in Fig. 2 *F*.

There are Likely at Least Two Epitopes within MBP Residues 87-104 that Can Induce EAE. To distinguish whether pM87-98 and pM91-104 represented distinct epitopes within mouse MBP residues, 87-104, the SML-4 line, was subcultured by restimulating with pM87-98. Both this line and clones isolated from this line reacted to pM87-98, but not to pM91-104 (Fig. 4).

Some of the peptides appeared to be toxic to lines and clones at higher concentration. Since hybridomas were less sensitive to this effect, T cell hybridomas were derived from the pM87-98-stimulated SML-4 line. The reactivity of these hybridomas to the peptides were similar to that found with the T cell clones (Fig. 5).

Since it is possible that subculturing the line and clones may have resulted in the selection of a non-crossreactive epitope of pM87-98, LNC proliferation after in vivo pM87-98 priming was tested. Again, there was reactivity against pM87-98, weaker responses against the truncated peptides pM88-98 and pM89-98, and no crossreactivity to pM91-104 (Fig. 6). Four additional encephalitogenic T cell lines, each derived from a different mouse immunized with pM87-98, showed no crossreactivity with pM91-104 (Table III).

The reciprocal experiment of LNC proliferation to the various peptides after in vivo priming with pM91-104 was also done (Fig. 7). The LNC response was the

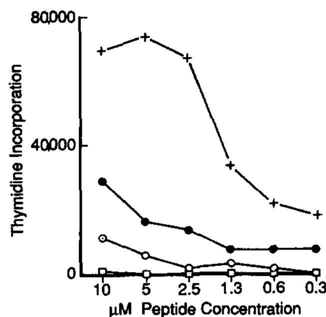

FIGURE 5. HT-2 assay of SML-4-derived T cell hybrid 17.1 specific for pM87-98. 10^5 hybrid 17.1 cells and 5×10^5 irradiated SJL/J spleen cells with varying concentration of antigen per 200 µl of DME growth media were cultured for 48 h. 50 µl of supernatant was transferred to 5×10^3 HT-2 cells in 50 µl of growth media. 1 µCi of [^{3}H]thymidine was added after 24 h and the cells were harvested 6 h later. Thymidine uptake is expressed as: (cpm with antigen) − (cpm with media alone). pM87-114 (●), pM87-98 (+), pM91-104 (□), pM88-98 (O).

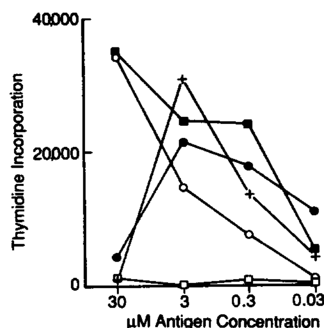

FIGURE 6. The pM87-98-primed LNC proliferative response to encephalitogenic synthetic peptides. SJL/J mice were immunized subcutaneously with an emulsion of equal volumes of 20 nM of pM87-98 in PBS and CFA supplemented with killed H37Ra. 10 d later 4 × 10^5 draining LNC were added to varying concentrations of antigen in 200 µl of synthetic media, Ventrex HL-1. 4 d later, 1 µCi of [^{3}H]thymidine was added and cells were harvested after 16 h. Thymidine uptake is expressed as: (cpm with antigen) − (cpm with media alone). pM87-98 (+), pM87-114 (O), pM91-104 (□), pM88-98 (●), pM89-98 (■). Results are representative of three separate experiments.

greatest with pM91-104; however, pM87-98 had a good, although lower response. The truncated peptides of pM87-98 could all elicit a response, except pM92-98, which was completely nonstimulatory. These observations suggest that the major response of pM91-104 is directed to an epitope also contained within pM87-98, which is likely the eight-residue peptide, pM91-98. The differences in magnitude may be due in part to changes in reactivity due to the shorter lengths of these truncated peptides. It is also possible that there are epitopes in pM91-104 that are not present in pM87-98.

(SJL/J × PL/J)F1 Develop EAE to pM87-98. Since previous reports were not clear about whether (SJL/J × PL/J)F_1 mice develop EAE to the COOH-terminal peptic fragment of MBP (9, 10), we also tested (SJL/J × PL/J)F_1 mice with one of the encephalitogenic peptides, pM87-98. Two of four mice developed EAE with an average grade of three after immunization with this peptide. The day of onset for each was 12 and 15.

pM87-98 Appears to be a Minor Epitope of Rat and Rabbit MBP for SJL/J Mice. We observed that rat MBP-primed LNC had a much greater response to rat MBP than rabbit MBP and similarly, rabbit MBP-primed LNC responded much more to rabbit MBP (Fig. 8). Since the encephalitogenic determinant has the exact same sequence in both rat and rabbit MBP, this implied that the xenogenic T cell epitope(s) was much more immunodominant than the encephalitogenic T cell epitope(s). In addi-

TABLE III
Response of pM87-98-derived Encephalitogenic T Cell Lines to M87-98 and pM91-104

Lines	cpm control	cpm pM87-98*	cpm pM91-104
SML-12.1	9,352	179,033	10,698
SML-12.3	1,442	59,380	1,883
SML-12.4	794	115,827	1,178
SML-12.5	812	58,155	340

See Table II for details of proliferation assay conditions. Values are the mean of triplicate experiments. T cell lines were tested for encephalitogenic potential by intravenous injection of 10^7 cells into SJL/J mice and followed for the development of paralysis.

* The final concentration of peptide used for the assay was 8 µM.

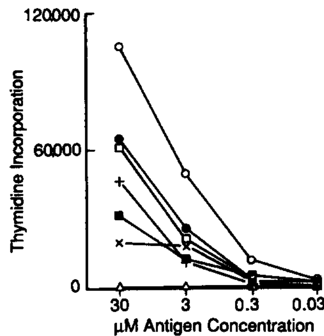

FIGURE 7. The pM91-104-primed LNC proliferative response to encephalitogenic synthetic peptides. The details of this procedure are in the legend to Fig. 5. Thymidine uptake is expressed as: (cpm with antigen) − (cpm with media alone). pM87-98 (+), pM91-104 (○), pM88-98 (□), pM89-98 (●), pM90-98 (■), pM91-98 (X), pM92-98 (Δ). Results are representative of three separate experiments.

tion, when mice primed with rat MBP were tested for recall proliferation to several peptides, the response of LNC to native MBP was much greater than to the encephalitogenic peptides, pM87-114 and pM87-98, for which the response was not significantly different than the control (Fig. 8). In contrast to this, when mice were immunized with 50 nM of pM87-114, the LNC proliferative response was present for pM87-114, pM87-98, and pM91-104, as well as the truncated peptides of pM87-98 (Fig. 9). There was also a good response with rat MBP (data not shown). Thus, these peptide determinants appear to be minor epitopes of rat MBP.

Discussion

In this study, we were able to identify in SJL/J mice two overlapping encephalitogenic peptides within the COOH-terminal region of small mouse MBP by testing for the ability of six overlapping synthetic peptides to induce EAE. Both of these pathogenic peptides could elicit a T cell response, whereas the other nonencephalitogenic peptides in this region did not. To further characterize these peptides as distinct epitopes, we generated T cell lines to one peptide, pM87-98. These lines can transfer EAE to naive mice, confirming that the disease is mediated by peptide antigen-specific T cells. The two peptide antigens appeared to define separate epitopes, since we could not demonstrate crossreactivity using lymph node cells primed in vivo to pM87-98, and T cell lines, clones, and hybrids directed against pM87-98. Thus, within a peptide determinant of 18 amino acids we are able to detect two encephalitogenic T cell epitopes.

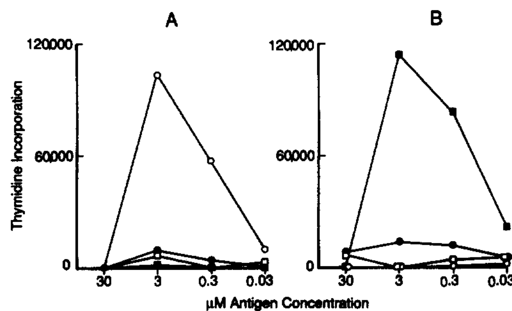

FIGURE 8. The rat (*A*) or rabbit (*B*) MBP-primed LNC proliferative response to MBP and encephalitogenic peptides. The details of this procedure are contained in the legend of Fig. 5. Thymidine uptake is expressed as: (cpm with antigen) − (cpm with media alone). Rat MBP (○), rabbit MBP (■), pM87-114 (●), pM87-98 (□). Results are representative of three separate experiments.

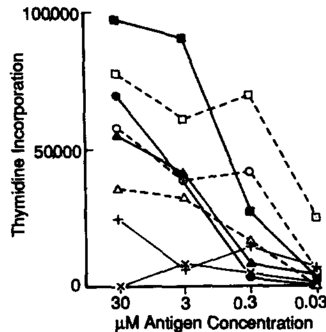

FIGURE 9. The pM89-114-primed LNC proliferative response to encephalitogenic synthetic peptides. The legend of Fig. 5 contains the details for the procedure. Thymidine uptake is expressed as: (cpm with antigen) − (cpm with media alone). pM87-114 (□), pM87-98 (○), pM91-104 (△), pM88-98 (■), pM89-98 (●), pM90-98 (▲), pM91-98 (+), pM92-98 (X). Results are representative of three separate experiments.

SJL/J mice do not express the I-E$^s_\alpha$ molecule due to a deletion of 650 bp in the promoter region (18, 19) and although intracytoplasmic I-E$^s_\alpha$ protein can be identified, there does not appear to be any significant expression of the I-E$^s_\alpha$ protein on the cell surface (18). Both encephalitogenic peptides must therefore bind to the I-A^s molecule. With the large region of overlapping amino acids, it is likely that these two peptides share sequences that bind to the I-A^s molecule, but have at least two different sets of residues recognized by T cells. One set requires sequences between residues 87 and 90 for recognition and the other may recognize a number of the shared residues between 91 and 98.

Several well-defined model systems using panels of cloned T cells have shown that there are a limited number of peptide determinants that can account for the murine T cell response to a native protein molecule. Proteins studies have included: cytochrome *c* (20–22), lysozyme (23, 24), myoglobin (25–27), ovalbumin (28, 29), staphylococcal nuclease (30), and the influenza virus hemagglutinin (31). For the autoantigen, MBP, a similar limited number of peptide determinants are found since both SJL/J and PL/J mice each develop EAE to a single, but different encephalitogenic peptide region. This limited number of determinants might be especially expected in the SJL/J strain because of both the deletion of approximately half of the T cell receptor V_β gene segments (32) and the inability to express the I-E protein dimer (18, 19).

In contrast to the PL/J encephalitogenic peptide region, pM1-9NAc, which appears to have a single encephalitogenic T cell epitope (3), the SJL/J encephalitogenic peptide region we have described here appears to have at least two distinct encephalitogenic T cell epitopes. These observations are consistent with the current model of peptide-Ia binding based on the crystallographic structure of the class I HLA-A2 (33, 34). According to this model the SJL/J encephalitogenic peptide would bind to the I-A^s molecule lengthwise in a groove that is $\sim$20 amino acid residues long. It is likely that the shared sequence between pM89-98 and 91-104 contains the residues that bind to the I-A^s molecule and that the two T cell epitopes are the result of T cells recognizing different sets of amino acids within MBP residues 89-104. Further mapping of T cell reactivity within this region may uncover additional epitopes. Careful mapping of peptide determinants for other nonautoantigens such as lysozyme (35), cytochrome *c* (36), myoglobin (37), and ovalbumin (38) have shown a similar heterogeneity of T cell epitopes for a peptide determinant. The apparent single encephalitogenic T cell epitope, pM1-9Ac found in the PL/J mice (3) may

be explained by its position at the NH_2-terminal of MBP and the use of rat MBP as the immunogen. Based on the model of peptide determinants for nonautoantigens (35–38), multiple T cell epitopes would be expected along an Ia-binding peptide region. Since murine MBP differs from rat MBP at residues 10 and 11, due to a deletion of histidine and glycine in murine MBP, immunization of PL/J mice with rat MBP will produce murine MBP-specific T cells restricted to reactivity to pM1-9Ac. As expected, immunization with rat MBP yields additional T cell epitopes, involving residues 10 and 11 of rat MBP, which are nonencephalitogenic most likely because they fail to recognize murine MBP (10). Immunization of PL/J mice with a longer NH_2-terminal peptide homologous to mouse MBP may uncover additional encephalitogenic epitopes, involving residues 10 and 11 of murine MBP. Although pM1-9Ac appears to be short enough to define a single epitope, fine mapping of pM1-9Ac by amino acid substitutions may reveal more epitope(s). The presence of multiple T cell epitopes may possibly play a role in relapsing disease. Additional truncated and single amino acid substituted peptides will allow us to map and assess the significance of these encephalitogenic epitopes in greater detail.

One of the peptides, pM87–98, was tested on (SJL/J × PL/J)F_1 mice and found to be highly encephalitogenic. Although only a small number of mice were studied, the incidence and severity of disease were similar to that induced in the SJL/J parental strain. Prior studies using peptic fragments of guinea pig MBP showed that these (SJL/J × PL/J)F_1 mice developed EAE with an incidence of 11/14 peptide fragment residues 1–37, but only 1/14 to residues 89–169 (9). In addition, MBP-specific encephalitogenic T cell clones derived from (SJL/J × PL/J)F_1 mice have all been reactive with the NH_2-terminal epitope, but not with the COOH-terminal peptide (39). The results of studies using preparations of COOH-terminal peptide fragments may have been affected by contamination with small amounts of NH_2-terminal peptides, and therefore, it was unclear whether the COOH-terminal peptide could induce any EAE in (SJL/J × PL/J)F_1 mice (10). Since we have found that (SJL/J × PL/J)F_1 mice develop EAE to the synthetic peptide pM87–98, it is unlikely that depletion of the T cell repertoire due to thymic selection (40) is the cause of the bias toward recognition of the NH_2-terminal peptide. It would appear from lymph node proliferation studies that antigen processing of MBP may be the major determinant of this bias (Kono, D., unpublished data).

This (SJL/J × PL/J)F_1 bias toward recognition of the NH_2-terminal peptide is consistent with our finding of the SJL/J MBP COOH-terminal peptides as being a minor or "subdominant" (41) determinant compared with the PL/J MBP NH_2-terminal peptide which is "immunodominant" (Kono, D., unpublished data). In addition, the observation that xenogeneic MBP T cell epitopes appear to be immunodominant may account for the difficulty in obtaining encephalitogenic T cell clones in SJL/J mice after immunization with xenogeneic MBP (14, 42, 43), and the success of obtaining encephalitogenic T cell clones by alternating MBP from different species to restimulate T cell lines before cloning (4). Alternating MBP from different species would select for T cells recognizing MBP determinants common to the different species that would also be homologous to mouse MBP. The fact that a minor peptide determinant can cause EAE raises the question of whether additional encephalitogenic minor peptide determinants of MBP are present that have escaped detection. To address this question, overlapping peptides encompassing the

whole MBP protein will likely have to be synthesized and characterized for their ability to solicit T cell responses and EAE. In addition, neonatal tolerance to synthetic peptides has been shown to be an effective method for tolerizing mice to a single specific epitope (44). This approach may prove useful for determining whether there are other encephalitogenic MBP-specific epitopes in SJL/J and PL/J mice. In future studies we plan to use murine MBP in order to avoid the bias in T cell repertoire caused by the immunodominant xenogenic T cell epitopes of rat MBP.

DeLisi and Berzofsky (45) have found that the majority of immunodominant T cell peptide epitopes tend to form amphipathic α-helical structures with periodical variations in their hydrophobicity so that hydrophobic residues are primarily on one side of the α helix and hydrophilic residues on the other. Based on this model, an algorithm was subsequently created that was able to identify 75% of a series of known T cell epitopes (46). When we applied this algorithm to the small mouse COOH terminal of MBP, the only region with sufficient amphipathic properties to score as immunodominant was one spanning MBP, residues 83–93. Both of the encephalitogenic peptides contain residues within this region. These data suggest that the amphipathic model of peptides capable of activating T cells may be helpful in identifying potential T cell determinants but it certainly does not predict their locations precisely.

The observations on the encephalitogenic determinants of MBP for both the SJL/J and PL/J mice show that the response to this autoantigen from a "privileged site" follows the same principles for T helper cell antigen recognition as any nonautoantigen. This may differ from autoantigens that are accessible to the thymus, in which there may be deletion of T cells recognizing these antigens. This suggests that interventions that suppress the T cell response to nonautoantigens may also be successful in suppressing the T cell response to autoantigens from privileged sites and may play a role in therapy since the T helper cell is of major importance in the pathogenesis of autoimmune disease.

T helper cells alone can transfer disease in several models of autoimmunity such as EAE (1–4), adjuvant arthritis (8), experimental allergic thyroiditis (6), and collagen arthritis (47). In addition, treatment of animals with anti-L3T4 has been successful in treating EAE (48), experimental allergic thyroiditis (6), murine lupus (5), and collagen arthritis (7). EAE is the prototype model of antigen-specific T helper cell–mediated autoimmune disease since it is the best characterized and the neurological damage seen in this disease may be directly mediated by T helper cells alone. Thus, principles derived from this model may have direct implications for the other animal models and human autoimmune diseases. Since the MHC–peptide epitope-T cell receptor complex interaction is one of the few pathogenic mechanisms with specificity, specific intervention directed at this complex may lead to specific therapy of autoimmune disease without suppression of the whole immune system. The finding now of a limited number of encephalitogenic T cell peptide determinants to MBP in two different strains of mice supports this possibility. Possibly antibodies directed against clonotypic determinants or even variable gene segments of the T cell receptor may provide specific treatment for EAE, since there appears to be a very limited variable gene segment usage for a single T cell epitope (49, 50). In fact, we have recently discovered a highly restricted use of variable gene segments in both the α and β chains of the T cell receptors recognizing the PL/J encephalitogenic epitope

(Urban, J., manuscript in preparation). However, our findings of at least two encephalitogenic T cell epitopes within a given peptide determinant and that minor or subdominant determinants are also encephalitogenic point to the complexity of the interactions and the potential difficulties that must be addressed when devising strategies toward specific therapy directed at the MHC-peptide epitope-T cell receptor interaction. Further studies to define and map the encephalitogenic T cell epitopes for murine MBP using synthetic peptides will provide a better understanding of how this interaction contributes to the development of autoimmunity.

Summary

Experimental allergic encephalomyelitis (EAE) is an autoimmune demyelinating disease of the central nervous system (CNS) that occurs after immunization of animals with myelin basic protein (MBP). The disease is a prototype model for the study of antigen-specific T helper cell-mediated autoimmune disease. In SJL/J mice, EAE is mediated by T helper cells directed against a 40-amino acid COOH-terminal peptic fragment of mouse small MBP. To identify the minimal T cell epitopes of MBP responsible for EAE, overlapping peptides completely encompassing the epitopes within this region were synthesized. A 28-residue peptide of mouse MBP spanning residues 87-114 (pM87-114) was able to elicit both a strong T cell response and chronic relapsing disease. To better localize the T cell epitopes, shorter peptides within this region were synthesized and two overlapping peptides, pM87-98 and pM91-104, were able to induce EAE. T cell clones and bulk lymph node cell populations reactive with pM87-98 did not respond to pM91-104. However, lymph node cells reactive with pM91-104 also reacted with pM87-98, thus showing that these two peptides represent contiguous, but distinct encephalitogenic epitopes and that both these epitopes may be contained within pM87-98. In addition, pM87-114 and pM87-98 were found to be minor determinants of the total T cell response to rat and rabbit MBP. The restricted response to MBP in SJL/J mice is similar to that of the PL/J mice in that each appears to have only a single peptide region in MBP that elicits encephalitogenic T cells. However, within the region studied, there were two if not more T cell epitopes. This differs from the single encephalitogenic PL/J epitope. These findings of a single encephalitogenic peptide region with multiple T cell epitopes and the fact that encephalitogenic T cell epitopes may be subdominant have implications for the design of treatments directed at the T cell receptor-MHC-peptide epitope complex in autoimmune disease.

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