IDENTIFICATION AND CHARACTERIZATION OF A SECOND ENCEPHALITOGENIC DETERMINANT OF MYELIN PROTEOLIPID PROTEIN (RESIDUES 178–191) FOR SJL MICE[1]

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We previously described a synthetic peptide of myelin proteolipid protein (PLP), peptide 139–151, which induces experimental allergic encephalomyelitis in SJL/J (H-2^s) mice. We have now identified an additional determinant, PLP residues 178–191, that is also a potent encephalitogen in this strain. When PLP peptide 178–191 was compared with peptide 139–151 on an equimolar basis, the day of onset of disease induced by PLP 178–191 was earlier, but the incidence, severity, and histologic features were indistinguishable. Lymph node cells from animals immunized with the whole PLP molecule responded to both PLP 178–191 and 139–151, suggesting immunologic codominance of the two epitopes. PLP 178–191 elicited stronger proliferative responses and this may relate to the earlier onset of disease induced with this peptide. Two CD4$^+$, peptide-specific, I-A^s-restricted T cell lines, selected by stimulation of lymph node cells with either PLP 178–191 or 139–151, were each encephalitogenic in naive syngeneic mice. The presence of multiple encephalitogenic codominant PLP epitopes within a single mouse strain emphasizes the complexity of the immune response to PLP and its potential as a target Ag in autoimmune demyelinating diseases.

EAE[3] is a T cell-mediated autoimmune disease that is widely used as a model for MS. It has long been known that EAE can be induced in laboratory animals by immunization with spinal cord homogenate, CNS myelin, or the major myelin protein constituents (1, 2). The encephalitogenic activity of MBP and MBP peptides has been studied extensively and has been the basis for therapeutic strategies for EAE and MS. However, MBP alone does not account for all of the encephalitogenic activity of myelin. More recently, PLP, the most abundant protein constituent of CNS myelin, has been accepted as a major encephalitogen (3–7). PLP is a hydrophobic, polytopic integral membrane protein with physical and chemical properties that have made it difficult to study. Synthetic peptides based on the PLP sequence have now begun to provide information on its immunologic properties.

Encephalitogenic PLP epitopes have been identified in strains of mice representing several H-2 haplotypes. PLP 139–151 (HCLGKWLGHPDKF) is encephalitogenic in SJL (H-2^s) mice (6), PLP 103–116 (YKTTICGKGLSATV) in SWR (H-2^q) mice (7), PLP 215–232 (GKVCGSNLLSICKTAEF) in C3H (H-2^k) mice (8), and PLP 43–64 (EKLIETYFSKNYQDYEYLINVI) in PL/J (H-2^u) mice (9). Thus far, only a single PLP determinant has been identified in any one mouse strain, thereby limiting the scope of the immunologic studies that can be carried out. The identification of additional encephalitogenic epitopes within a single strain and the characterization of the T cell responses to such determinants would permit more complete analysis of the immune response to the whole PLP molecule. This information would provide insight for understanding analogous responses in humans.

A previous study in SJL mice indirectly suggested an encephalitogenic epitope(s) in addition to PLP 139–151, in that some encephalitogenic PLP-specific T cell clones cross-reacted with DM-20 (5). DM-20 is an alternatively spliced form of PLP in which residues 116–150 are deleted (10). In our study, we report the identification and characterization of a second encephalitogenic epitope of PLP in SJL mice. PLP 178–191 (NTWTTCQSIAFPSK) has been found to induce severe EAE in this strain. Furthermore, mice immunized with the whole PLP molecule respond strongly to both peptides 139–151 and 178–191, suggesting a codominant recognition of these determinants. Some of the properties of encephalitogenic T cell lines specific for the two epitopes are also described.

MATERIALS AND METHODS

Mice. Female SJL/J (H-2^s) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. They were age matched for each experiment and were immunized at 10 to 14 wk of age. Various H-2^s congenic mice (A.TL, B10.HTT, and A.TH) were obtained from Dr. David Sachs (National Institutes of Health, Bethesda, MD).

PLP and PLP peptides. PLP was prepared by concentrating a washed total lipid extract of bovine white matter (11) and partially removing lipids by acetone extraction. The protein was then dissolved in chloroform-methanol-acetic acid (2:1:0.03 by volume) and the remaining lipids were removed by two passages through a Seph-

adex LH-60 column equilibrated and eluted with the same solvent mixture (12). Conversion of PLP to the aqueous form was carried out by evaporation of the organic solvent in a stream of nitrogen and gradual replacement with water (13). Finally, the aqueous solution was dialyzed at 4°C against three changes of double distilled deionized water to remove acid.

PLP peptides were prepared according to the sequence of murine PLP (14, 15). Peptides 103–116, 139–151, and 178–191 were synthesized in the laboratory of Dr. R. Laursen (Department of Chemistry, Boston University, Boston, MA) on a Milligen model 9050 synthesizer (Millipore Corp., Bedford, MA), using FMOC chemistry. Milligen PAL resins, which produce peptides with C-terminal amides, were used as the solid support. Peptides 170–189 and 180–199 were a generous gift from Dr. D. A. Hafler (Brigham and Womens Hospital, Boston, MA). The sequences of these peptides are shown in Table I.

Active induction of EAE. Mice were injected s.c. in the flank with 100 μg of whole PLP or PLP peptide(s) and 200 μg of *Mycobacteria tuberculosis* H37Ra (Difco Laboratories, Detroit, MI) in an emulsion consisting of equal volumes of water and CFA (Difco). Peptide 178–191 was dissolved in 0.02 M acetic acid, because it was not soluble in water. Each mouse was also injected i.v. on days 0 and 3 with 4.5 × 10⁹ *Bordetella pertussis* bacilli (pertussis vaccine, lot no. 264, Massachusetts Public Health Biologics Laboratories, Boston, MA), unless otherwise stated. Control mice received identical treatment as test animals, except that PLP or PLP peptides were not added.

Clinical and histologic evaluation. Mice were assessed clinically as previously described (4) according to the following criteria: 0, no disease; 1, decreased tail tone or slightly clumsy gait; 2, tail atony and/or moderately clumsy gait and/or poor righting ability; 3, limb weakness; 4, limb paralysis; and 5, moribund state. Animals were killed within 10 days of the initial appearance of clinical signs of disease. Mice that showed no clinical signs were killed 25 to 28 days after immunization. Brains and spinal cords were removed and fixed in 10% phosphate-buffered formalin, and paraffin embedded sections were stained with luxol fast blue-hematoxylin and eosin for light microscopy. Histologic disease was quantitated by counting the inflammatory foci in meninges and parenchyma as previously described (16).

Preparation of LNC and T cell lines. Pooled LNC were prepared from inguinal lymph nodes from two to three mice injected 8 days earlier with a single s.c. injection of 100 μg whole PLP or PLP peptide in CFA. Control animals were injected with an emulsion of CFA and water (or 0.02 M acetic acid).

Thereafter, LNC were used for proliferation assays, or were stimulated with PLP peptide 139–151 or 178–191 to produce Ag-specific T cell lines. T cell lines were maintained in RPMI containing T cell growth promoter (American Bio-Technologies Inc., Cambridge, MA) and stimulated two to three times at approximately 10- to 15-day intervals with 20 μg/ml PLP peptide, using mitomycin C-treated syngeneic splenocytes as APC.

mAb. The following cell lines producing mAb (with their respective specificities) were obtained from the American Type Culture Collection, Rockville, MD: TIB 105 (CD8), 14.4.4.S (I-Eᵏ), 10.2.16 (I-Aˢ), and HO.13.4 (Thy-1.2). mAb 145.2C11 (CD3), GK1.5 (CD4), and H57.597 (TCR-αβ) were obtained from Drs. Jeffrey Bluestone (University of Chicago, Chicago, IL), Frank Fitch (University of Chicago), and Ralph Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO), respectively. Antibodies were used as culture supernatants or, for anti-I-A antibody, as ascites.

Proliferative assay. The in vitro proliferative responses of LNC and T cell lines were assayed in triplicate in 96-well microtiter plates. LNC were used at a concentration of 3 × 10⁵ cells/well. T cell lines were used at 5 × 10⁴ cells/well with 5 × 10⁵ mitomycin C-treated splenocytes/well as APC. Cells were incubated for 72 h at 37°C in 5% CO₂ in the presence or absence of Ag. One μCi of [³H]TdR was added during the final 18 to 20 h of culture. The plates were harvested using a PHD cell harvester (Cambridge Technology, Cambridge, MA) and counted using standard liquid scintillation techniques in a Beckman scintillation counter model LS5000 (Beckman Instruments Inc., Fullerton, CA). In some experiments, various mAb were added to block the Ag-specific proliferative response of T cell

lines. These blocking assays were set up following the methods described previously (17). The data are expressed as stimulation indices (SI) that were determined by the formula:

$$SI = \frac{\text{mean cpm of Ag-containing triplicate wells}}{\text{mean cpm of control triplicate wells}}$$

All SD were <15% of the mean.

Flow cytometric analysis. Samples containing at least 1 × 10⁶ cells were incubated on ice for 30 min with 20 to 50 μl of hybridoma culture supernatant and washed two or three times with PBS containing 0.1% BSA and 0.1% NaN₃. These samples were then incubated for 30 min with 25 μl of the appropriate FITC-conjugated second antibody reagent. Second step reagents included a 1/50 dilution of FITC-conjugated rabbit anti-hamster Ig, goat anti-rat Ig, or goat anti-mouse Ig (Cooper Biomedicals, Organon Teknica, Malvern, PA). After the final incubation, samples were washed twice, fixed in 1% paraformaldehyde, and analyzed on a FACS SCAN (Coulter Corp., Hialeah, FL).

Passive transfer of EAE. Cell lines were cultured in the presence of 20 μg/ml of the specific peptide. After 3 days, dead cells were removed on a Ficoll gradient. Live cells were washed twice and 2 × 10⁶ cells were injected i.v. into naive recipient mice.

RESULTS

Induction of disease with peptide 178–191. In the course of attempts to produce antibodies against defined regions of PLP, we immunized pairs of SJL mice with either PLP peptide 170–189 or 180–199 (100 μg peptide/mouse in CFA). These mice did not receive pertussis vaccine. Nevertheless, both mice receiving PLP 170–189 and one mouse receiving PLP 180–199 developed severe EAE 13 to 15 days postimmunization with clinical disease scores of 3 to 4.

Inasmuch as the two peptides overlap and both cause disease, it seemed likely that the overlapping region corresponded to an encephalitogenic epitope. To confirm this observation, eight SJL mice were immunized with PLP peptide 178–191 by the standard protocol described in *Materials and Methods.* Four control mice received the same treatment, except that peptide was not included. All mice receiving injections of PLP 178–191 developed EAE 11–13 (12.4 ± 0.7) days postinjection. Clinical signs were severe, with a mean clinical score of 4.8. None of the control mice developed clinical signs of EAE.

Histologically, meningeal inflammatory infiltrates were present over the entire neuraxis of mice immunized with PLP 178–191 (Fig. 1a). Perivascular, predominantly perivenous, inflammation was present in optic nerves and in cerebral periventricular, cerebellar, brain stem, and spinal cord white matter. Infiltrates consisted of mixtures of mononuclear inflammatory cells and neutrophils with more neutrophils in the parenchyma than in the meninges. No inflammation was present in the choroid plexus, spinal nerves, or other cranial nerve roots. Small foci of perivascular demyelination were present adjacent to dorsal root entry zones in the spinal cords. None of the control mice had histologic disease.

Comparison of disease induction by equimolar amounts of two encephalitogenic peptides. To compare the encephalitogenic activity of the two SJL determinants, PLP 139–151 and 178–191, groups of mice were immunized with equimolar amounts of each peptide and then evaluated for the development of EAE. Twelve of 12 animals immunized with PLP 178–191 and 10/12 mice immunized with PLP 139–151 developed clinical signs (Table II). At peptide concentrations ranging from 10 to 50 nmol, disease was severe in both groups. Mice immu-

TABLE I

Sequences of PLP peptides used[a]

Peptide	Sequence	M.W.
PLP 103-116	YKTTICGKGLSATV	1439
PLP 139-151	HSLGKWLGHPDKF	1519
PLP 170-189	AVPVYIYFNTWTTCQSIAFP	2966
PLP 178-191	NTWTTCQSIAFPSK	1573
PLP 180-199	WTTCQSIAFPSKTSASIGSL	2090

[a] Sequences shown are based on murine PLP (14, 15). S was substituted for the native C at position 140 in peptide 139-151 (6).
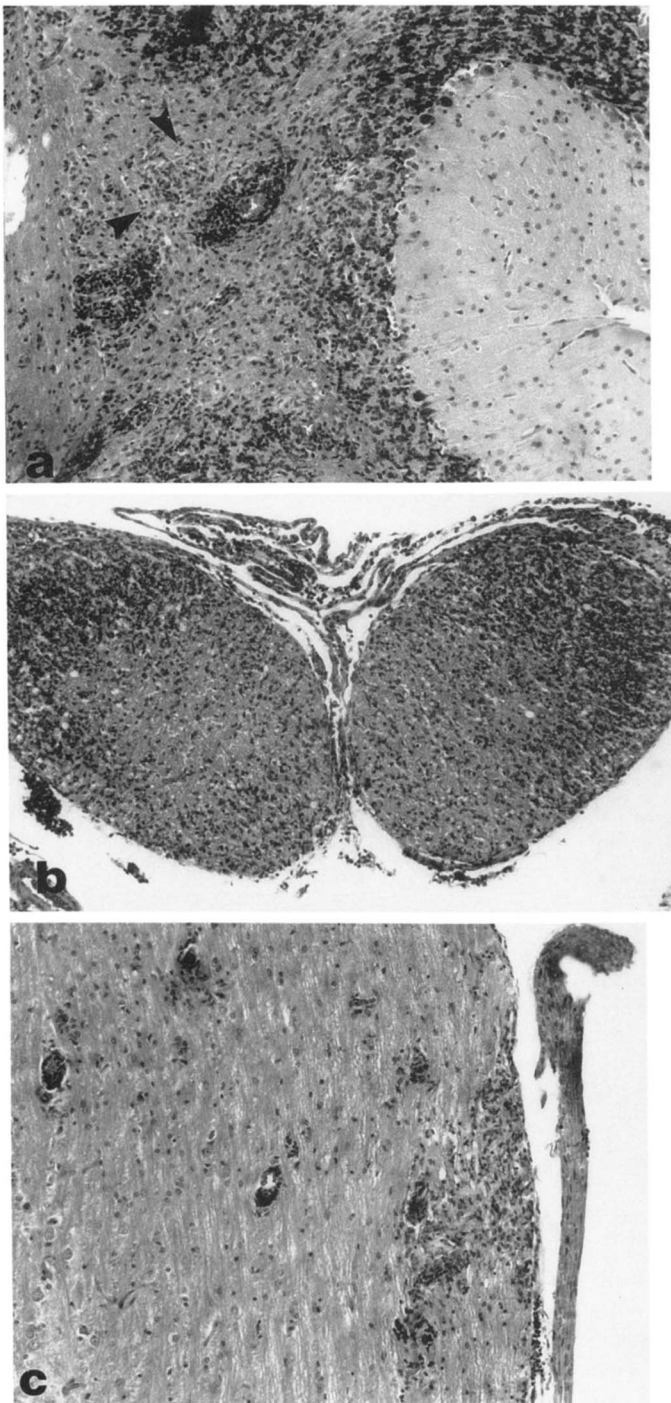
Figure 1. Histologic features of EAE in PLP 178–191 immunized SJL mice. *a*, Perivascular mononuclear cell infiltrates with associated mild demyelination (*arrowheads*) in the cerebellar white matter. *b*, Infiltration of neutrophils and mononuclear cells in the optic nerves. *c*, Mononuclear cell infiltrate in the spinal cord. Sections are from CNS tissue of mice that had been sensitized with 25 to 64 nmol (39–100 μg) PLP peptide 178–191, 12 to 15 days previously and had clinical scores of 5. Luxol fast blue-hematoxylin and eosin, 125×.

nized with PLP 178–191 consistently developed EAE earlier than those immunized with 139–151 (12.8 ± 0.8 vs 19.4 ± 3.9 days; $p < 0.001$), but the maximal clinical scores and histologic features of the disease were essentially the same in the two groups. Histologically, typical EAE lesions, with associated demyelination in some cases, were observed in all mice immunized with the various doses of PLP 178–191 (Fig. 1, *b* and *c*), and in all

except one immunized with PLP 139–151. Control mice, which received either no peptide or an irrelevant PLP peptide, showed no clinical or histologic disease.

Proliferative responses of LNC from mice immunized with whole PLP or peptide 178–191. LNC from mice injected with PLP 178–191 responded strongly to the immunizing peptide in proliferative assays (Fig. 2*a*). The stimulation indices ranged from 6 to 17, depending on peptide concentration. The response of these LNC to PLP 139–151 was no more than two to three times the background count and there was no response to the control peptide 103–116. The LNC from mice immunized with whole PLP responded to both of the encephalitogenic peptides, but the response to peptide 178–191 was consistently greater than that to peptide 139–151, when tested at concentrations of 25 or 50 μg/ml (Fig. 2*b*). LNC from control mice did not respond to any of the peptides.

Two possible reasons for the enhanced response to PLP 178–191 by LNC from mice immunized with whole PLP are that PLP 178–191 is more immunogenic, or that the presence of DM-20 in the PLP preparation increases the amount of this peptide in the immunizing material. To test the latter, mice were immunized with mixtures of the two peptides. LNC from mice immunized with equimolar amounts of the two peptides responded more strongly to PLP 178–191 than to 139–151 (Fig. 3*a*), and the responses were comparable to those observed with whole PLP-immunized mice (Fig. 2*b*). Next, the relative immunogenicity of the two peptides was tested by immunizing mice with mixtures of the peptides in which the molar amount of PLP 139–151 was kept constant but the amount of PLP 178–191 was decreased. The proliferative responses of the LNC to peptides 139–151 and 178–191 were approximately equal when the mice were immunized with 2.5 times more PLP 139–151 (Fig. 3*b*). Even when 10 times more PLP 139–151 was used, significant proliferative responses to PLP 178–191 were observed (data not shown).

Production and characterization of T cell lines. T cell lines were derived from the LNC of SJL mice after immunization with PLP peptides 139–151 or 178–191. Line E-13.S, specific for PLP 139–151, responded to PLP peptide 139–151, but not to 178–191. Similarly, line P-17.S, specific for PLP 178–191, responded to this peptide, but not to 139–151 (Fig. 4). Flow cytometric analysis showed that both lines were Thy-1.2$^+$, CD3$^+$, CD4$^+$, CD8$^-$, and TCR-$\alpha\beta^+$ (data not shown).

To further characterize the T cell lines, the effect of adding mAb specific for CD4, CD8, I-A^s, and I-E^k on the stimulation of lines E-13.S and P-17.S by their respective Ag was investigated. Anti-CD4 mAb blocked the proliferative response of both lines (94% inhibition of the response of E-13.S to PLP 139–151, and 77% inhibition of the response of P-17.S to PLP 178–191), whereas anti-CD8 and anti-I-E^k antibodies did not block (5–20% inhibition). Anti-I-A^s mAb partially blocked the proliferative responses of both lines (approximately 50% inhibition for each line). It was not clear from this result whether the anti-I-A^s mAb was inefficient under the experimental conditions, or whether the lines were restricted by elements other than I-A^s. Therefore, to determine the MHC restriction of the T cell lines, splenocytes from congenic strains of mice that differ from SJL at various H-2 loci were used as potential APC in proliferative assays. APC

TABLE II

Comparison of EAE induction with equimolar amounts of PLP peptides 139-151 and 178-191

Peptide[a]	Clinical EAE			Histologic EAE	
	Incidence	Day of onset[b]	Score[c]	Incidence	No. foci[d]
178-191					
10 nmol	4/4	13.0 (12–14)	3.8 (1–5)	4/4	111 ± 77
25 nmol	4/4	12.5 (11–14)	4.0 (3–5)	4/4	49 ± 36
50 nmol	4/4	12.8 (12–13)	4.3 (3–5)	4/4	185 ± 97
139-151					
10 nmol	3/4	16.6 (14–18)	3.0 (2–5)	3/4	139 ± 20
25 nmol	3/4	20.7 (16–26)	3.7 (2–5)	4/4	102 ± 45
50 nmol	4/4	20.5 (16–26)	4.0 (4)	4/4	88 ± 41
Controls					
103-116					
50 nmol	0/2		0	0/2	0
no peptide	0/4		0	0/4	0

[a] Mice were immunized with 10, 25 or 50 nmol of PLP 139-151 or PLP 178-191, and 50 nmol of PLP 103-116 as described in Materials and Methods.
[b] Values are mean day after inoculation and ranges for each group.
[c] Mean maximum clinical score and range of animals with disease.
[d] Mean ± SD of number of inflammatory foci in meninges and parenchyma of mice with histologic EAE or control mice.
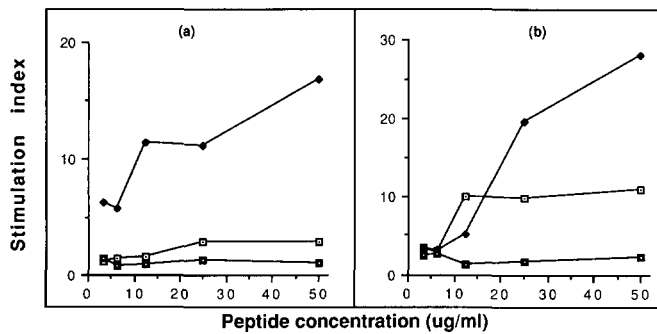

Figure 2. Proliferative response of LNC from mice immunized with 100 μg of PLP peptide 178-191 (a) or 100 μg of whole PLP (b). LNC were obtained from two or three mice 8 days after immunization and were tested for their ability to proliferate to peptides. LNC (3 × 10⁵/well) were incubated for 72 h with various concentrations of PLP 139-151 (▣), PLP 178-191 (♦), or PLP 103-116 (▨). One μCi of [³H]TdR was added during the last 16 to 18 h of incubation. Data are presented as mean stimulation indices from a representative experiment. Background cpm were: a, 1212 and b, 1749.
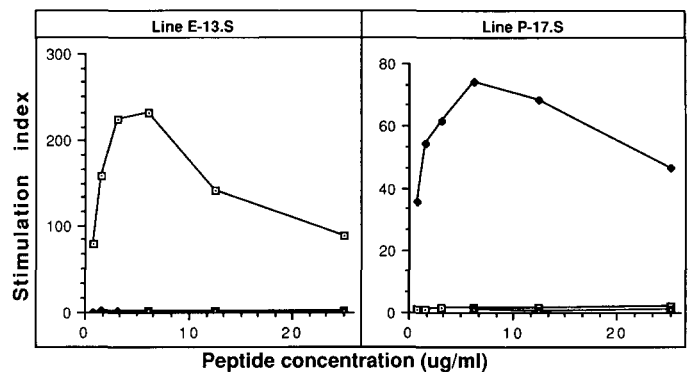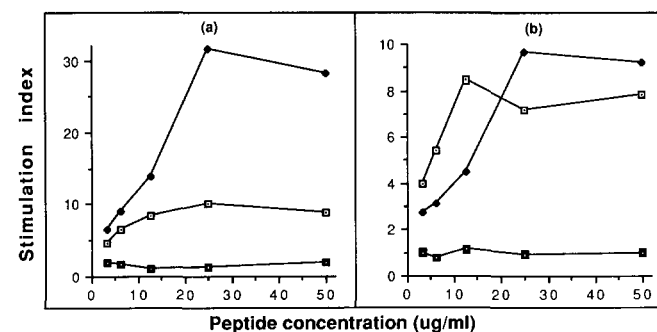

Figure 3. Proliferative responses of LNC from mice immunized with equimolar amounts (50 nmol) of PLP peptides 139-151 + 178-191 (a) or 50 nmol of PLP 139-151 + 20 nmol of PLP 178-191 (b). LNC were prepared and tested as described in the legend to Figure 2. Data are presented as mean stimulation indices from a representative experiment. Background cpm were: a, 660 and b, 2894.

from SJL, A.TH and B10.HTT mice, all of which express I-A^s, could present Ag to both T cell lines and induce specific proliferative responses (Table III). In contrast, splenocytes from A.TL mice, which express I-A^k, were unable to present antigen to either cell line. Therefore, the T cell lines recognize their respective Ag in the context of I-A^s. The lower stimulation indices for B10.HTT mice

Figure 4. Proliferative responses of T cell lines E-13.S and P-17.S to Ag. T cells (5 × 10⁴/well) and syngeneic APC (5 × 10⁵/well) were incubated for 72 h with various concentrations of PLP 139-151 (▣), PLP 178-191 (♦) or PLP 103-116 (▨). One μCi of [³H]TdR was added during the last 16 to 18 h of incubation. Data are presented as mean stimulation indices from a representative experiment. Background cpm = 1131 for E-13.S and 3413 for P-17.S.

TABLE III

Proliferative responses of T cell lines using APC from mice with different MHC haplotypes

T Cell Line[a]	Peptide[b]	APC Donor Mice[c]			
		SJL (K^sA^sD^s)	A.TH (K^sA^sD^d)	B10.HTT (K^sA^sE^kD^k)	A.TL (K^sA^kE^kD^d)
E-13.S	139–151	184.1[d]	182.9	14.3	0.8
	178–191	1.4	0.9	1.0	1.1
	103–116	1.4	1.2	1.1	0.9
P-17.S	139–151	1.0	1.6	1.0	0.8
	178–191	279.6	457.5	17.2	0.8
	103–116	0.9	1.2	0.8	0.9

[a] T cell lines E-13.S and P-17.S were selected and grown in vitro as described in the text.
[b] Peptides were added to proliferation assays at a concentration of 5 μg/ml.
[c] APC were in the form of mitomycin C-treated splenocytes from the strains of mice indicated.
[d] Values represent stimulation indices. Background cpm were 500 to 2,000 when SJL and A.TH splenocytes were used as APC, and 40,000 to 60,000 when B10.HTT or A.TL were used.

reflect higher background cpm (40,000–60,000) for cultures containing B10.HTT splenocytes as APC, compared to background cpm of 500 to 2000 with SJL or A.TH splenocytes. Despite this, significant proliferative responses were still observed with antigen. High back-

ground cpm also occurred when A.TL splenocytes were used as APC. This effect was probably due to alloreactivity of TCR Vβ17 bearing T cells to I-E molecules expressed on APC from the B10.HTT and A.TL mice (18).

The ability of lines E-13.S and P-17.S to induce EAE after transfer in vivo was tested. Both lines were found to be encephalitogenic when injected i.v. into naive SJL mice (Table IV). Mice that received line P-17S developed disease more rapidly than did those that received line E-13.S. However, the latter group had more severe disease. Histologically, the adoptively transferred disease was identical to that induced by active sensitization with PLP peptides 139–151 and 178–191.

DISCUSSION

This is the first report of multiple encephalitogenic PLP epitopes within a single strain of mice. However, more than one PLP epitope for SJL mice was indirectly suggested by two previous studies. Van der Veen et al. (19) derived several PLP-specific T cell clones from SJL mice. Only some of the clones were specific for residues 139–151, thereby implying the existence of an additional epitope. Satoh et al. (5) found that some encephalitogenic T cell lines from PLP-immunized mice responded to DM-20, as well as to PLP, implying that an additional encephalitogenic epitope is present outside the 116–150 region. We have shown that PLP peptide 178–191 is encephalitogenic in SJL mice and that disease can be passively transferred by a T cell line responding to that peptide.

Recently, Endoh et al. (8) reported that sensitization with bovine DM-20 did not induce EAE in SJL mice. In view of our results, an encephalitogenic response might have been expected. The apparent discrepancy could be related to sequence differences between bovine and mouse PLP. Although the sequence of PLP is highly conserved, there are several differences between the bovine and mouse molecules. In the mouse, residue 188 is phenylalanine, whereas it is alanine in the bovine sequence (20). The peptide that we found to be encephalitogenic was synthesized according to the mouse sequence, and it is possible that the phenylalanine residue is critical for disease induction. Endoh et al. (8) also reported that a mixture of bovine PLP peptides (181–211 + 212–276) failed to induce EAE. This could be due to the differences between the bovine and mouse sequences or the absence of a critical residue(s) at the N-terminus. Furthermore, it is possible that the mixture of the two large peptides may contain suppressor epitopes that prevent EAE. We have previously found that whereas peptide 139–151 induces severe disease in SJL mice, the larger peptide 137–154 is not as effective (6). More recent studies show that the presence of additional residues flanking PLP 139–151

results in a tolerogenic response (21) and/or induction of Ts cells (V. K. Kuchroo, unpublished observations).

Our results indicate little difference in the incidence, severity, or histologic features of EAE induced by PLP peptides 139–151 and 178–191. However, PLP 178–191 induced disease more rapidly, and this may relate to its greater immunogenicity. We considered that the stronger proliferative response to PLP 178–191 in whole PLP-immunized mice might be attributable to the presence of DM-20 in the PLP preparation. Peptide 178–191 is present in both PLP and DM-20, whereas peptide 139–151 is only in PLP. DM-20 is not separated from PLP by the methods used in our study and, as a consequence, there is more 178–191 than 139–151 in the immunizing preparation. Nevertheless, when equimolar amounts of the two synthetic peptides were injected together, PLP 178–191 still induced a stronger response and the responses were comparable only when there was at least 2.5 times more peptide 139–151. This difference could be due to more efficient binding to the I-A^s molecule or to recruitment of a larger T cell repertoire. Alternatively, the relative insolubility of PLP 178–191 under nonacidic conditions may lead to its aggregation, thereby increasing the efficiency of presentation by APC to Ag-specific T cells.

The codominant recognition of PLP 139–151 and 178–191 described herein is in direct contrast to observations on MBP epitopes. EAE studies involving MBP have shown multiple immunogenic and encephalitogenic epitopes within single mouse strains (22–24). However, after immunization with the whole MBP molecule, the response is predominantly directed against a single epitope (24–26). Similarly, when (PL/J × SJL)F$_1$ mice are immunized with whole MBP, a T cell response is observed only to the PL/J determinant (MBP peptide Ac1–9) but not to the SJL determinant (MBP 89–101), further emphasizing the dominance of a single MBP determinant in inducing a T cell response (27). To some extent, the immunodominance of a single epitope may account for the relatively limited or restricted TCR usage in the response to MBP (28–31) and the success of TCR and peptide-based therapies in preventing MBP-induced EAE (30–34).

We have recently described that, in contrast to MBP, TCR usage by T cells recognizing PLP 139–151 in SJL mice is diverse (17). The existence of multiple codominant determinants within this strain may further increase the diversity of the T cell response against the whole PLP molecule. It is also possible that a comparable complexity of T cell responses to PLP exists in humans. Indeed, T cells from some MS patients have been shown to respond to several PLP peptides (35), suggesting that the response to PLP in humans may also be directed against multiple determinants rather than a single dominant epitope. This raises the question as to whether therapy directed against one of the PLP epitopes would be sufficient to protect against the encephalitogenic effects of other determinants.

TABLE IV

Adoptive transfer of EAE

T Cell Line	Clinical EAE			Histologic EAE[a]
	Incidence	Day of onset[b]	Score[c]	No. foci[d]
E-13.S	3/3	13.3 (12–14)	4.5	50.0 ± 16.3
P-17.S	5/5	7.6 (6–9)	2.5	32.4 ± 8.8

[a] Histologic disease was observed in all mice.
[b] Values are mean day after transfer and ranges for each group.
[c] Mean maximum clinical score.
[d] Mean ± SE of number of inflammatory foci in meninges and parenchyma.

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