Pathogenicity of T Cells Responsive to Diverse Cryptic Epitopes of Myelin Basic Protein in the Lewis Rat

Felix Mor and Irun R. Cohen[1]

The cellular immunology of experimental autoimmune encephalomyelitis, a model for multiple sclerosis, has been studied, for the most part, using T cells directed to dominant epitopes of the Ag myelin basic protein (MBP). To characterize T cells reactive to cryptic epitopes of MBP, we immunized Lewis rats with each of 17 overlapping peptides of the 18.5-kDa isoform of rat MBP. We found that, in addition to the known 71–90 epitope, six other peptides induced active encephalomyelitis in the majority of the injected rats. T cell lines raised to six different MBP epitopes were encephalitogenic upon adoptive transfer to naive rats. In contrast to the T cells specific for the dominant 71–90 peptide, the T cell lines reactive to cryptic epitopes were not restricted in their TCR genes to Vβ8.2, and some of the lines caused prolonged disease. Thus, T cells of different specificities and TCR usage can be pathogenic. *The Journal of Immunology,* **1995, 155: 3693–3699.**

The peptide epitopes of an Ag molecule recognized by T cells may be divided functionally into two types: dominant and cryptic (1). Dominant epitopes activate specific T cells upon immunization with the whole Ag molecule; cryptic epitopes activate their specific T cells primarily upon immunization with the particular peptide, not when the whole Ag is the immunogen (1). The immune responses to cryptic and dominant epitopes are reported to be regulated differently. Induction of tolerance to hen egg lysozyme by transgenic expression of the protein in mice showed that tolerance was induced to the immunodominant epitope, but not to subdominant epitopes (2). An important question then is whether there exists a fundamental difference between dominant and cryptic epitopes of self Ags in autoimmune conditions in which immunologic regulation is critical.

Experimental autoimmune encephalomyelitis (EAE)[2] is a prototype autoimmune disease inducible in rats and other species by immunization to the myelin basic protein (MBP) molecule in a suitable adjuvant (3). In the Lewis rat, EAE is marked by the dominance of an epitope in the peptide 71–90 (p71–90) of the MBP sequence (4); by a restricted usage of TCR genes (Vβ8.2 and Vα2 or Vα4) in the responding T cells that proliferate strongly to p71–90 (3); and by a severe, but limited, course of paralysis of up to 7 days. Induction of EAE in Lewis rats, using human MBP as the immunogen, revealed an additional pathogenic epitope, p87–99. The epitope was found to be cryptic since T cells reactive to this peptide manifested low responses to whole rat MBP (5). In addition to these two encephalitogenic epitopes, an additional nonpathogenic reactivity was found in induction of EAE using guinea pig MBP (GpMBP), p55–68 (6). T cells reactive to this peptide did not respond to rat MBP and did not cause EAE (6).

The questions that we posed in designing this study were whether there exist cryptic epitopes for rat T cells in the rat MBP molecule; whether the responses to these epitopes can cause EAE; whether the responding T cells use restricted TCR V genes; and whether the EAE caused by T cells to cryptic epitopes is severe and acute. Recent work has documented the existence of multiple cryptic epitopes of MBP in mouse strains; however, the in vivo functional relevance of these T cells was not reported (7). To study those questions, we prepared 17 peptides of 14 to 20 amino acids each to cover the sequence of the 18.5-kDa isoform of rat MBP, with a 10-amino-acid overlap between each of the peptides. Despite the fact that most of the literature on EAE in the Lewis rat has used GpMBP as the immunogen, we chose to use overlapping peptides of the rat MBP sequence to focus on autoimmunity to MBP epitopes. We found that, in addition to the well characterized GpMBP-dominant epitope, there were multiple cryptic epitopes in rat MBP, and that some of these epitopes were encephalitogenic. Both active immunization with the peptides in CFA and inoculation of T cell lines reactive to several cryptic MBP epitopes caused EAE in recipient rats. These findings have both basic and practical implications.

Materials and Methods

Rats

Inbred female Lewis rats were supplied monthly by Harlan Olac (Bicester, UK) and were used at 2 to 3 mo of age. Rats were matched for age in each experiment.

Antigens

MBP from the spinal cords of guinea pigs or rats was prepared as described (8). *Mycobacterium tuberculosis* H37Ra (MT) was purchased from Difco (Detroit, MI). Peptides of MBP were synthesized using the F-moc technique with an automatic multiple peptide synthesizer (AMS 422; ABIMED, Langenfeld, Germany). Peptide sequences were all of the rat 18.5-kDa isoform of MBP, except p71–90, which was of the guinea pig sequence (serine instead of threonine in position 82; it is known that there is cross-reactivity at the T cell level between the p71–90 of rat and guinea pig origins (6)). The purity of the peptides was analyzed by HPLC and amino acid composition.

Induction of experimental autoimmune encephalomyelitis

Groups of rats were injected in both hind footpads with a 50-μl emulsion of mineral oil containing 100 μg of peptide or 250 μg of rat MBP and 200 μg of *M. tuberculosis* (CFA) per rat. Pertussis was injected i.v. on day 0 and i.p. on day 2 (9).

Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

Received for publication December 28, 1994. Accepted for publication July 14, 1994.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Irun R. Cohen, Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

[2] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; GpMBP, guinea pig myelin basic protein; MS, multiple sclerosis.

0022-1767/95/$02.00

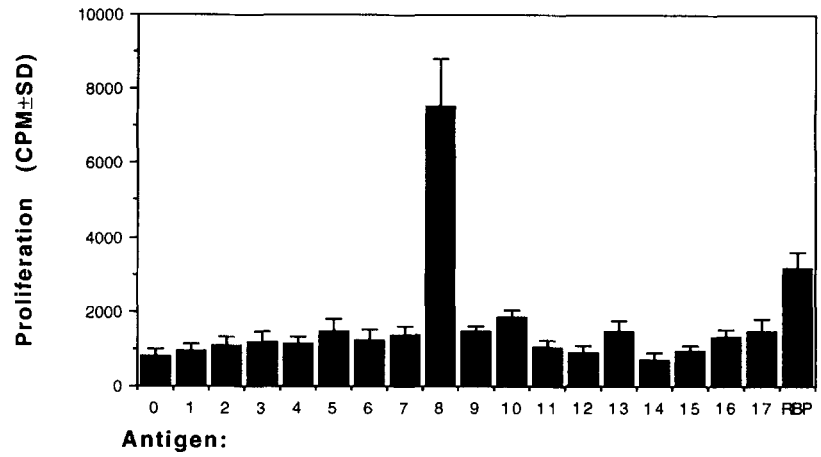

FIGURE 1. Proliferative responses of lymph node cells primed in vivo with rat MBP in CFA. Popliteal lymph node cells were obtained on day 15 of MBP/CFA footpad inoculation. Following one in vitro stimulation with rat MBP, the cells were tested in a proliferation assay (10^5 cells with 5×10^5 irradiated thymocytes as APCs). Proliferation is shown as cpm ± SD; 0 refers to background wells (cells and APCs without Ag). Peptide sequences are shown in Table I.

Passive EAE was transferred adoptively by i.p. injection of 2×10^7 peptide-activated cells of the lines, as described (10). Some rats were irradiated with 550 R (cobalt^{60}, Gamma Beam 150; Nordion, Canada) before inoculation with line cells. Clinical EAE was observed in immunized rats 10 to 14 days after peptide/CFA induction and 4 to 7 days following administration of T cell lines. Clinical scoring was: +1, paralysis of tail; +1.5, paresis of posterior paws and ataxia; +2, paraplegia; +3, paralysis extending to thoracic spine; and +4, a moribund state (10).

Histology

Histologic assessment of sections stained with hematoxylin and eosin was performed on the lumbar spinal cord obtained from rats killed 2 days following the peak of clinical signs or as indicated. Grading of lesions was as follows: mild, mononuclear infiltrates at the meningeal surface; moderate, scattered parenchymal inflammatory infiltrates; and severe, multiple perivascular parenchymal infiltrates (11).

T cell lines

Ag-specific T cell lines were established from lymph node cells that had been stimulated with peptide (5 μg/ml) for 3 days in stimulation medium composed of DMEM supplemented with 2 mercaptoethanol (5×10^{-5} M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 u/ml), streptomycin (100 μ g/ml), nonessential amino acids (1 ml/100 ml; Bio Lab Jerusalem, Israel), and 1% (v/v) autologous serum (10). Following stimulation, the T cell blasts were isolated on Lympho-prep (Nycomed Pharma, Oslo, Norway) and seeded in propagation medium (identical with stimulation medium without autologous serum, but supplemented with 10% (v/v) FCS and T cell growth factors from the supernatant of 10% (v/v) Con A-stimulated spleen cells (10)). Five days after seeding, the cells (5×10^5/ml) were restimulated with peptide (5 μg/ml) and irradiated thymocytes as APCs (10^7/ml) for 3 days in stimulation medium. Lines were expanded by repeated stimulation with peptides and irradiated thymocytes as APCs every 10 to 12 days (10). Following four to six rounds of stimulation, the cells were analyzed for their specificity to MBP peptides in a proliferation assay, and for their virulence by adoptive transfer.

T cell proliferation assay

T cell proliferation assay of popliteal lymph node cells from animals primed 15 days previously with the specific peptide in CFA was performed by seeding 1×10^5 cells in stimulation medium for 3 days. When T cell lines reached adequate numbers at the end of a rest phase, 5×10^4 line cells were seeded in 96 round-bottom microtiter wells (Greiner, Nurtingen, FRG) with 5×10^5 irradiated (2500 R) thymocytes or 2.5×10^5 spleen cells as accessory cells (10). Rat MBP was added at 10 μg/ml, and MBP peptides were added at 5 μg/ml, or as indicated in dose-response experiments. The proliferation was performed in stimulation medium, as described above. The cultures were incubated in quadruplicate for 72 h at 37°C in humidified air containing 7% CO_2. Each well was pulsed with 1 μCi of [^{3}H]thymidine (10 Ci/mmol sp. act.; Nuclear Research, Negev, Israel) for the final 18 h. The cultures were then harvested using a Micro-Mate 196 cell harvester, and cpm was determined using a Matrix 96 direct beta counter with use of avalanche gas (98.7% helium; 1.3% C_4H_{10}) ionization detectors (Packard Instrument Company, Meriden, CT). The results of proliferation are expressed as Δ cpm, calculated by subtraction of control wells cpm (containing line cells and APCs) from experimental wells (containing line cells, APCs, and Ags). In some experiments, the results are expressed as a stimulation index, calculated by dividing the mean cpm of experimental wells by the mean cpm of the control wells.

Determination of MHC restriction pattern

To determine MHC restrictions of responding T cell lines, monoclonal anti-I-A or anti-I-E Abs (MRC-OX6 or MRC-OX17, respectively; obtained from Serotec, Oxford, UK) were added to T cell proliferation wells. In the lines that were exclusively I-A or I-E restricted, addition of the mAb resulted in more than 80% inhibition of proliferation relative to control cultures; in the lines with a mixed restriction pattern, addition of either Ab led to approximately 50% inhibition of proliferation.

Flow cytometry

Line cells were incubated at 4°C for 45 min with an anti-Vβ8.2 Ab that was obtained from PharMingen (San Diego, CA; clone R78). Secondary rabbit anti-mouse FITC-conjugated Abs were incubated at 4°C for 30 min. The cells were then washed, and fluorescence was measured using the FACScan (Becton Dickinson, Mountain View, CA). Analysis of the results was performed using the Lysis II software.

Results

Definition of dominant and cryptic epitopes upon immunization with rat myelin basic protein

When GpMBP is used to induce EAE, the immunodominant epitope is consistently the 71–90 peptide (3, 4, 10). To characterize the T cells responsive to rat MBP, we immunized Lewis rats with a 10-fold higher dose of rat MBP (250 μg/rat) to produce EAE of similar severity, and examined the proliferative response of day 15 popliteal lymph node cells to whole rat MBP and to 17 overlapping peptides spanning the entire molecule. The cells were examined after one stimulation with rat MBP (10 μg/ml) and propagation for 7 days in IL-2-containing medium. The results of the proliferation are shown in Figure 1. The only peptide that demonstrated significant proliferation was 71–90; thus, the immunodominant peptide of rat MBP is also 71–90, and the rest are cryptic.

Multiple rat myelin basic protein peptides induce active encephalomyelitis

To characterize the immune response to the different MBP peptides, Lewis rats were immunized with 100 μg of each of 17 MBP peptides in CFA. To render them more susceptible to clinical EAE, some rats were also treated with pertussis toxin as part of the immunization protocol (12). Pertussis toxin is known for 30 yr to augment the encephalitogenic potential of MBP in both mice and rats (12, 13). In mice, the toxin was reported to increase the proliferative response to the autoantigen both in vivo and in vitro by a mechanism different from that operating with superantigens (13).

Table I. *Incidence and clinical score of peptide-induced encephalomyelitis*

Peptide	Sequence	Incidence of Active EAE	Mean Maximal Score	EAE with Pertussis	Mean Maximal Score	Histologic Lesions	Stimulation Index
1–20	ASQKRPSQRHGSKYLATAST	0/6		4/4	1	Mild	1.17
11–30	GSKYLATASTMDHARHGFLP	0/6		0/4		No	1.58
21–40	MDHARHGFLPRHRDTGILDS	1/6	1	1/4	1	Mild	1.15
31–50	RHRDTGILDSIGRFFSGDRG	0/6		3/4	1	Mild	1.2
41–60	IGRFFSGDRGAPKRGSGKDS	0/6		1/4	1	Mild	1.0
51–70	APKRGSGKDSH--TRTTHYG	0/6		1/4	1	Mild	1.1
61–80	H--TRTTHYGSLPQKSQ---	1/6	1	0/4		No	1.33
71–90	SLPQKSQ---RSQDENPVVH	10/10	4	2/2	4	Severe	3.8
81–100	RTQDENPVVHFFKNIVTPRT	5/6	1.5	4/4	3	Moderate	3.2
91–110	FFKNIVTPRTPPPSQGKGRG	5/6	1.5	4/4	2.5	Moderate	2.7
101–120	PPPSQGKGRGLSLSRFSWGA	4/9	1	2/4	1	Mild	1.7
111–130	LSLSRFSWGAEGQKPGFGYG	2/6	1	1/4	1	Mild	1.66
121–140	EGQKPGFGYGGRASDYKSAH	7/8	1.5	3/4	2	Moderate	1.0
131–150	GRASDYKSAHKGFKG-A-DA	2/6	1	3/4	1.5	Mild	1.16
141–160	KGFKG-A-DAQGTLSKIFKL	0/6		0/4		No	1.0
151–170	QGTLSKIFKLGGR----DSR	0/6		2/4	1.5	Mild	1.17
161–177	GGR----DSRSGSPMARR	0/5		0/4		No	1.1
Whole rat MBP		10/10	3	ND		Severe	6.7

The rats were scored for the development of clinical EAE, T cell proliferation to the peptide, and the histologic grade of inflammation in the spinal cords. The results are tabulated in Table I. The dominant epitope, p71–90, caused severe EAE in all rats, irrespective of the administration of pertussis. Among the other peptides, three induced active EAE in the majority of rats injected: p81–100, p91–110, and p121–140. Two of these peptides, p81–100 and p91–110, probably contain the 87–99 epitope previously reported (6). Several additional peptides induced EAE in the rats that were treated with pertussis: p1–20, p31–50, and p131–150. Thus, a total of seven peptides were encephalitogenic in a majority of the rats. The degree of inflammatory infiltration seen on histologic examination of the spinal cords correlated with the degree of EAE scored clinically.

Although rats immunized with whole MBP manifest strong T cell proliferative responses to MBP and to p71–90 (10), rats immunized with the various MBP peptides showed little or no T cell proliferation detected in the popliteal lymph nodes draining the site of immunization, even in rats clinically ill with EAE. The T cell proliferative response measured as a stimulation index in MBP-immunized rats to whole MBP was 6.7, the response to p71–90 in the rats immunized to that peptide was 3.8, and the response to the other peptides in the specifically immunized rats was lower or negligible (Table I). Therefore, a strong proliferative response in vitro may not detect a pathogenic population. Previous work by Mannie and colleagues (14) using substituted peptides pointed to a possible distinction between EAE induction and T cell proliferation in the Lewis rat.

Establishment of T cell lines to cryptic myelin basic protein epitopes

To further investigate peptide-induced EAE, we attempted to raise T cell lines specific for eight nonoverlapping peptides from the peptide-immunized rats. Although the T cell proliferative responses to some of the peptides was initially very weak, we succeeded in isolating six strongly proliferative T cell lines by repeated stimulation of the T cells with the respective peptide. Figure 2 shows the proliferation profiles of five of these T cell lines to each of the 17 overlapping MBP peptides (the specificity assay was not done for line 131–150). As can be seen, all of the lines exhibited peptide-specific proliferation. Although each line also manifested a proliferative response to whole rat MBP, the proliferation to whole rat MBP was much lower in the cryptic lines than was the response of the dominant 71–90 line (data not shown). This indicates that the epitopes other than p71–90 could be classified as latent cryptic determinants (1).

Crypticity index

The crypticity of a peptide is reflected in the magnitude of the response to the peptide relative to the magnitude of the response to the whole molecule. The degree of crypticity varied between the lines. Table II shows the proliferation of each line to its specific peptide and to whole rat MBP, and the ratio between the two as a crypticity index. The least cryptic line was anti-p71–90 (the dominant epitope), with a crypticity index of 0.96; the next least cryptic line was anti-p51–70; and the most cryptic was anti-p151–170 (Table II). The cryptic nature of the lines explains the difficulty of obtaining these T cell specificities when the whole Ag is used as the immunogen. However, we could obtain lines reactive to the least cryptic determinants, p71–90 and p51–70 of GpMBP, by using limiting dilution cultures (10).

Crypticity does not vary with the concentration of Ag nor the source of APC

To test crypticity as a function of Ag dose, we performed a dose-response analysis of various lines with their specific peptide and with whole rat MBP. We chose three different lines to represent different ranges of crypticity: anti-p91–110, high; anti-p11–30, medium; and anti-p51–70, low. As can be seen in Figure 3, the degree of crypticity was similar for the range of peptide and MBP concentrations tested. Thus, the reduced response to whole MBP relative to specific peptide was not overcome for any of the three lines by using a higher concentration of Ag.

It has been suggested that APCs from different organs or of different lineage may vary in the processing of the same Ag molecules (1). To examine the effect of different APCs on crypticity, we performed proliferation assays comparing thymus with spleen APCs from naive Lewis rats. We also used APCs from rats recovered from active EAE, since we found these rats to have significant Ab titers to multiple MBP epitopes, and thus a potential to use B cells as APCs. Figure 4 depicts the results of the assay for line

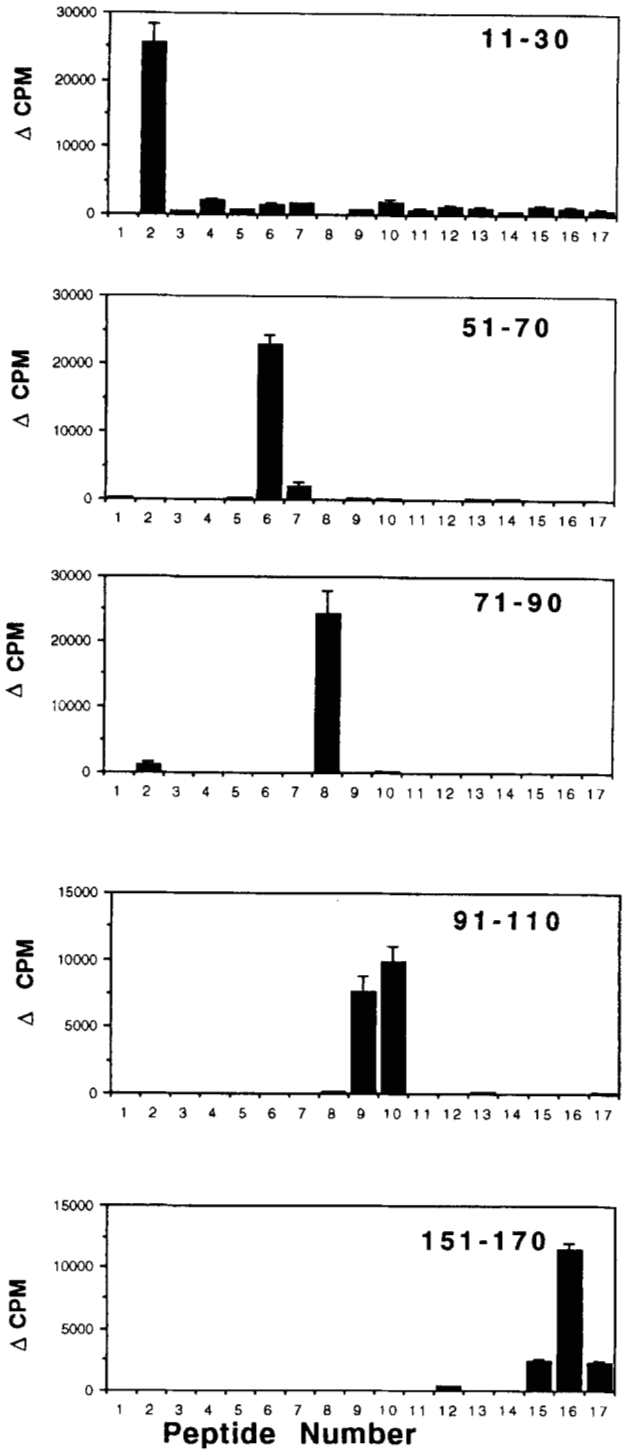

FIGURE 2. Proliferative response of T cell lines to MBP peptides. T cell lines were tested in a standard proliferation assay, with irradiated thymocytes as APCs and MBP peptides. The results are shown as Δ cpm ± SD. Peptide numbers designate each of the 17 overlapping peptides tested. The background proliferation for each line was: 11–30, 3,845 cpm; 51–70, 178 cpm; 71–90, 68 cpm; 91–110, 55 cpm; and 151–170, 24,098 cpm.

anti-p11–30. This assay shows that spleen APCs stimulated stronger proliferation than did thymus APCs. However, the proliferation profile of the line was not influenced by the source of APCs or whether the APCs had originated from rats recovered from EAE. Similar results were obtained for anti-p51–70, anti-p71–90, and anti-p91–110 lines. To test the possibility that purified APC populations might be more efficient in presenting whole MBP to cryptic lines, we performed dose-response experiments with anti-p11–30, anti-p51–70, and anti-p71–90, using as APCs Con A (100 μg/rat)- induced peritoneal macrophages (200,000 per well) or activated spleen B cells (dextran sulfate, 20 μg/ml, and LPS, 10 μg/ml for 48 h, 100,000 per well). The lines were incubated with graded doses of peptide and MBP. These purified APCs were less efficient in MBP presentation to the cryptic lines, while their efficacy in MBP presentation to the dominant line was similar to unfractionated thymocytes (data not shown). Thus, variation in the source of APCs did not abolish crypticity.

Table II. *The relative proliferation of peptide and MBP*

Line	Δ CPM Peptide (Optimal Conc. μg/ml)[a]	Δ CPM MBP (Optimal Conc. μg/ml)	Crypticity Index
Anti p11–30	31601 (50)	6871 (25)	4.6
Anti p51–70	42634 (25)	24662 (25)	1.7
Anti p71–90	38834 (25)	40602 (50)	0.96
Anti p91–110	29268 (50)	2963 (10)	9.9
Anti p131–150	24732 (50)	5259 (50)	4.7
Anti p151–170	37972 (50)	1289 (50)	29.5

[a] The background proliferation (line + APC, no Ag added) for the lines were 11–30: 1310 cpm, 51–70: 1294 cpm, 71–90: 693 cpm, 91–110: 337 cpm, 131–150: 22937 cpm, and 151–170: 4258 cpm.

T cell lines to cryptic epitopes are encephalitogenic and differ in V gene usage and MHC restriction

The six lines were analyzed for the percentage of cells bearing the Vβ8.2 TCR marker, for their MHC class II restriction (I-A or I-E), and for their capacity to adoptively transfer EAE in naive or irradiated recipient rats. Table III shows that all but one of the lines (anti-p131–150) were able to cause clinical EAE in naive rats, and all six lines caused EAE in irradiated recipients. The enhancing effect of irradiation on passive transfer was noted previously in experimental autoimmune thyroiditis (15) and in adjuvant arthritis (16). The line reactive to the dominant epitope, anti-p71–90, responded to its target peptide in the context of the MHC I-A molecule (I-A restricted), responded strongly in the proliferative assay to whole MBP and to isolated p71–90, and was composed largely of T cells expressing TCR Vβ8.2. Functionally, line anti-p71–90 caused an acute self-limited disease; the disease remitted spontaneously within 7 days. Histologic examination of the brain and spinal cord on day 6 showed typical perivascular mononuclear infiltrates (Fig. 5*A*) that were absent upon histologic evaluation on day 38 after inoculation (Fig. 5*C*). These properties are typical of T cells induced in response to immunization with whole MBP. Thus, immunization with the dominant p71–90 epitope appeared to be functionally equivalent to immunization with whole MBP.

The T cell lines to the cryptic epitopes differed. Three of these lines were either exclusively I-E restricted (anti-p91–110) or contained T cells with an I-E restriction (anti-p11–30 and anti-p151–170). Two of the lines contained no T cells expressing Vβ8.2 (anti-p11–30 and anti-p91–110), and the other three lines were largely free of Vβ8.2 (anti-p51–70, anti-p131–150, and anti-p151–170). Previous work on T cell lines reactive to the 87–99 peptide (probably equivalent to our 91–110 line that also reacts to 81–100) revealed that these cells do not use the Vβ8.2 in their TCRs (17), and are I-E restricted (6). Note that line anti-p11–30 was pathogenic, despite the fact that active immunization with p11–30 failed to induce EAE (see Table I). Most importantly, two of the cryptic

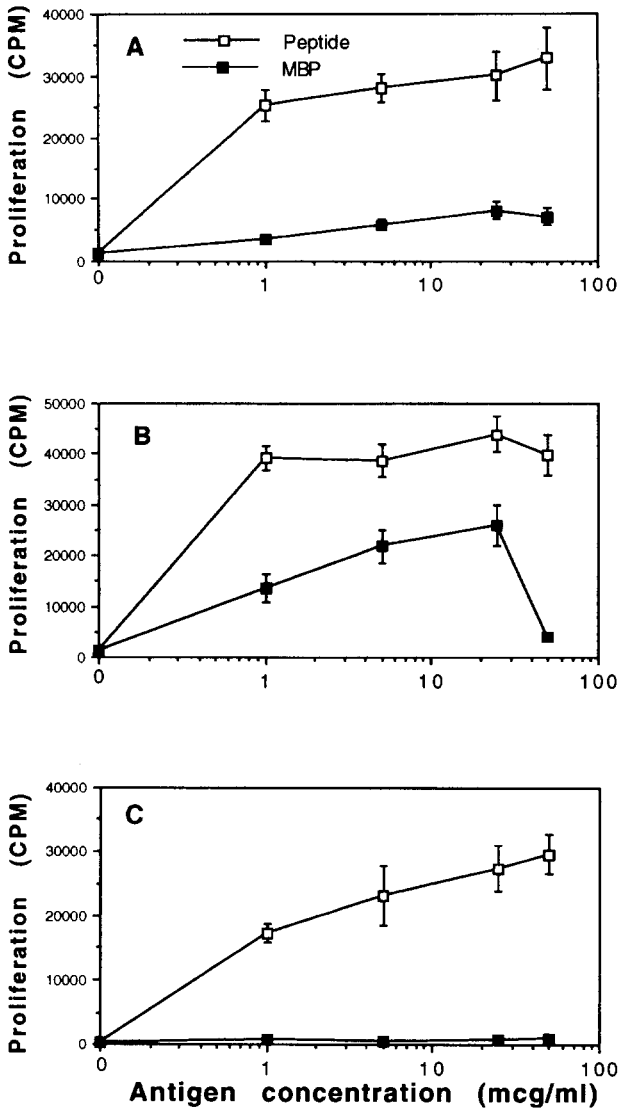

FIGURE 3. Dose response of cryptic T cell lines (*A* is anti-p11–30, *B* is anti-p51–70, and *C* is anti-p91–110) to peptide (open squares) and to rat MBP (designated MBP, filled squares). The results are shown as cpm ± SD. Irradiated thymocytes were used as APCs.

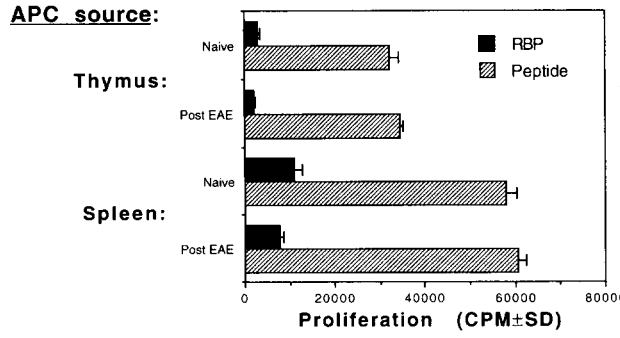

FIGURE 4. The effect of variation in the source of APCs on crypticity. Proliferative response of line anti-p11–30 to peptide (Peptide) and to rat MBP (RBP) using APCs from naive thymocytes and spleen cells, and thymocytes and spleen cells from rats 40 days post active EAE.

lines (anti-p51–70 and anti-p11–30) caused prolonged EAE. In two separate experiments, we compared the clinical course of EAE produced by line anti-p71–90 with that produced by line anti-p51–70 (four rats per group in each experiment). We found that while clinical EAE resolved within 7 days in the recipients of the anti-p71–90 line, the anti-p51–70 line caused clinical paralysis of the tail, with weakness of the hind limbs that persisted for at least 90 days. Histologic examination of the spinal cords from two rats with prolonged EAE, 38 days following injection of anti-p51–70, showed mononuclear cell infiltration in the lumbar, thoracic, and cervical spinal cord areas (Fig. 5*D*). In contrast, the rats that had been injected with anti-p71–90 showed normal histology by day 38 (Fig. 5*C*). Thus, T cells responsive to cryptic epitopes may have the ability to cause persisting lesions and prolonged paralysis.

Discussion

The main conclusions of our study are: 1) The T cell repertoire to MBP in the Lewis rat includes a diversity of T cells reactive to cryptic peptides in addition to the known and restricted dominant epitopes; thus, the methodology used for analyzing the T cell repertoire, using the whole MBP or using synthetic peptides, can influence the dimensions of the demonstrable repertoire. 2) T cells directed to cryptic determinants can be encephalitogenic, indicating that in vivo APCs are able to present cryptic peptides, and that the effector function of such T cells to these peptides includes the capacity to induce inflammation and paralysis. 3) While T cells reactive to the dominant p71–90 use Vβ8.2, T cells reactive to cryptic determinants use other Vβ genes. 4) The clinical pattern of EAE caused by some cryptic T cells may be prolonged.

EAE is thought by many to be a model of multiple sclerosis (MS), and, following EAE, MS has been probed immunologically by the proliferative T cell responses of patients to the dominant peptide epitopes of MBP and by the TCR genes used by patients' T cells (18). However, as reported in this work, T cells capable of mediating chronic lesions, the scourge of MS, may be directed to cryptic epitopes rather than to dominant epitopes, may use diverse TCR V genes, and may go undetected by T cell proliferation assays unless isolated as T cell lines. Our results in EAE suggest the possibility that the dominant MBP peptides may play a role in acute disease, but the chronic, clinically aggressive phases of disease may be produced by T cells responsive to cryptic epitopes. Indeed, in mouse EAE, spreading from dominant to cryptic epitopes was demonstrated (19).

At the molecular level, the phenomenon of epitope dominance has been attributed to favored processing and presentation of the dominant peptides, such as p71–90, compared with the other, cryptic peptides of MBP (1). However, our results suggest that cryptic epitopes must be available to T cells in vivo; otherwise, we cannot explain the pathogenicity of T cells specific for cryptic peptides. An epitope that is pathogenic in vivo must be an epitope that is presented in vivo.

Early work using enzymatic cleavage fragments of MBP of various species pointed to the encephalitogenic potential of regions of the molecule outside of the well known 71–90 peptide (20, 21). Thus, McFarlin and colleagues found that while bovine MBP was encephalitogenic, the 45–89 peptide was not pathogenic, suggesting that the encephalitogenic determinant of bovine MBP is located elsewhere in the molecule (20). Using similar methodology, Martenson, Levine, and Sowinski located the encephalitogenic potential of bovine MBP to regions 1–42 and 37–88 of the molecule, and further described an additional determinant at region 89–152 (21). However, the fact that relatively large peptides were used precluded the identification of defined T cell epitopes in these studies. Moreover, since T cell proliferations were not performed and heterologous MBP was used in these

Table III. *Characterization of T cell lines to rat MBP peptides*

				T Cell Line-Mediated EAE				
		T Cell Line		Naive recipients			Irradiated recipients	
Peptide	Active EAE	MHC rest.	Vβ 8.2 (%)	Incidence	Mean max. score	Duration	Incidence	Mean max. score
p11–30	None	IA + IE	0	3/4	1.5	24 days	4/4	3.25
p51–70	Mild	IA	7.5	4/4	1.5	90 days	4/4	3.0
p71–90	Severe	IA	86	4/4	2.5	7 days	4/4	4.0
p91–110	Moderate	IE	0	3/4	1.5	7 days	4/4	2.0
p131–150	Mild	ND	11	0/4			3/4	1.0
p151–170	Mild	IA + IE	10	2/4	1.0	5 days	2/4	1.0

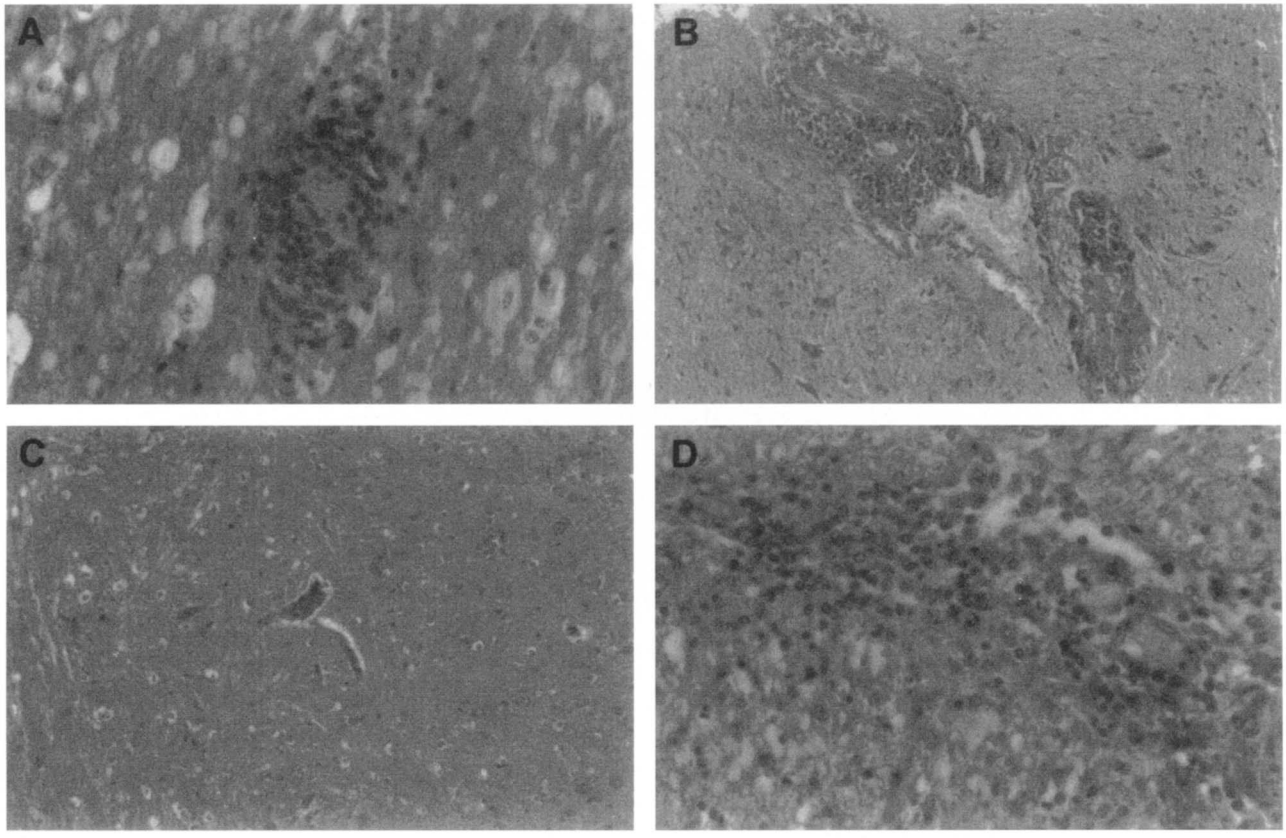

FIGURE 5. Photomicrographs of spinal cord sections stained with hematoxylin and eosin. *A*, Spinal cord of a rat injected with T cell line reactive to p71–90 6 days earlier, showing perivascular mononuclear cell infiltration. *B*, Spinal cord of a rat injected with the anti-p51–70 T cell line 8 days earlier, demonstrating a large interstitial and perivascular infiltrate. *C*, Spinal cord 38 days following injection of anti-p71–90 line, showing normal morphology. *D*, Spinal cord 38 days following injection of anti-p51–70 line, showing perivascular mononuclear infiltrates. Magnification: *A* and *D*, ×430; *B* and *C*, ×200.

early studies, it is unclear whether the investigators were describing dominant or cryptic epitopes; it is possible that upon injection of bovine MBP, 71–90 is not presented and other epitopes become immunodominant. To circumvent these problems, we used short peptides of the self-rat MBP sequence. The notion that cryptic T cells have encephalitogenic potential was recently suggested by Lehmann and colleagues, who described the phenomenon of determinant spreading in chronic relapsing EAE in mice (19). Thus, following the induced immune response to the immunodominant peptide, mice develop proliferative responses to cryptic determinants probably as a result of an in vivo immune response to MBP. In addition, the study by Clayton and associates (22) revealed that induction of neonatal tolerance with the immunodominant epitope 1–9 in B10.PL mice did not prevent EAE induced by whole MBP, suggesting the presence of other encephalitogenic epitopes. In a recent study by the same group (7), several cryptic epitopes were found in the same mouse strain upon immunization with peptides, including 31–50, 101–120, 121–140, and 131–150. The same mice responded only to 1–9 after MBP immunization (7).

The discrepancy in the duration of paralysis between the 71–90 line and the cryptic 51–70 line is consistent with the idea that the dominant repertoire to MBP is under efficient T cell regulation, while the cryptic response might continue unabated due to deficient regulation (1, 2). In patients with MS, the disease is often relapsing or protracted, suggesting a defect in the regulation of the

pathogenic T cell population. The results of our study suggest that the pathogenic human T cells in the chronic phase of MS might emerge from the cryptic T cell pool. Thus, T cells to cryptic epitopes of MBP have a functionally important pathogenic role in autoimmune encephalomyelitis.

Acknowledgments

We thank Dr. D. Teitelbaum for the gift of pertussis toxin. I. R. Cohen is the incumbent of the Mauerberger Chair in Immunology and head of the Robert Koch Minerva Center for the Study of Autoimmune Disease.

References

1. Sercarz, E. E., P. V. Lehmann, A. Amenti, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11:729.*
2. Cibotti, R., J. M. Kanellopoulos, J. P. Cabaniols, O. Halle-Panenko, K. Kosmatopoulos, and E. Sercarz. 1992. Tolerance to a self protein involves its immunodominant but does not involve its subdominant determinants. *Proc. Natl. Acad. Sci. USA 89:416.*
3. Acha-Orbea, H., L. Steinman, and H. O. McDevitt. 1989. T cell receptors in murine autoimmune diseases. *Annu. Rev. Immunol. 7:371.*
4. Chou, C. H. J., F. C. H. Chou, T. J. Kowalski, R. Shapira, and R. F. Keibler. 1977. The major site of guinea-pig myelin basic protein encephalitogenic in Lewis rats. *J. Neurochem. 28:115.*
5. Vandenbark, A. A., G. A. Hashim, B. Celnick, A. Galang, X. Li, E. Heber-Katz, and H. Offner. 1989. Determinants of human myelin basic protein that induce encephalitogenic T cells in Lewis rats. *J. Immunol. 142:3512.*
6. Offner, H., G. A. Hashim, B. Celnick, A. Galang, X. Li, F. R. Burns, N. Shen, E. Heber-Katz, and A. A. Vandenbark. 1989. T cell determinants of myelin basic protein include a unique encephalitogenic IE restricted epitope for Lewis rats. *J. Exp. Med. 170:355.*
7. Bhardwaj, V., V. Kumar, I. S. Grewal, T. Dao, P. V. Lehmann, H. M. Geysen, and E. E. Sercarz. 1994. T cell determinant structure of myelin basic protein in B10.PL, SJL/J, and their F1s. *J. Immunol. 152:3711.*
8. Hirshfeld, H., D. Teitelbaum, R. Arnon, and M. Sela. 1970. Basic encephalitogenic protein: a simplified purification on sulfoethyl-Sephadex. *FEBS Lett. 7:317.*
9. Aharoni, R., D. Teitelbaum, and R. Arnon. 1993. T suppressor hybridomas and interleukin-2 dependent lines induced by copolymer 1 or by spinal cord homogenate down-regulate experimental allergic encephalomyelitis. *Eur. J. Immunol. 23:17.*
10. Mor, F., and I. R. Cohen. 1993. Shifts in the epitopes of myelin basic protein recognized by Lewis rat T cells before, during, and after the induction of experimental autoimmune encephalomyelitis. *J. Clin. Invest. 92:2199.*
11. Sobel, R. A., B. W. Blanchette, A. K. Bhan, and R. B. Colvin. 1984. The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells in situ. *J. Immunol. 132:2393.*
12. Levine, S., and E. J. Wenk. 1965. A hyperacute form of allergic encephalomyelitis. *Am. J. Pathol. 47:61.*
13. Kamradt, T., P. D. Soloway, D. L. Perkins, and M. L. Gefter. 1991. Pertussis toxin prevents the induction of peripheral T cell anergy and enhances the T cell response to an encephalitogenic peptide of myelin basic protein. *J. Immunol. 147:3296.*
14. Mannie, M. D., P. Y. Paterson, D. C. Uprichard, and G. Fluoret. 1989. Encephalitogenic and proliferative responses of Lewis rat lymphocytes distinguished by position 75 and 80-substituted peptides of myelin basic protein. *J. Immunol. 142:2608.*
15. Williams, W. V., M. Kyriakos, G. C. Sharp, and H. Braley-Mullen. 1987. Augmentation of transfer of experimental autoimmune thyroiditis (EAT) in mice by irradiation of recipients. *Cell. Immunol. 109:397.*
16. Holoshitz, J., Y. Naparstek, A. Ben-Nun, and I. R. Cohen. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science 219:56.*
17. Sun, D., D. P. Gold, L. Smith, S. Brostoff, and C. Coleclough. 1992. Characterization of rat encephalitogenic T cells bearing non-vβ8 T cell receptors. *Eur. J. Immunol. 22:591.*
18. Ota, K., M. Matsui, E. L. Milford, G. A. Mackin, H. L. Weiner, and D. A. Hafler. 1990. T cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature 346:183.*
19. Lehmann, P. V., T. Forsthuber, A. Miller, and E. E. Sercarz. 1992. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature 358:155.*
20. McFarlin, D. E., S. E. Blank, and R. F. Kibler. 1973. Experimental allergic encephalomyelitis in the rat: response to encephalitogenic proteins and peptides. *Science 179:478.*
21. Martenson, R. E., S. Levine, and R. Sowinski. 1975. The location of regions in guinea pig and bovine myelin basic proteins which induce experimental allergic encephalomyelitis in Lewis rats. *J. Immunol. 114:592.*
22. Clayton, J. P., G. M. Gammon, D. G. Ando, D. H. Kono, L. Hood, and E. E. Sercarz. 1989. Peptide specific prevention of experimental allergic encephalomyelitis, neonatal tolerance induced to the dominant T cell determinant of myelin basic protein. *J. Exp. Med. 169:1681.*