

Shifts in the Epitopes of Myelin Basic Protein Recognized by Lewis Rat T Cells before, during, and after the Induction of Experimental Autoimmune Encephalomyelitis

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Abstract

An epitope present in the 71–90 sequence of basic protein (BP) has been identified as the dominant epitope recognized by most Lewis rat encephalitogenic T cells isolated during experimental autoimmune encephalomyelitis (EAE). In the present study, we investigated the BP epitopes recognized by Lewis rat T cells in naive rats, in rats suffering from acute EAE, and in recovered rats. T cells isolated from the spinal cord lesions and from the lymph nodes were studied using T cell lines and bulk cultures. Virulence of the T cells was assayed by adoptive transfer. We now report that naive and recovered Lewis rats are populated with T cells reactive to a variety of BP epitopes and only a minority are specific for the 71–90 epitope. In contrast, the induction of EAE was associated with a predominance of T cells reactive to the 71–90 epitope. T cells recovered from naive, diseased, or recovered rats were found to be virulent upon passive transfer. Some of these virulent T cells were specific to BP epitopes other than the 71–90 epitope. There was no major difference in the BP specificities of T cells isolated from the lesions and from the lymph nodes. Thus, natural T cell reactivity to BP is heterogeneous and pathogenicity is not confined to one particular epitope, active disease is characterized by a dominant response to the 71–90 epitope, and recovery is marked by a return to heterogeneity. (*J. Clin. Invest.* 1993. 92:2199–2206.) Key words: T cell lines • autoimmune disease • encephalitogenic peptides • immunodominant epitopes • T cell repertoire

Introduction

Experimental autoimmune encephalomyelitis (EAE)¹ is an inflammatory disease of the central nervous system inducible in susceptible strains of rats and mice by active immunization with neuroantigens in adjuvant (1) or by the adoptive transfer of encephalitogenic T cell lines or clones (2). The introduction of T cell lines and clones to the study of EAE has made it possible to analyze the epitope specificity of encephalitogenic T cells. Virulent T cells obtained from Lewis rats developing ac-

tive EAE were found to recognize primarily an epitope in the 71–90 portion of the guinea pig and rat basic protein (BP) sequences (1, 3). However, potentially virulent anti-BP T cells have also been isolated from naive Lewis rats (4, 5) and from rats recovered from acute EAE and resistant to reinduction of disease (6, 7). The presence of such T cells in animals free of disease may be viewed as a form of benign autoimmunity. The development of EAE, therefore, involves a transition from benign to pernicious autoimmunity and, in the case of recovery, back again to benign autoimmunity (8).

To begin to understand the evolution of anti-BP autoimmunity, we need to have more information about the BP specificities of the T cells present before, during, and after the disease. Are there shifts in repertoire as disease evolves? Moreover, are there differences in the BP epitopes recognized by T cells in the spinal cord lesions and by T cells in the lymph nodes? We investigated these questions by raising T cell lines from limiting numbers of cells as well as by studying the responses of lymphocytes in bulk cultures. The isolated lines made it possible to detect anti-BP specificities that otherwise might have been obscured by regulatory mechanisms or by overgrowth of dominant clones in bulk culture.

We found that the natural T cell repertoire to BP was heterogeneous in disease-free rats, but the response became dominated by the 71–90 epitope in the acute phase of EAE. There was no difference between the BP specificities of T cells obtained from the spinal cord and the lymph nodes. Thus, the onset of EAE is marked by a contraction of the T cell response to a single dominant epitope, whereas benign autoimmunity both before and after disease is characterized by epitope heterogeneity.

Methods

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

Antigens. BP from the spinal cords of guinea pigs or rats was prepared as described (9). *Mycobacterium tuberculosis* H37Ra (MT) was purchased from Difco Laboratories (Detroit, MI). Peptides of myelin BP were generously provided by Dr. O. Lider (Weizmann Institute, Israel) (amino acids [aa] 71–90: SLPQKSQ—RSQDENPVVHF, aa 88–101: VHFFKNIVTPRTPP), Dr. L. Steinman (Stanford University Medical Center) (aa 1–9: ASQKRPSQR, aa 1–20: ASQKRPSQR—HGSKYLATAST, aa 17–27: TASTMDKARHG), and Dr. A. Vandenberg (VA Medical Center, Portland, OR) (aa 35–52: TGILDSLGRFFSGDRGAP, aa 50–69: GAPKRGSGKDSHHAA—RTTHY, aa 68–86: HYGSLPQKSQ—RSQDENP). Sequences 1–52 and 88–101 were of the rat BP sequence and 50–91 was of the guinea pig BP sequence (10).

Induction of EAE. The disease was induced by injecting both hind foot pads with 0.05 ml containing 25 µg guinea pig BP and 200 µg MT emulsified in equal volumes of incomplete Freund's adjuvant and PBS (9). Passive EAE was adoptively transferred by intraperitoneal injection of BP-activated cells of the lines or clones as described (9). Clinical

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1. *Abbreviations used in this paper:* BP, basic protein; EAE, experimental allergic encephalomyelitis; MT, *Mycobacterium tuberculosis* H37Ra.

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EAE was observed in > 90% of immunized rats 10–12 d after BP/CFA induction and 4–5 d after administration of virulent lines or clones. Severity of disease was graded as follows: +1, paralysis of tail; +2, paralysis of hind legs; +3, paralysis extending to the thoracic spine; +4, moribund state.

Preparation of cell suspensions. Rats were killed by ether anesthesia, and cell suspensions were prepared from lymph nodes and thymuses by pressing the organs through a fine wire mesh (9). Spinal cords were extruded from the vertebral column aseptically by passing a wooden applicator through the spinal canal (11). Spinal cord lymphocytes were obtained by gentle grinding of spinal cord tissue with a 15-ml tissue grinder (Dounce model 357544; Kontes Glass Co., Wheaton, NJ) in 10 ml of PBS. The homogenate was then subjected to two to three cycles of Ficoll gradient separation. The nervous tissue remained at the interface and the lymphocytes were recovered from the pellet.

T cell lines from lymph nodes. Antigen-specific T cell lines were established from lymph node cells that had been stimulated with Con A (1.2 µg/ml) for 2 or 3 d in stimulation medium composed of DME supplemented with 2-mercaptoethanol (5×10^{-5} M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), nonessential amino acids (1 ml/100 ml; Bio Lab, Jerusalem, Israel), and autologous serum 1% (vol/vol) (12). The T cells were seeded in 96-well round-bottomed microtiter plates (Greiner, Nürtingen, Germany) at 500 cells per well, in propagation medium (identical to stimulation medium without autologous serum, supplemented with FCS 10% [vol/vol] and T cell growth factors from the supernatant of Con A-stimulated spleen cells 10% [vol/vol] [9]). 5 d after seeding, the cells were restimulated with guinea pig BP (10 µg/ml) and irradiated thymocytes as antigen-presenting cells (10^5 /well) for 3 d in stimulation medium. Wells showing positive growth were individually expanded in propagation medium and transferred after one or two BP stimulations to 24-well plates (Falcon, Becton Dickinson, Plymouth, England) and later to 10-ml (100 × 20-mm) plates (Falcon, Becton Dickinson). Lines were expanded by repeated stimulation (2.5×10^5 /ml) with guinea pig BP and irradiated thymocytes as antigen-presenting cells (5×10^6 /ml in 24-well plates and 10^7 /ml in 10-ml plates) every 10–12 d (9). After five to seven rounds of stimulation, the cells were analyzed for their specificity to BP epitopes in a proliferation assay and for their virulence by adoptive transfer.

T cell lines from spinal cord. After the harvest of spinal cord cells during acute EAE, the cells were seeded in 96-well round-bottomed microtiter plates, 250–500 cells per well, in propagation medium with irradiated thymocytes (2,500 rad, 10^5 /well) and BP (10 µg/ml) (11). These cultures were maintained for 7 d. After this stage the wells showing the highest proliferation were transferred to 24-well plates to be expanded in a manner identical to lines from lymph-node cells.

T cell clones. A line generated from popliteal lymph node cells 10 d after BP/CFA injection (designated BP10) was cloned by limiting dilution (2, 11) to one, two, and five cells per well in 96-well plates, on the third day of the fourth stimulation of the bulk line. The clones were expanded and characterized as was done with the lines.

T cell proliferation assay. When T cell lines reached adequate numbers at the end of a rest phase, 5×10^4 line cells were seeded in 96 round-bottomed microtiter wells (Greiner) with 5×10^5 irradiated (2,500 rad) thymocytes as accessory cells (9). The lymph node bulk proliferations were tested for reactivity to BP and to BP peptides. Since these cells gave low proliferative responses to the antigens tested, we repeated the proliferation assay after a Con A stimulation and 5 d of culture in propagation medium. At the end of this culture the cells were tested in a proliferation assay (10^5 cells with 5×10^5 irradiated thymocytes). The basis for this procedure is that mitogen stimulation preferentially amplifies the T cells that were recently activated *in vivo* (12). Guinea pig and rat BP were added at 10 µg/ml, and BP peptides were added at 5 µg/ml. 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% CO₂. Each well was pulsed with 1 µCi of [³H]thymidine (10 ci/mmol sp act; Nuclear Research, Negev, Israel) for the final 18 h. The cultures were then harvested (MicroMate 196 cell harvester; Packard Instrument Co., Meri-

den, CT) and cpm were determined (Matrix 96 direct beta counter, using avalanche gas [98.7% helium; 1.3% C₄H₁₀] ionization detectors; Packard Instrument Co.). The proliferations of the spinal cord-derived T cell lines were harvested using liquid scintillation vials and read with a liquid scintillation counter (GAMMAmatic B; Kontron Instruments, Zurich, Switzerland). This form of harvesting usually yielded background readings (line with thymocytes without antigen) that were 20-fold higher than the Matrix 96 direct beta counter (average 3,000 cpm, compared with 150 cpm).

Adoptive transfer of EAE. Anti-BP T cell lines were injected intraperitoneally in the numbers indicated to groups of four rats (2, 9). The rats were observed daily for the clinical signs of EAE. Line-mediated EAE appeared 4–6 d after injection and lasted for 3–5 d.

Flow cytometry. Line cells were stained at 4°C for 45 min with the following monoclonal antibodies at a 1:100 dilution: w3/25 for CD4, MRC ox-8 for CD8, and R7.3 for αβ TCR. All antibodies were purchased from Serotec, (Oxford, England). Secondary rabbit anti-mouse FITC-conjugated antibodies were used at a 1:50 dilution at 4°C for 30 min. The cells were then washed and fluorescence was measured using the FACScan® (Becton Dickinson & Co., Mountain View, CA).

Results

Naive animals: heterogeneity in the response of T cell lines to BP. The immune response to BP in the naive rat has not been studied previously in detail. Schluesener and Wekerle (5) reported that it was possible to isolate encephalitogenic T cell lines from unprimed Lewis rats. These lines reacted to an epitope in the 68–88 region of BP, equivalent to the 71–90 peptide. To increase the chances of isolating T cells reactive to other epitopes in addition to the dominant epitope in the 71–90 sequence of BP, we seeded each well with only 500 T cells from bulk cultures of T cell blasts that had been first stimulated with the T cell mitogen Con A. 17 lines were raised from this low initial number of blasts. Therefore, we can estimate the approximate frequency of BP-responsive T cell blasts in the naive animal to be 1 in 2,800 (17 of 96 wells divided by 500 cells seeded per well). Table I and Fig. 1 show the proliferation profiles of these lines. The classical encephalitogenic epitope (71–90) was found in 4 of 17 lines (23%; lines N1, N2, N17, and N18); 5 of 17 lines reacted to the 50–69 peptide (29%; lines N8, N9, N10, N15, and N16). One line (N3) reacted to the 88–101 peptide (6%), and 7 of 17 lines reacted to other BP epitopes. Lines N4, N5, N14, and N19 did not respond to any of the epitopes examined. N6 was reactive to the 12–20 peptide, but had some degree of autoreactivity; it responded to irradiated thymocytes in the absence of antigen expressed as an elevated background. A similar autoreactive pattern was seen in lines N7 and N11.

Some of these lines were analyzed for encephalitogenicity by intraperitoneal injection to groups of naive rats. As shown in Table II, three types of pathogenic T cells were observed. Three of the four 71–90-reactive lines were encephalitogenic (N2, N17, and N18). Line N3, which responded to the 88–101 epitope, and line N19, which responded to whole BP but not to any of the BP epitopes, were also pathogenic. The five lines reactive to the 50–69 epitope were not pathogenic.

Acute EAE: lines responsive to the 71–90 epitope dominate. In acute EAE, we generated T cell lines from spinal cord infiltrates using the limiting dilution technique, and from the draining lymph nodes using bulk cultures that were cloned after four stimulations. Table III shows 10 lines obtained from animals on day 12 of EAE induction; these lines were described in part in a previous study (11). A second experiment (not shown) was done using donor rats on day 11 after induction of

Table I. Proliferation Profiles of BP Lines from Naive Rats

Line	BG	Peptide							GpBP
		1-9	12-20	35-52	50-69	68-86	71-90	88-101	
N1	211	165	167	256	1,096	<u>4,068</u>	<u>13,687</u>	193	14,699
N2	227	194	150	380	153	390	<u>3,743</u>	276	6,391
N3	654	726	949	851	916	730	650	<u>1,735</u>	2,131
N4	74	63	67	64	66	123	65	48	228
N5	81	56	37	80	70	76	67	64	1,631
N6	870	769	<u>1,533</u>	580	726	670	778	970	1,412
N7	3,053	2,933	3,425	5,008	3,951	4,619	5,697	3,680	13,265
N8	259	218	590	1,425	<u>61,957</u>	2,314	418	493	20,025
N9	177	124	477	182	<u>40,052</u>	312	1,132	149	9,343
N10	85	61	56	388	<u>36,983</u>	116	101	119	13,651
N11	3,178	2,879	2,948	3,590	2,015	5,122	<u>7,733</u>	2,919	5,394
N14	303	341	200	257	294	214	284	178	5,766
N15	268	500	271	303	<u>16,475</u>	249	317	216	12,644
N16	200	343	344	277	<u>714</u>	249	337	235	907
N17	348	297	268	285	271	<u>1,102</u>	636	269	1,602
N18	118	119	182	118	164	<u>1,570</u>	1,571	171	3,875
N19	251	271	178	188	272	101	143	148	1,247

Anti-BP T cell lines isolated from the lymph nodes of naive Lewis rats were assayed for their proliferative responses to various BP peptides and to whole guinea pig BP (GpBP). The background (BG) refers to the cpm of controls cultured in the absence of antigens. The results are shown as the mean cpm of quadruplicate cultures. The standard errors were not >10% of the mean. Underlined numbers represent the dominant proliferative response among the peptides tested.

EAE; 12 additional lines were derived and produced results similar to those shown in Table III. Most of the lines isolated from the spinal cord were reactive to the 71-90 epitope. A minority of lines reacted to other unidentified epitopes. Seven lines were tested for their ability to mediate EAE; the six lines

that responded to the 71-90 peptide all produced EAE (lines 7, 11, 12, 14, 18, and 23). One line (SC19) that responded to whole BP but to none of the peptides tested (not shown) did cause EAE upon transfer.

Our second approach to study BP responses in acute EAE was to clone an early line started in the conventional method: cells were obtained from the popliteal lymph node draining the site of guinea pig BP/CFA inoculation. We cloned the cells after four passages to preserve any heterogeneity that might be lost during prolonged culture by overgrowth of a dominant clone. The initial bulk culture showed a vigorous response to the 71-90 peptide and a weak response to the 50-69 peptide (data not shown). The results of the proliferation assays of the clones isolated from this bulk culture are shown in Table IV and Fig. 2. All the clones demonstrated strong proliferative responses to guinea pig BP, the antigen used for their in vitro expansion. 17 of the 22 clones had a dominant response to the 71-90 epitope (77%) and 2 of the clones (9%) reacted to the 50-69 peptide (clones 47 and 62). Two clones did not respond to any of the epitopes examined (clones 55 and 81) and one clone (clone 60) was autoreactive. Interestingly, there was heterogeneity in the response of the 71-90 clones to BP. Some clones showed strong proliferation to BP of both guinea pig and rat origin (11 of 21) and others responded well to guinea pig BP but showed very low or no responses to rat BP (clones 4, 26, 38, 39, and 47). Analysis of the encephalitogenicity of these clones showed that clones 4, 19, 23, 26, 28, 30, 33, 36, 43, and 46 were pathogenic. Apparently, the low response of clones 4 and 26 to rat BP was sufficient to endow them with pathogenicity. However, the most pathogenic clones (33 and 30) demonstrated strong proliferation to rat BP.

Epitope diversity of lines obtained from rats recovered from EAE. To analyze the T cell repertoire after clinical recovery from EAE, we started limiting dilution lines from lymph nodes of rats 40 days after EAE induction. Table V and Fig. 1 show

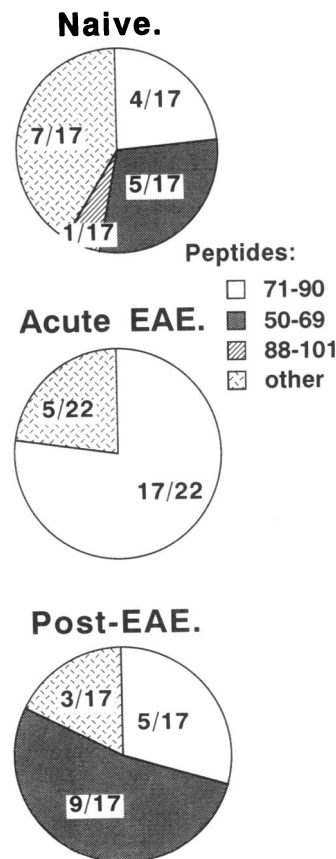


Figure 1. Histograms of distributions of BP specificities recognized by anti-BP T cell lines originating from lymph nodes of naive Lewis rats (top), spinal cords of rats suffering from EAE (middle), and lymph nodes of rats recovered from EAE (bottom). The fractions of responding lines are included in the histograms.

Table II. Adoptive Transfer of EAE by T Cell Lines

Origin	Line	Epitope specificity	Number of cells injected	EAE maximal clinical score
Naive				
lymph node	N1	71-90	4.8×10^6	0
	N2	71-90	15.6×10^6	+3
	N3	88-101	5×10^6	+3
	N8	50-69	3×10^6	0
	N10	50-69	2×10^6	0
	N14	Other	10^7	0
	N15	50-69	10^7	0
	N16	50-69	10^7	0
	N17	71-90	10^7	+3
	N18	71-90	10^7	+4
N19	Other	10^7	+4	
Acute EAE				
spinal cord	SC7	71-90	2×10^7	+1
	SC8	Other	2×10^7	0
	SC11	71-90	2×10^7	+1
	SC12	71-90	2×10^7	+2
	SC14	71-90	2×10^7	+3
	SC18	71-90	2×10^7	+3
	SC19	Other	10^7	+3
	SC21	71-90	2×10^7	0
	SC22	71-90	2×10^7	0
	SC23	71-90	2×10^7	+3
Post-EAE				
lymph node	B2	71-90	12.9×10^6	+2
	B3	71-90	4.8×10^6	+2
	B4	71-90	2×10^6	+2
	B7	50-69, 71-90	23×10^6	+3
	B9	50-69	18.5×10^6	+3
	B11	50-69	14.6×10^6	+2
	B12	50-69	14.6×10^6	+2
	B12	50-69	4.1×10^6	+2
	B15	71-90, 50-69	4×10^6	0
	B16	50-69	4.9×10^6	+2
	B17	50-69	10^7	+3
	B18	Autoreactive	5.6×10^6	+2
	B23	Other	2×10^6	0

T cell lines were isolated from naive rats, from spinal cords of rats with EAE, and from lymph nodes of recovered rats. The lines were injected intraperitoneally into naive rats. Clinical EAE was scored as indicated in the Methods section. 21 of the 34 lines were individually analyzed by FACS[®] and all lines were found to be >90% CD4⁺, <15% CD8⁺, and >90% αβ⁺ (data not shown).

the results of proliferation assays of these lines. 5 of 17 lines responded to the 71-90 peptide (29%), 9 of 17 (53%) responded to the 50-69 peptide, and 3 of 17 (18%) had other specificities. Two of the lines that had strong proliferative responses to both the 50-69 and 71-90 peptides (B7 and B15) were included in both groups (Fig. 1). Among the 71-90 lines, three of the four tested were encephalitogenic (B2, B3, and B4). In contrast to the avirulent cells isolated from the naive animals, the lines isolated from recovered rats with responses predominantly (B7, B9, B11, and B12) or exclusively to the 50-69 peptide (B16 and B17) mediated EAE. Note, however, that we analyzed T cell lines and not clones in the naive and recovered rats. Thus, we cannot ascribe the encephalitogenic potential of a line to the proliferative response stimulated by a

single known epitope; the same line could contain pathogenic T cells reactive to an unidentified peptide as well as to the known peptide. Indeed, the lines reactive to the 50-69 peptide, some of which were pathogenic, may serve as an example of this problem. In trying to prove the encephalitogenic potential of this peptide, we isolated clones from a 50-69-reactive line. However, none of the T cell clones reactive to this peptide were encephalitogenic (data not shown). Moreover, immunization with the 50-69 peptide in CFA did not result in EAE. The pathogenicity of lines such as B16 and B17 could be explained most easily by the presence of encephalitogenic T cells reactive to unknown peptides.

Analysis of BP epitopes using bulk cultures. Conclusions about the T cell repertoire to BP based solely on analysis of lines and clones could be misleading; line and clone technology favors T cells that grow well in culture. Therefore, we also studied the responses of bulk cell populations to BP and its peptides in the various stages of EAE. Figs. 3-5 depict the proliferation results naive rats and of rats 11, 16, and 140 days after EAE induction. The bulk culture of naive rat lymph node cells demonstrated no appreciable proliferative response to either BP or to any of the BP peptides examined (Fig. 3), although we were able to isolate virulent anti-BP lines from naive rats (Table I). Thus, it appears to be easier to isolate anti-BP T cells from naive rats using limiting numbers of cells than it is to detect the presence of the T cells in bulk cultures. In contrast to the lymph node cells from the naive animals, lymph node cells obtained on day 11 of EAE induction revealed a response to the classical encephalitogenic 71-90 epitope as well as to guinea pig BP and rat BP. On day 16 of EAE induction (Fig. 4), the cells responded to four peptides, including 35-52, 50-69, 68-86, and 88-101. The responses to BP epitopes were more marked after the Con A enrichment protocol (12). A similar pattern of response was observed on day 140 of EAE induction, 4 mo after recovery. Thus, recovery from EAE was associated with a return to T cell heterogeneity to BP.

Discussion

Most studies of the anti-BP response profile of Lewis rats have used a similar technique to generate pathogenic T cell lines: the repeated stimulation in bulk culture of popliteal lymph node cells draining the site of BP/CFA injection on day 10 of EAE induction (1, 2, 3, 9, 13). The results of those studies indicated that the anti-BP T cell repertoire is dominated by cells responding to the 71-90 peptide. This study was designed to facilitate the detection of heterogeneity in the response to BP and involved the generation of 54 T cell lines and 22 T cell clones from unprimed rats, from rats with acute EAE, and from rats after recovery. Schluessener and Wekerle (5) isolated from naive rats pathogenic anti-BP T cells specific for the 68-88 (71-90) segment of BP. Our findings indicate that the anti-BP T cells present in naive, healthy rats are directed to a variety of epitopes of the BP molecule; only about one-quarter of the T cells were specific to the 71-90 peptide. However, these 71-90 lines were virulent and could cause EAE in naive recipients (Table II). The anti-BP T cells responsive to other epitopes tended not to be pathogenic. Thus, the immune system's natural picture of BP, the immunological homunculus (14, 15), includes both virulent and avirulent T cells.

The fact that anti-BP T cells were detectable as isolated lines but not in bulk culture (Fig. 3) suggests that the lymphoid populations might contain regulatory cells that can suppress

Table III. Proliferation Profiles of Spinal Cord-derived BP Lines

Line	BG	1-9	71-90	87-98	84-102	GpBP
SC6	2,739	3,336	<u>75,208</u>	3,955	2,419	127,228
SC7	4,008	3,500	<u>74,181</u>	4,355	2,461	94,434
SC8	2,266	1,016	2,510	1,174	1,713	53,888
SC11	4,244	3,480	<u>42,110</u>	3,469	2,741	78,864
SC12	4,976	3,328	<u>33,326</u>	3,668	2,174	124,519
SC14	3,265	4,769	<u>101,043</u>	4,158	1,994	116,323
SC18	4,565	4,625	<u>57,210</u>	7,471	3,186	155,454
SC21	2,730	<u>25,709</u>	<u>91,818</u>	3,014	3,041	119,650
SC22	2,325	<u>27,429</u>	<u>129,244</u>	2,003	2,604	120,886
SC23	8,303	5,225	<u>130,778</u>	5,770	3,216	86,775

T cell lines isolated from the spinal cord infiltrates on day 12 of EAE induction were analyzed in a proliferation assay against MBP peptides and whole guinea pig BP (GpBP). These proliferations were harvested using liquid scintillation vials and read with a liquid scintillation counter (Kontron).

the response of the anti-BP T cells (16). Apparently, the regulatory cells are lost in the process of raising lines. Note, however, that lines isolated from naive rats could produce EAE upon inoculation into other naive rats; thus, the putative regulatory cells present in naive rats are of limited effectiveness and can be overcome by administration of a sufficient number of activated anti-BP T cells. Alternatively, it is possible that the anti-BP T cells in naive rats do not grow in bulk culture and do not cause EAE because they exist in too low a frequency. This naturally low frequency of anti-BP T cells in naive rats could have precluded their detection in a proliferation assay, whereas the derivation of T cell lines was not affected.

Immunization to BP/CFA led to the predominance of the 71-90 T cells over the T cells of the other specificities that are

present in the natural anti-BP repertoire. These 71-90 T cells are usually pathogenic; few T cells specific to other epitopes caused EAE. However, we do not yet know why immunization with BP/CFA favors the 71-90 T cells, and why 71-90 T cells tend to be pathogenic. The nonpathogenic anti-BP T cells responsive to other epitopes may serve a protective function; they could regulate the pathogenic 71-90 T cells by the secretion of TGF- β or other suppressive cytokines (17). Suppressor T cells have been shown to occur after induction of oral tolerance to BP (18).

In addition to anti-BP suppressor T cells (19), antiidiotypic T cells (20, 21) might also be invoked to explain how anti-BP T cells can exist in naive rats without causing disease; but such mechanisms do not explain why the 71-90 T cells

Table IV. Proliferative Responses of T Cell Clones Isolated from Line BP10

Clone	BG	Thymus APC	50-69	71-90	GpBP	RBP
4	269	385	257	<u>13,250</u>	24,910	466
19	85	80	115	<u>6,102</u>	19,367	8,144
23	89	121	87	<u>590</u>	21,911	5,342
26	172	208	143	<u>7,800</u>	15,192	363
28	177	200	171	<u>18,384</u>	35,349	924
30	196	694	572	<u>27,326</u>	28,523	11,200
32	79	129	85	<u>3,028</u>	9,073	1,111
33	90	105	92	<u>11,764</u>	25,975	14,588
36	194	268	223	<u>33,922</u>	32,975	11,207
38	107	128	85	<u>2,809</u>	6,304	213
39	170	153	135	<u>2,573</u>	13,543	274
43	133	159	82	<u>43,511</u>	49,059	9,890
45	287	291	186	<u>29,000</u>	72,100	4,010
46	380	580	541	<u>24,177</u>	29,401	1,998
47	152	203	<u>7,473</u>	583	3,173	149
48	160	172	168	<u>8,399</u>	20,810	824
54	224	366	129	<u>2,248</u>	7,337	709
55	125	127	139	159	9,250	962
58	351	349	238	<u>754</u>	1,230	602
60	66	5,801	5,816	<u>6,627</u>	5,626	4,575
62	107	152	<u>29,287</u>	130	11,705	97
81	143	167	153	212	10,408	6,387

T cell clones from line BP10 were generated from the popliteal lymph nodes of day 10 after EAE induction. Proliferative responses were assayed in the absence of antigen and thymocytes (BG), with thymocytes (Thymus APC) alone or with APC and BP peptides, guinea pig BP (GpBP) or rat BP (RBP).

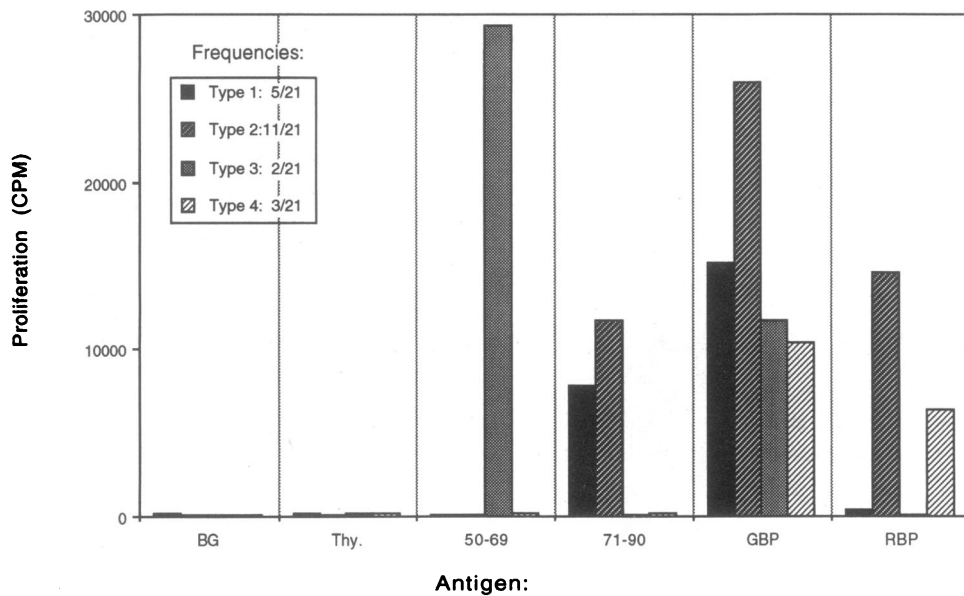


Figure 2. T cell clones were derived from the BP10 line after four stimulations in bulk culture. The clones showed different proliferation profiles to BP peptides and to rat BP (RBP). The bar graph depicts the type of proliferative response and the frequencies of proliferation profiles are as shown.

tend to be more pathogenic than other anti-BP T cells or why immunization with BP/CFA enhances their dominance. The administration of BP or of CFA alone does not cause EAE; on the contrary, BP (22) or CFA (23) augments resistance. Therefore, one may reason that it is the combination of BP with CFA that is critical in activating the 71-90 T cells to dominate the anti-BP response leading to overt disease.

It has been proposed that mixing self-antigens together with CFA makes the self-antigens autoimmunogenic by providing them with a context of infection (14, 15, 24). The context is bogus, but it suffices to trick the immune system, at least once, into interpreting the self antigen as part of an infection. The present study suggests that the 71-90 epitope is especially favored by the CFA context. It is conceivable that the processing and presentation of 71-90 is enhanced relative to other BP epitopes in antigen-presenting cells concomitantly activated by the CFA. It is also possible that the 71-90 epitope is mimicked

by foreign antigens or infectious agents with which the rat has already been primed (25). Such priming might explain how the 71-90 epitope triggers T cells to produce inflammatory effects with greater frequency than do other BP epitopes that may be free of any past association with the signals of infection. The T cells responsive to the other epitopes may be primed by the encounter with self BP a context free of the adjuvant signals of infection. These naive T cells, in contrast to the naive 71-90 T cells, would tend to be nonpathogenic.

It is interesting that spontaneous recovery from EAE is associated with a return to a greater heterogeneity in the anti-BP repertoire (Fig. 1 and Table V). Similar findings of clonal diversity after EAE were also reported by Vainiene et al. (7). Recovery from EAE has been found to be accompanied by antiidiotypic T cells specific to the virulent anti-BP T cells (6, 20). The amplification of antiidiotypic regulation by the disease itself may account for the resistance to repeated EAE asso-

Table V. Proliferation Profiles of BP Lines Started from Popliteal Lymph Node Cells 40 d after EAE Induction

Line	BG	1-9	12-20	35-52	50-69	68-86	71-90	88-101	GpBP
B1	107	113	75	81	65	123	118	105	2,010
B2	136	169	171	114	149	333	1,469	114	3,625
B3	106	151	160	70	117	1,563	7,845	62	13,760
B4	54	62	161	46	55	1,051	6,256	49	7,522
B7	55	65	66	59	9,577	101	7,376	54	10,493
B9	72	98	74	80	16,011	214	2,818	59	13,309
B11	231	230	359	209	30,482	839	3,301	245	12,522
B12	348	253	237	241	27,311	715	1,068	284	11,404
B15	151	374	494	446	3,551	1,238	4,618	272	4,040
B16	68	84	94	98	8,686	75	68	82	6,922
B17	178	191	186	187	38,076	149	132	110	19,114
B18	3,828	2,578	3,237	1,447	5,758	2,264	1,014	3,150	5,240
B21	163	127	165	115	5,051	112	162	120	1,596
B23	58	65	54	51	36	50	50	37	6,469
B32	144	148	86	124	33,992	66	63	86	16,867

Anti-BP T cell lines isolated from the draining lymph nodes of rats on day 40 of EAE induction were analyzed for their proliferative responses to BP peptides and to guinea pig BP (GpBP).

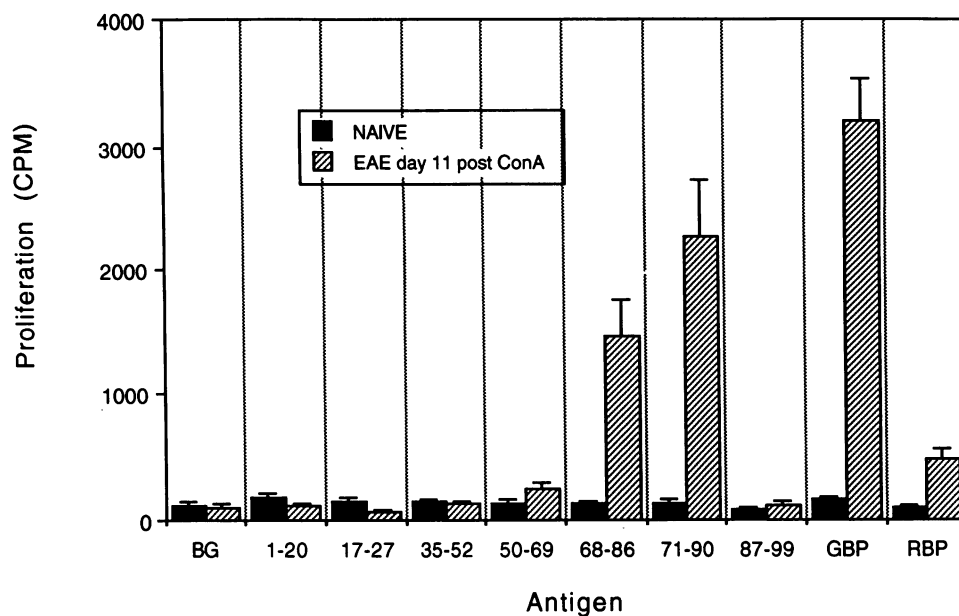


Figure 3. Proliferative responses of popliteal lymph node cells (10^5) isolated from naive Lewis rats (black bars) and from rats 11 d after induction of active EAE (hatched bars). The lymphocytes from sick rats were first stimulated with Con A for 2 d followed by 4 d of propagation in IL-2-containing medium before testing in proliferation assay (designated EAE day 11 post-Con A). The cells were analyzed for responses to BP peptides and to guinea pig (GBP) and rat BP (RBP). Background counts are from cultures in the absence of antigen (BG). Error bars indicate standard deviations.

ciated with recovery (14, 15). The antiidiotypic T cells could suppress the virulent 71-90 T cells and allow the other heterogeneous T cells to reappear. Thus, the resistance after recovery could be explained by enhanced regulation boosted by a bout of the disease itself.

The present findings also indicate that the T cells isolated from the EAE lesion are not enriched for any particular BP epitope compared with the T cell repertoire populating the lymphoid organs (Tables III and IV). This conclusion is contrary to the report by Bourdette et al. (26), who found that spinal cord T cell lines reacted only to encephalitogenic epitopes (72-89 and 87-99). Recently, Gold et al. (27) characterized T cell clones isolated from Lewis rats with acute EAE induced by immunization with the peptide 87-99. The peripheral response was found to be heterogeneous (8 different $V\beta$ genes were expressed by 12 clones), but $V\beta$ 6 was predominant among the spinal cord clones. We are now analyzing our sam-

ple of lines from both the spinal cord and the periphery for the $V\beta$ gene usage to address this issue.

This work provides a basis for interpreting the anti-BP repertoires found in patients with multiple sclerosis and in normal individuals. A diversity of epitopes were found to be recognized by T cells from healthy humans but fewer epitopes were recognized by T cells of patients with multiple sclerosis (28). It is conceivable that the more restricted repertoire seen in multiple sclerosis patients is the result of epitope domination of the autoimmune response during disease, in analogy to the situation we see in Lewis rats with acute EAE. Hence the time of sampling of T cells from patients with multiple sclerosis may influence the results of an analysis of the repertoire. T cell lines generated from a patient during an acute attack may show immunodominance of one or a few epitopes, whereas lines generated during a remission may reveal a diverse pattern of peptide recognition.

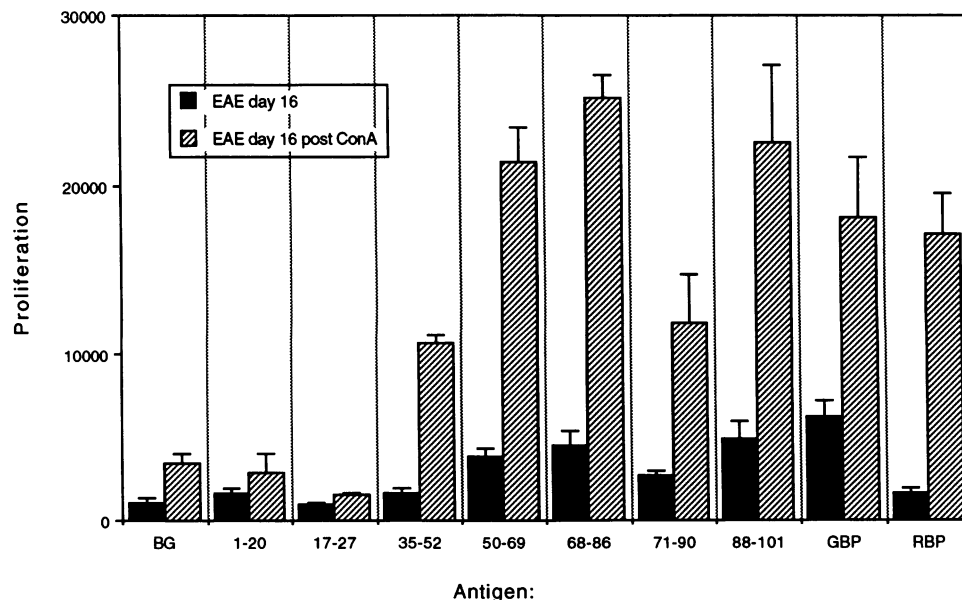


Figure 4. Proliferative responses of popliteal lymph node cells (10^5) isolated from Lewis rats on day 16 of EAE induction (black bars, EAE day 16) and from the same cells that were first stimulated with Con A and propagated in IL-2 medium for 5 d (hatched bars, EAE day 16 post-Con A).

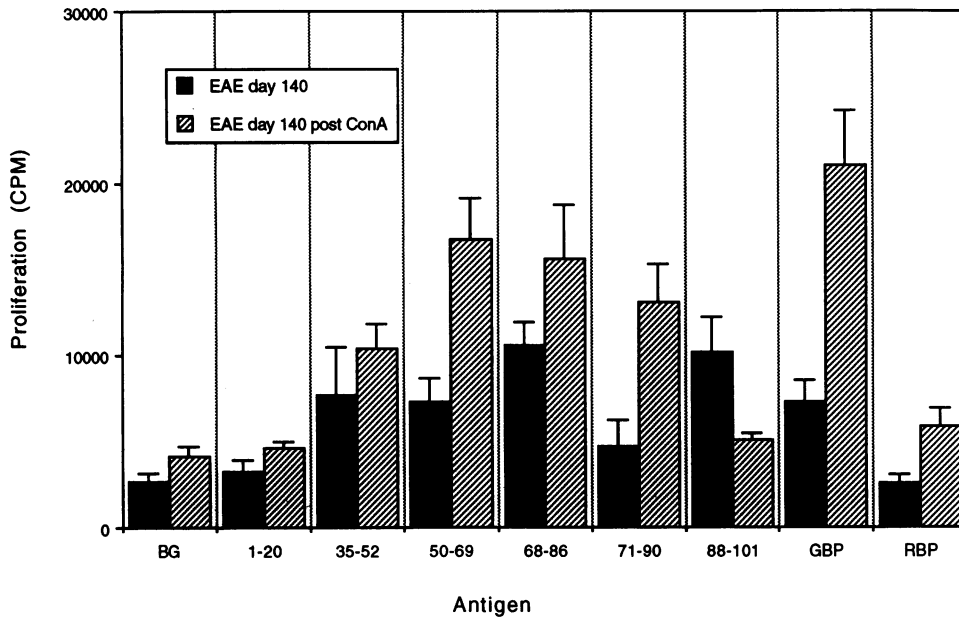


Figure 5. Comparative analysis of proliferation to BP peptides from popliteal lymph node cells obtained from rats on day 140 after active induction of EAE. Clinical recovery occurred by day 18.

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