

T CELL DETERMINANTS OF MYELIN BASIC PROTEIN
INCLUDE A UNIQUE ENCEPHALITOGENIC
I-E-RESTRICTED EPITOPE FOR LEWIS RATS

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Experimental autoimmune encephalomyelitis (EAE)¹ is a paralytic and inflammatory disease of the central nervous system mediated by Th cells specific for myelin basic protein (BP) (1). The encephalitogenic epitope(s) of BP differ among animal strains and are restricted by the available MHC class II molecules, predominantly I-A (2-5), and in one case, the B.10/PL mouse, by I-E (6). The major encephalitogenic epitope for the Lewis rat is the 72-89 sequence of guinea pig (GP)- or rat (Rt)-BP, restricted by I-A. T cell lines and clones selected with whole BP in vitro from lymph nodes collected 9 d after immunization with GP-BP respond only to the 72-89 determinant (7-9) and utilize preferentially the rat homologs of the murine V α 2:V β 8 gene combination in their TCR (10). Other determinants of GP-BP (i.e., the 44-68 sequence) may be immunogenic in the absence of the immunodominant 72-89 epitope (11), however, and it has been unclear why BP-selected T cell lines do not contain T cell specificities to epitopes other than the 72-89 sequence.

In this report, we have evaluated the T cell specificities present in lines selected from lymph nodes of Lewis rats before and after recovery from EAE induced with either GP- or Rt-BP. With both BPs, a second T cell specificity appeared later than the primary 72-89-specific response and persisted in the lines. Evidence is presented to show that the secondary GP-BP determinant, first detected 14 d after immunization, was included in the 55-68 sequence and was restricted by I-A. T cells specific for this determinant did not respond to the corresponding Rt-BP sequence, which differs by three residues at positions 61-63, and were not encephalitogenic in spite of their ability to transfer delayed-type hypersensitivity (DTH) reactions in vivo. The secondary Rt-BP epitope was found in the 87-99 sequence and was restricted

This work was supported by the Veterans Administration, U. S. Public Health Service grants NS-23444, NS-23221, NS-21466, and NS-11036, by grant BNS-11133 from the National Science Foundation, and by grant NMS RG-15934A-1 from the National Multiple Sclerosis Society.

¹ *Abbreviations used in this paper:* BP, basic protein; CDR, complementarity-determining region; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; GP, guinea pig; Rt, rat.

by I-E. Rat T cell lines and clones responsive to the 87-99 determinant were encephalitogenic and also transferred DTH reactions, and active immunization with the 87-99 peptide in CFA induced EAE. T cell clones specific for the 87-99 peptide restricted by I-E utilized the same TCR V α 2:V β 8 gene combination as clones specific for the GP-BP 72-89 sequence restricted by I-A (8, 10). This is the first demonstration of I-E-restricted encephalitogenic T cells in Lewis rats. These results support the conclusion that the I-E class II locus is involved in autoimmune diseases.

Materials and Methods

Rats. Lewis female rats, ~8 wk old, were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN, and were housed and cared for in the Animal Resource Facility at the Portland Veterans Administration Medical Center.

Antigens. GP- and Rt-BP were prepared according to the method of Eylar et al. (12). Proteolytic cleavage fragments of GP-BP (kindly provided by Dr. C. H.-J. Chou, Emory University, Atlanta, GA) were obtained and purified to contain >95% of the desired peptide by ion-exchange chromatography as described previously by Chou et al. (13). Myelin BP peptides were synthesized by solid phase techniques (14) according to the sequences for GP- or Rt-BP (15). All preparations were >95% pure as determined by HPLC and amino acid analyses. The sequences of peptides used in this study are shown in Table I.

T Cell Lines and Clones. T cell lines were selected as described previously (1) from lymph nodes of rats immunized either with GP- or Rt-BP, with peptides obtained by proteolysis of GP-BP, or with synthetic peptides emulsified in CFA containing 400 μ g H37Ra (Difco Laboratories Inc., Detroit, MI). T cell clones were isolated from the lines by the soft agar technique described earlier (8). Designations given to T cell lines and clones are shown in Table II. Activation of T cells was measured by [³H]Tdy uptake. 5×10^5 lymph node cells or 2×10^4 line cells in the presence of 10^6 irradiated thymic accessory cells were incubated with culture medium, antigens, and in some experiments with mAbs specific for I-A (OX-6) or I-E (OX-17) in microtiter wells for 18 h before the addition of 0.5 μ Ci labeled thymidine. The cell cultures were harvested onto glass fiber filters and counted by liquid scintillation techniques. Mean cpm was calculated from triplicate cultures. SD from replicate cultures varied <10% from the mean value.

Induction of EAE and DTH. Active EAE was induced by injecting BP or peptide emulsified with CFA. Each rat received a single dose of 100 μ g antigen and 100 μ g *Mycobacterium tuberculosis* or *M. butyricum*. Passive EAE was induced by injecting intraperitoneally $6-10 \times 10^6$ T line cells, which were activated with specific antigen presented by syngeneic thymic accessory cells for 3 d. Clinical signs were monitored daily on a scale of 0 to 4: 0, no signs; 0.5, lethargy and weight loss; 1, limp tail; 2, hind leg weakness; 3, hemiparalysis; 4, paralysis of front and hind limbs/moribund condition. Brain and spinal cord tissues were collected within 4 d of recovery from EAE or after 30 d and evaluated for histological evidence of EAE. DTH reactions were measured by the ear swelling assay (16) 24 and 48 h after injection intradermally of 20 μ g antigen.

TCR V Gene Expression. T cell RNA extracts were evaluated for the presence of specific V α 2 and V β 8 sequences using cDNA probes V α 510 and V β 510 (10). The V α 510 probe is 135 bp with Eco RI and Pst I restriction enzyme treatment, and the V β 510 probe is 240 bp with Eco RI and Alu I treatment and includes 6 bp of D region sequence. Northern blot hybridizations were performed using ³²P-labeled probes as described previously (10).

Results

Secondary T Cell Epitope of GP-BP. As we have shown previously, a GP-BP-specific T cell line, GP-BP(d9), and the D9 clone selected from Lewis rat lymph nodes 9 d after immunization recognized only the encephalitogenic S72-89 peptide sequence, and failed to respond to other epitopes present on GP-BP (Table III). The response

TABLE I
BP Peptide Sequences

Peptide	Single-letter amino acid sequence
GP-BP	
44-68	FGSDRAAPKRGSGKDSHHAARTTHY
GP-BP	
S39-54	SIGRFFGSDRAAPKRG
GP-BP	
S55-74	SGKDSH - - TRTTHYGSLPQK
Rt-BP	
S55-74 (modified)	SGKDSHHATRTHYGSLPQK
GP-BP	
S72-89	PQKSQ--RTQDENPVVHF
Rt-BP	
S72-89	PQKSQ--STQDENPVVHF
Rt-BP	
S87-99	VHFFKNIVTPRTP
Rt-BP	
S88-99	HFFKNIVTPRTP
Rt-BP	
S90-99	FKNIVTPRTP
Rt-BP	
S91-99	KNIVTPRTP

Numbering system conforms to the bovine BP numbering system of Eylar et al. (12). S indicates synthetic peptide.

to S72-89 of both the GP-BP(d9) line and the D9 clone was inhibited by the OX-6 mAb specific for I-A, but not by OX-17 specific for I-E (Table III).

We evaluated rats after recovery from EAE to determine if additional T cell specificities were present in GP-BP-selected T cell lines. As shown in Table III, T cell lines selected with whole GP-BP 28 d after immunization with GP-BP/CFA responded not only to the S72-89 peptide (as expected), but also to the 44-68 fragment of GP-BP. Responses to other fragments of GP-BP did not persist during line

TABLE II
T Cell Line Designations

T cell line/clone	Immunizing antigen	Line initiation	Selecting antigen	Specificity
GP-BP(d9)	GP-BP	Day 9	GP-BP	GP-BP S72-89
Clone D9	GP-BP	Day 9	GP-BP	GP-BP S72-89
GP-BP(d14-28)	GP-BP	Days 14-28	GP-BP	GP-BP S72-89
GP-BP ₄₄₋₆₈	GP-BP	Days 14-28	GP-BP 44-68	GP-BP 55-68
GP-BP(44-68)	GP-BP 44-68	Day 12	GP-BP 44-68	GP-BP 55-68
Rt-BP(d9)	Rt-BP	Day 9	Rt-BP	Rt-BP S72-89
Rt-BP(d20)	Rt-BP	Day 20	Rt-BP	Rt-BP S72-89
Rt-BP ₈₇₋₉₉	Rt-BP	Day 20	Rt-BP S87-99	Rt-BP S87-99
Rt-BP(87-99)	Rt-BP S87-99	Day 12	Rt-BP S87-99	Rt-BP S87-99
Clones C-3, C-6, and C-10	Rt-BP S87-99	Day 12	Rt-BP S87-99	Rt-BP S87-99

TABLE III
*Specificity of GP-BP-specific T Cell Lines from Lewis Rats
 before and after Recovery from GP-BP-induced EAE*

Stimulant	Proliferation response of T cell line/clone:		
	GP-BP(d9)	D9 clone	GP-BP(d28)
	<i>cpm/1,000</i>		
None	10 ± 1	2 ± 0	6 ± 1
GP-BP	153 ± 2	110 ± 5	228 ± 18
Rt-BP	68 ± 5	68 ± 2	72 ± 8
1-37	8 ± 1	1 ± 1	6 ± 1
44-89	111 ± 8	158 ± 12	158 ± 15
44-68	10 ± 1	1 ± 0	58 ± 4
+ OX-6 (α I-A)	ND	ND	4 ± 1 (100)
+ OX-17 (α I-E)	ND	ND	59 ± 5 (0)
S72-89	109 ± 9	167 ± 4	137 ± 12
+ OX-6 (α I-A)	10 ± 2	2 ± 0	5 ± 1 (100)
+ OX-17 (α I-E)	100 ± 11	155 ± 8	127 ± 10 (8)
90-169	13 ± 1	2 ± 1	7 ± 1
PPD	9 ± 1	1 ± 0	5 ± 1

20,000 T line cells or clones were stimulated for 3 d in the presence of antigens and 10^6 syngeneic thymic accessory cells. Proliferation was measured by uptake of [3 H]Tdy during the last 18 h of culture. mAbs specific for I-A or I-E were added at the beginning of the culture period to establish MHC class II restriction. Parentheses indicate percent inhibition. PPD, purified protein derivative of *M. tuberculosis*.

selection. The responses to both S72-89 and 44-68 peptides were completely inhibited by OX-6 but not OX-17, indicating that both were restricted by I-A.

The T cell population specific for the GP-BP 44-68 determinant could be detected in lymph node cell populations as early as 14 d after immunization with GP-BP (Table IV). The 44-68-specific response persisted in four different GP-BP-selected T cell lines produced from lymph nodes collected on days 14, 17, 24, and 28 after immunization with GP-BP (Table IV), and was consistently 20-40% as large as the response to the S72-89 epitope.

TABLE IV
*Response to GP-BP 44-68 Fragment Remains Stable after
 Appearing on Day 14 after Immunization*

Stimulant	Proliferation response									
	Day 9		Day 14		Day 17		Day 24		Day 28	
	LN	Line	LN	Line	LN	Line	LN	Line	LN	Line
	<i>cpm/1,000 - background</i>									
44-68	0	0	17	24	12	35	10	18	4	52
S72-89	14	99	41	119	34	100	28	58	13	131
44-68/72-89 response*	0	0	41	20	35	35	36	31	31	40

* Data represent percent.

To enrich for 44-68-specific T cells, a line containing T cells of both 72-89 and 44-68 specificities was subselected by restimulating three times with a highly purified 44-68 fragment of GP-BP (Table V). This subline, designated GP-BP₄₄₋₆₈, responded selectively to the 44-68 fragment in the context of I-A, and retained responsiveness to the parent 44-89 fragment and to whole GP-BP. However, the GP-BP₄₄₋₆₈ subline did not respond either to the 72-89 peptide of GP-BP or to whole Rt-BP (Table V). A second T cell line specific for the 44-68 fragment of GP-BP, designated GP-BP(44-68), was selected from lymph nodes of Lewis rats immunized with the 44-68 fragment in CFA, using only the 44-68 peptide as antigen. This T cell line was specific for the 44-68 fragment and was I-A restricted, recognized the parent 44-89 fragment and GP-BP, but failed to recognize either the GP-BP S72-89 peptide or Rt-BP (Table V).

To delineate further the T cell epitope involved, we stimulated the GP-BP(44-68) line with synthetic peptides corresponding to sequences in GP- and Rt-BP (Table V). No stimulation was observed to the GP-BP S39-54 or S72-89 sequences, or to the Rt-BP S55-74 sequence, which has a two-amino acid deletion (HA) relative to the GP sequence at positions 61 and 62, and a T for A substitution at position 63 (see Table I). However, a modified rat sequence with T at position 63 and the HA insertion at positions 61 and 62 was partially stimulatory for the 44-68-specific T cells (Table V). Thus, the shortest sequence recognized by the T cells included residues 55-68 of GP-BP.

Secondary T Cell Epitope of Rt-BP. In a manner similar to that described for GP-BP, we evaluated T cell responses in rats before and after recovery from Rt-BP-induced EAE. Lewis rats were immunized for 9 or 20 d with Rt-BP in CFA, and T cell lines

TABLE V
Characterization of GP-BP 44-68-specific T Cell Lines

Stimulant	Proliferation response of T cell line:	
	GP-BP ₄₄₋₆₈	GP-BP(44-68)
	<i>cpm/1,000</i>	
None	12 ± 2	15 ± 4
GP-BP	215 ± 20	124 ± 6
Rt-BP	8 ± 2	15 ± 2
1-37	10 ± 1	10 ± 1
44-89	200 ± 21	124 ± 14
44-68	171 ± 14	141 ± 21
+ OX-6 (α I-A)	8 ± 2 (100)	10 ± 2 (100)
+ OX-17 (α I-E)	166 ± 12 (3)	134 ± 9 (6)
GP-BP S39-54	ND	12 ± 1
Rt-BP S55-74	ND	12 ± 0
Rt-BP S55-74	ND	60 ± 3
S72-89	10 ± 1	15 ± 2
90-169	11 ± 1	12 ± 2

20,000 T line cells or clones were stimulated for 3 d in the presence of antigens and 10⁶ syngeneic thymic accessory cells. Proliferation was measured by uptake of [³H]Tdy during the last 18 h of culture. mAbs specific for I-A or I-E were added at the beginning of the culture period to establish MHC class II restriction. Parentheses indicate percent inhibition.

TABLE VI
*Specificity of Rt-BP-specific T Cell Lines from Lewis Rats
 before and after Recovery from Rt-BP-induced EAE*

Stimulant	Proliferation response of T cell line:	
	Rt-BP(d9)	Rt-BP(d20)
	<i>cpm/1,000</i>	
None	2 ± 0	6 ± 0
Rt-BP	80 ± 6	129 ± 10
44-68	2 ± 0	7 ± 2
S72-89	81 ± 4	57 ± 5
+ OX-6 (α I-A)	2 ± 0 (100)	5 ± 1 (100)
+ OX-17 (α I-E)	81 ± 3 (0)	52 ± 3 (10)
S87-99	2 ± 0	66 ± 8
+ OX-6 (α I-A)	ND	62 ± 4 (7)
+ OX-17 (α I-E)	ND	6 ± 1 (100)
PPD	2 ± 0	6 ± 1

20,000 T line cells or clones were stimulated for 3 d in the presence of antigens and 10^6 syngeneic thymic accessory cells. Proliferation was measured by uptake of [3 H]Tdy during the last 18 h of culture. mAbs specific for I-A or I-E were added at the beginning of the culture period to establish MHC class II restriction. Parentheses indicate percent inhibition.

were selected from lymph nodes using whole Rt-BP. As is shown in Table VI, Rt-BP-specific T cell lines selected 9 d after immunization responded to Rt-BP and to the immunodominant S72-89 peptide of Rt-BP, but not to the 44-68 or S87-99 sequences of BP. Consistent with our previous results (9), the Rt-BP(d9) response to S72-89 was inhibited by OX-6 but not OX-17 mAb, indicating restriction by I-A MHC molecules.

In contrast to the Rt-BP(d9) T cell line, a second T cell line selected from recovered rats 20 d after immunization, designated Rt-BP(d20), responded almost equally to both the S72-89 and the S87-99 sequences of Rt-BP (Table VI). As expected, the response of the Rt-BP(d20) line to S72-89 was I-A restricted. Surprisingly, the response of this line to S87-99 was inhibited by OX-17 but not OX-6, indicating that the S87-99-specific T cells were restricted by I-E (Table VI).

Three approaches were used to enrich for S87-99-specific T cells. First, an S87-99-specific T cell line was subselected from the Rt-BP(d20) line that contained both the S72-89 and the S87-99 T cell specificities by restimulating the line four times with only the S87-99 peptide. This subline, designated Rt-BP₈₇₋₉₉, lost its response to the S72-89 peptide of Rt-BP, but retained reactivity to the S87-99 sequence and to whole Rt-BP (Table VII). The response to S87-99 was inhibited completely by OX-17, but not OX-6 (Table VII). Thus, unlike any of the BP-specific T cell lines characterized to date, the Rt-BP₈₇₋₉₉ subline was restricted by I-E but not by I-A MHC class II molecules.

Second, an S87-99 specific T cell line, designated Rt-BP(S87-99), was selected from the lymph nodes of rats immunized for 12 d with the S87-99 peptide in CFA. Similar to the Rt-BP₈₇₋₉₉ subline described above, the Rt-BP(S87-99) line responded selectively to the S87-99 peptide and to a lesser degree to Rt-BP, but not to the 44-68 or S72-89 peptides (Table VII). Again, the 87-99 response was restricted

TABLE VII
 Characterization of Rt-BP S87-99-specific T Cell Lines and Clones

Stimulant	Proliferation response of T cell line/clone:				
	Rt-BP ₈₇₋₉₉	Rt-BP(87-99)	Clone C-3	Clone C-6	Clone C-10
	<i>cpm/1,000</i>				
None	4 ± 0	4 ± 0	0 ± 0	0 ± 0	1 ± 0
Rt-BP	78 ± 6	14 ± 1	4 ± 0	10 ± 1	8 ± 1
44-68	3 ± 0	4 ± 0	0 ± 0	0 ± 0	1 ± 0
S72-89	4 ± 0	2 ± 0	0 ± 0	0 ± 0	1 ± 0
S87-99	70 ± 6	115 ± 3	37 ± 2	51 ± 4	46 ± 2
+ OX-6 (α I-A)	ND	119 ± 7 (0)	41 ± 1 (0)	48 ± 3 (6)	46 ± 1 (0)
+ OX-17 (α I-E)	ND	42 ± 6 (66)	1 ± 1 (97)	6 ± 2 (88)	5 ± 1 (89)
PPD	4 ± 1	4 ± 0	0 ± 0	0 ± 0	1 ± 0

20,000 T line cells or clones were stimulated for 3 d in the presence of antigens and 10^6 syngeneic thymic accessory cells. Proliferation was measured by uptake of [^3H]TdR during the last 18 h of culture. mAbs specific for I-A or I-E were added at the beginning of the culture period to establish MHC class II restriction. Parentheses indicate percent inhibition.

by I-E but not by I-A (Table VII). Further analysis of the fine specificity indicated that the Rt-BP(S87-99) line responded equally to the S87-99 and S88-99 peptides, but had significantly reduced responses to the S90-99 sequence, and virtually no response to the S91-99 peptide (Fig. 1).

Third, three T cell clones (C-3, C-6, and C10) were isolated from the Rt-BP(87-99) line using the soft agar technique described previously (8). Each of these clones responded selectively to the S87-99 peptide, and to a lesser degree to Rt-BP, but not to the 44-68 or S72-89 peptides (Table VII). Consistent with the Rt-BP₈₇₋₉₉

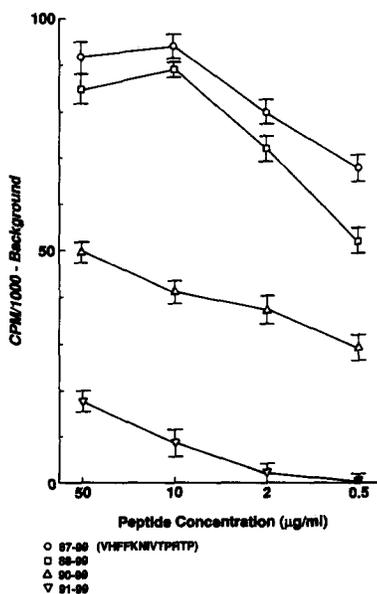


FIGURE 1. Response of the S87-99-specific T cell line to S87-99 and other synthetic peptides of decreasing length. 2×10^4 T cells were stimulated for 3 d with syngeneic thymic accessory cells and varying concentrations of peptides before an 18-h pulse with [^3H]Tdy.

and Rt-BP(87-99) lines, the response of all three of the S87-99-specific clones was I-E but not I-A restricted.

Encephalitogenicity of T Cells and BP Epitopes In Vivo. To evaluate encephalitogenic T cell activity in vivo, we injected $6-10 \times 10^6$ activated T cell lines and clones into naive Lewis rats. As is shown in Table VIII, the GP-BP 44-68-specific T cells were unable to induce clinical or histological EAE, although they were capable of transferring potent 44-68-specific delayed hypersensitivity reactions. By comparison, the same number of activated T cells specific for the 72-89 determinant of GP-BP transferred both EAE and 72-89-specific DTH responses (Table VIII). Additional experiments (not shown) indicated that rats receiving GP-BP 44-68-specific T cells were not resistant to a subsequent encephalitogenic challenge with GP-BP in CFA, nor did the presence of activated 44-68-specific T cells within the GP-BP(d28) line influence in any detectable manner the encephalitogenic activity of 72-89-specific T cells (Table VIII).

In contrast, injection of $6-10 \times 10^6$ activated Rt-BP₈₇₋₉₉ or Rt-BP(S87-99) T line cells induced clinical and histological EAE in a total of 20 of 23 recipients, as well as strong DTH responses (Table VIII). Consistent with the Rt-BP(S87-99) line, the C-3 and C-6 T cell clones derived from this line also transferred comparable signs of EAE in three of three recipient rats, as well as strong 87-99-specific DTH responses (Table VIII).

The encephalitogenic activity demonstrated in the S87-99-specific T cell lines and clones C-3 and C-6 suggested that the S87-99 peptide itself would likely be encephalitogenic as well. To evaluate encephalitogenic activity, rats were injected with various synthetic peptides in CFA. As is shown in Table IX, S87-99, S88-99, and S90-99 peptides all possessed encephalitogenic activity. The S91-99 peptide, which did not stimulate the Rt-BP(S87-99) T cell line, however, was only mildly encephalitogenic (Table IX).

TCR. Previously, we reported that encephalitogenic T cell lines and clones and T cell hybridomas specific for the 72-89 peptide of GP-BP restricted by I-A prefer-

TABLE VIII
Activity of T Cell Lines and Clones In Vivo

Specificity of T cell line	Stimulant	Maximum EAE severity*	DTH (Δmm^{-2})		
			44-68	72-89	87-99
GP-BP(d9)	GP-BP	3.0 ± 0.7 (6/6)†	0 ± 0	14 ± 2	
GP-BP(d28)	GP-BP	3.2 ± 0.8 (6/6)	7 ± 1	15 ± 1	
GP-BP ₄₄₋₆₈	44-68	0 ± 0 (0/6)	18 ± 2	2 ± 1	
GP-BP(44-68)	44-68	0 ± 0 (0/6)	21 ± 3	1 ± 1	
Rt-BP(d9)	Rt-BP	3.5 ± 1.0 (8/8)		11 ± 2	0 ± 0
Rt-BP(d28)	Rt-BP	2.7 ± 0.4 (6/6)		15 ± 1	14 ± 2
Rt-BP ₈₇₋₉₉	87-99	1.8 ± 0.9 (12/15)		1 ± 1	22 ± 3
Rt-BP(87-99)	87-99	1.5 ± 0.3 (8/8)		3 ± 1	24 ± 4
Rt-BP clone C-3	87-99	1.5 ± 0.0 (3/3)		ND	ND
Rt-BP clone C-6	87-99	1.7 ± 0.3 (3/3)		ND	ND

* Clinical EAE: 0, no signs; 1, limp tail, weight loss; 2, hind leg weakness; 3, hind quarter paralysis, incontinence.

† Number positive/total number.

TABLE IX
Induction of EAE with Synthetic Peptides from the 87-99 Region of Rt-BP

Peptide	Dose $\mu\text{g}/\text{CFA}$	Maximum clinical EAE*
Rt-BP S87-99	100	2.0 \pm 1.1 (6/6) [†]
Rt-BP S87-99	100	2.0 \pm 1.1 (4/4)
Rt-BP S88-99	100	2.0 \pm 1.1 (4/4)
Rt-BP S90-99	100	3.0 \pm 0.0 (4/4)
Rt-BP S91-99	100	0.5 \pm 0.0 (4/4)

* Clinical EAE: 0, no signs; 0.5, lethargy, weight loss; 1, limp tail; 2, hind leg weakness; 3, hind quarter paralysis, incontinence.

[†] Number positive/total number.

TABLE X
TCR V Gene Use

T cell line	Specificity	Restriction	Encephalogenic	V α 2	V β 8.2
GP-BP(d9)	GP-BP S72-89	I-A	+	+	+
GP-BP(d28)	GP-BP 44-68	I-A	+	+	+
	GP-BP S72-89	I-A	+	+	+
GP-BP ₄₄₋₆₈	GP-BP 44-68	I-A	-	-	-
Rt-BP(87-99)	Rt-BP S87-99	I-E	+	+	+
Clone C-3	Rt-BP S87-99	I-E	+	+	+
Clone C-6	Rt-BP S87-99	I-E	+	+	+
Clone C-10	Rt-BP S87-99	I-E	ND	+	+

RNA extracted from the T cell lines and clones was blotted onto nitrocellulose and hybridized with the V α 510 and V β 510 probes as described in Materials and Methods.

entially used TCR V genes from the rat homologs of the murine V α 2 and V β 8 families. Using the same V α and V β probes, we examined mRNA from the various T cell lines and clones described above. As expected, the GP-BP-selected T cell line specific for the 72-89 peptide sequence expressed message for both the V α 2 and the V β 8 genes (Table X). However, the nonencephalitogenic GP-BP₄₄₋₆₈ T cell line did not utilize either the V α 2 or the V β 8 gene.

The encephalitogenic I-E-restricted Rt-BP(S87-99) T cell line, and the C-3, C-6, and C-10 clones derived from this line, were all positive for both the TCR V α 2 and V β 8 genes (Table X). The presence of both V α 2 and V β 8 message in each of the clones indicated that these two genes were utilized in combination in the TCR specific for the S87-99 peptide restricted by I-E.

Discussion

In this work, we describe for the first time the delayed appearance in immunized Lewis rats of T cells specific for additional discrete determinants of GP- or Rt-BP. The secondary GP-BP determinant, first detected 14 d after immunization, included the 55-68 sequence and was restricted by I-A. T cells specific for this determinant

did not respond to the corresponding Rt-BP sequence, which differs by three residues at positions 61–63 (15), and were not encephalitogenic in spite of their ability to transfer DTH reactions *in vivo*.

Further, this study describes a unique encephalitogenic I-E-restricted T cell epitope for Lewis rats located within the 87–99 sequence of Rt-BP. Rat T cell lines and clones that were responsive to this determinant were able to passively transfer clinical EAE and specific DTH reactions *in vivo*, and utilized the same $V\alpha 2:V\beta 8$ gene combination in their antigen receptors as T cells specific for the GP-BP S72–89 determinant restricted by I-A. Immunization of naive rats with peptides as short as the S90–99 sequence induced active EAE, demonstrating conclusively the encephalitogenicity of this I-E-restricted epitope.

The 87–99 sequence is highly conserved in BP from many species, including Rt, mouse, GP, and human BP, and it is of great interest that this sequence is also a major encephalitogenic determinant for the SJL/J(H-2^s) mouse (5, 17). We demonstrated previously that encephalitogenic SJL/J T cell clones were specific for the S87–99 peptide, and were restricted by I-A MHC molecules (17), perhaps due to the absence of functional I-E molecules. Thus, the 87–99 determinant of BP would appear to be somewhat unusual in its ability to form an immunogenic and encephalitogenic complex with both rat I-E and mouse I-A restriction molecules. However, unlike T cells from rat (Fig. 1), mouse T cells responded fully to both the S90–99 and S91–99 peptides (17), suggesting differences in fine specificity to this determinant.

The 87–99 sequence of BP, which induced an I-E-restricted T cell response, represents the third determinant of BP capable of inducing encephalitogenic T cells in the Lewis rat. Previous studies have shown that T cells specific for the major encephalitogenic epitope corresponding to the 72–89 sequence of GP- or Rt-BP were restricted by I-A (8). Also, a variant T cell specificity directed at the 72–84 sequence of Rt-BP was restricted by I-A (9). Thus, the presence of multiple discrete encephalitogenic determinants on BP, restricted by either I-A or I-E molecules, demonstrates clearly the diversity of the encephalitogenic process in a given genetic setting, and supports the notion that both of the human homologs of rodent I-A and I-E molecules, HLA-DQ and HLA-DR, respectively (18), may be capable of participating in human encephalitogenic processes (19). In this regard we have observed that both of these class II loci restrict discrete epitopes of human MBP in patients with neurologic diseases, including multiple sclerosis (19).

Considering the putative binding function of the TCR with an MHC/peptide complex, the preferential use of the $V\alpha 2:V\beta 8$ gene combination by two discrete encephalitogenic T cell specificities would appear to be unexpected. To the contrary, the current results support our previous unusual finding that both B.10/PL mouse T cells specific for the BP peptide 1–11 and Lewis rat T cells specific for S72–89 use this same TCR $V\alpha 2:V\beta 8$ gene combination as well. According to models proposed by Davis and Bjorkman (20) and Claverie et al. (21), the complementarity determining regions (CDR) 1 and 2 of the TCR V genes would most likely interact with the MHC restriction molecule, whereas the CDR3 region that spans the V-J (α chain) or V-D-J (β chain) junction region would most likely interact with the antigenic peptide bound by the MHC molecule. Based on this model, one would expect TCRs using the same $V\alpha:V\beta$ gene combination to recognize the same MHC restriction molecule, and possibly a portion of the same antigenic peptide, depending on selection of D and

J genes and how much of the CDR3 region was associated with the V gene product. The T cell specificities described above do not recognize either the same peptide or the same MHC molecule, and thus suggest interaction of the TCR V gene products with conserved regions of different MHC, or similarities in peptide-binding properties not discerned from primary sequences.

The appearance of T cells of a single dominant specificity for the 72-89 sequence of BP is well established in lines selected with whole GP- or Rt-BP 9 d after immunization (8). As demonstrated in this report, lines selected 14-28 d after immunization contained T cells of two different specificities, and in the GP-BP selected line, both specificities were restricted by I-A. During the line selection process, the relative response to each epitope remained constant, and generally reflected the relative response to each epitope observed in the immunized lymph node population (Table IV). Taken together, these results demonstrate that the process of T cell line selection did not alter substantially the composition of BP-reactive T cells present initially in the lymph node. Thus, the emergence of a single dominant T cell specificity in lines selected 9 d after immunization appears to be a reflection of a relatively high 72-89 peptide-specific T cell frequency and a low GP-BP 55-68 or Rt-BP 87-99 frequency. In the GP-BP-selected line, where both the 72-89 and 55-68 T cell specificities persisted, it would appear that competition between the two processed peptides for binding to the available I-A restriction molecules (22, 23) did not play a significant role in establishing immunodominant T cell specificities.

Summary

The major encephalitogenic epitope for Lewis rats is the 72-89 sequence of guinea pig basic protein (GP-BP) or rat basic protein (Rt-BP). T cells responsive to this epitope are I-A restricted and preferentially express the V α 2:V β 8 gene combination in their TCR. In this work, we describe for the first time the delayed appearance of T cells specific for additional discrete determinant of BP, the nonencephalitogenic 55-68 sequence of GP-BP restricted by I-A, and the encephalitogenic 87-99 sequence of Rt-BP restricted by I-E. The TCR V α 2:V β 8 gene combination was expressed by both encephalitogenic GP-BP S72-89 and Rt-BP S87-99 T cell specificities but not by GP-BP 44-68-specific T cells. This is the first demonstration of I-E-restricted encephalitogenic T cells in Lewis rats and supports the conclusion that the I-E class II locus is involved in autoimmune diseases.

We thank Karin Coffee for help in manuscript preparation.

Received for publication 3 April 1989.

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