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T CELL LINES SPECIFIC FOR AN IMMUNODOMINANT EPITOPE OF HUMAN BASIC PROTEIN DEFINE AN ENCEPHALITOGENIC DETERMINANT FOR EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS-RESISTANT LOU/M RATS[1]

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The LOU/M rat (RT-1^w) haplotype, although resistant to an encephalitogenic challenge of guinea pig myelin basic protein (Gp-BP)/CFA and unresponsive to Gp-BP, responded strongly to human (Hu)-BP. Both T cell and antibody responses focused on the 110-129 determinant of Hu-BP, and T cells specific for this epitope transferred clinical and histologic experimental autoimmune encephalomyelitis (EAE) to naive LOU/M rats. Moreover, EAE could be induced actively with Hu-BP and a synthetic Hu-S110-129 peptide in CFA, but only with co-immunomodulation by pertussis toxin or cyclophosphamide. Analysis of TCR V region genes revealed the predominant use of the Vβ8.5-Jβ2.3 gene combination, with extensive N region additions to both Dβ1 and Dβ2. These results define the Hu-BP 110-129 peptide sequence as the major encephalitogenic epitope for the LOU/M strain of rat previously considered resistant to EAE, and support the idea that the encephalitogenic property of BP and other CNS Ag for a given MHC is encompassed within immunodominant T cell epitopes. Furthermore, the TCR sequence data indicate the predominant use of a different Vβ8 subfamily member (Vβ8.5) than the Vβ8.2 gene used preferentially by several other rat strains and the PL/J mouse in the T cell response to BP.

Gp-BP[3] is highly encephalitogenic in a variety of rat and mouse strains when injected with adjuvants. The encephalitogenic determinants of Gp-BP are different among these strains (1–7), and are influenced by the association of processed BP peptides with strain-specific class II MHC molecules (8–11). The prototype EAE model in rat is in the Lewis strain (RT-1^l), in which the immunodominant and encephalitogenic region of Gp-BP is subsumed within the 69-89 amino acid sequence (12). Rt-BP, which differs in this region by a threonine for serine substitution at position 80, has approximately 10-fold lower encephalitogenic activity and a reduced capacity to stimulate proliferation of T cells specific for the Gp-BP sequence (13). Hu-BP and bovine BP have even greater sequence differences in this region, creating a defective, non-encephalitogenic T cell epitope for Lewis rats (14). The adjoining 87-99 sequence, which is conserved in all BP, is also encephalitogenic for Lewis rats (15), and accounts for the encephalitogenic activity observed with Hu- and bovine-BP.

The encephalitogenic capacity of a given epitope is related both to its ability to stimulate specific T cells and to its sequence homology with the autologous BP. Thus, the 55-74 sequence of Gp-BP is capable of inducing highly specific T cell lines in Lewis rats that can transfer delayed type hypersensitivity reactions, but not clinical EAE or central nervous system lesions (15). The lack of encephalitogenic activity can be accounted for by the inability of the Gp-55-74 specific T cells to recognize Rt-BP or the Rt-55-74 peptide that has substantial variation from the Gp-BP sequence.

Much of our understanding of encephalitogenic epitopes has been derived from analysis of BP-specific T cell lines. Immunization with whole BP activates a spectrum of T cell specificities (15, 16), and further selection with BP in vitro allows the emergence of clones responsive to immunodominant epitopes. These epitopes can be identified in vitro by assessing the specificity of the BP-specific T cells using highly purified, enzymatically cleaved or synthetic peptides that correspond to sequences within the encephalitogen (15). Encephalitogenic activity can then be confirmed by using the peptides to activate the T cell lines, and by active injection of peptides with adjuvants. In our hands this strategy has been useful in defining encephalitogenic epitopes for LEW (12, 13), F344 (12), BN (12), BUF (6) (R. E. Jones. Manuscript in preparation), and ACI (7) rats, and for SJL/J mice (2).

In this manuscript, we use BP-specific T cell lines to define a major encephalitogenic determinant for the

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[3] Abbreviations used in this paper: Gp-BP, guinea pig myelin basic protein; Rt-BP, rat BP; Hu-BP, human BP; EAE, Experimental autoimmune encephalomyelitis.

EAE-resistant LOU/M rat, which is of the (RT-1^w) haplotype and is distinct from other rat strains studied previously. Biologic activity was not found in Gp-BP, but resided within the 110-129 sequence of Hu-BP, which differs from Gp-BP by a single amino acid residue at position 123. In addition, we found that within the Vβ8 family, Vβ8.5 is preferentially expressed in the encephalitogenic Hu-BP lines, unlike the prototypic EAE Lewis rat that primarily uses Vβ8.2 in response to GP-BP (15, 17, 18).

MATERIALS AND METHODS

Animals. LOU/M and LEW rats were obtained from Harlan Sprague Dawley, Inc. Indianapolis, IN. The rats were used after they reached 10 to 12 wk of age. All animal procedures were in accordance with institutional and federal guidelines.

Preparation of BP and synthetic peptide Ag. Gp, Rt, or Hu BP was extracted and purified according to the method of Eylar et al. (19). All peptides used in this study were synthesized in our laboratory by the Merrifield solid phase method as detailed elsewhere (20). The peptides were purified by Sephadex G-10 column chromatography and by HPLC, and their composition verified by amino acid analysis. The sequences used include: Gp-S49S (69-84) = GSLPQKSQ--RSQDEN; Hu-S49S = GSLPQKS-HGRTQDEN; S87-99 (conserved in all BP) = VHFFKNIVTPRTP; Hu-S102-129 = PSQGKGRGLSLSRFSWGAEGQRPGFGYG; Hu-S110-129 = LSLSRFSWGAEGQRPGFGYG.

Induction of EAE. BP or peptides were dissolved in saline and emulsified with an equal volume of CFA, and 0.1 ml of the emulsion was injected s.c. into one hind footpad. Some groups received an i.v. injection of 3.5 μg *Bordetella pertussis* toxin (product no. 180, List Biological Laboratories, Inc., Campbell, CA) on the day of BP challenge and 48 h later. Animals were scored daily for clinical signs of EAE according to the following scale: 0 = no signs; 1 = limp tail, weight loss; 2 = hind leg weakness; 3 = hind leg paralysis, incontinence; 4 = moribund condition. Animals with no signs or animals recovered from EAE were euthanized 25 to 30 days after challenge, and the serum, brain, and spinal cord were removed for analysis of antibodies and for histologic signs of EAE.

Histologic evaluation. The entire brain and spinal cord tissues were fixed in 10% formalin in PBS. The paraffin-embedded tissues were processed and serial sections (8 μ) were prepared and stained with hematoxylin-eosin for light microscopy. Histologic lesions were counted and scored as follows: 0 = no lesions; 1 = 2 lesions; 2 = 4 lesions; 3 = 6 lesions; 4 = 8 lesions in the sagittal sections of the brain and in longitudinal sections of the entire spinal cord.

T cell proliferation assay. Lymph nodes draining the site of injection of BP/CFA were removed aseptically, and 5×10^5 cells from a single cell suspension were cultured in RPMI medium containing added glutamine, antibiotics, and 1% fresh rat serum in flat-bottomed 96-well microtiter plates with Ag and mitogens (1). The cultures were incubated at 37°C in a 5% CO_2 atmosphere for 3 days, and proliferation was measured by uptake of ^{3}H-thymidine (0.5 μBq added per well) during the last day of culture. The cells were harvested on glass fiber filters, and labeled thymidine uptake counted by standard liquid scintillation techniques.

T cell lines. Lymph node cells were cultured with Hu- or Gp-BP (33 μg/ml) in 6-ml petri plates at a concentration of 7×10^6 cells/ml RPMI medium described above. After 3 days culture, the cells were resuspended in RPMI medium supplemented with 10% horse serum and 10% IL-2-enriched supernatant from 2-day Con A-stimulated splenocytes. When growth slowed in the IL-2-enriched medium, 5×10^6 cells were restimulated with 50 μg BP in the presence of 100×10^6 irradiated normal thymocytes in 10-ml petri plates in medium without IL-2 (1). Thereafter, the T cells were cycled between IL-2 growth and restimulation with BP. Specificity of the T cell line was assessed by proliferation as described above, culturing 20,000 T cells and 10^6 thymocytes per microtiter well for 3 days.

Antibody assay. LOU/M serum antibody reactivity to BP and peptides was measured as described previously (14) using an adaptation of the direct ELISA.

Determination of TCR β-chain gene usage. Total cellular RNA was isolated from a Hu-BP-specific T cell line by lysis in guanidinium isothicyanate and centrifugation through a cesium chloride cushion (21). RNA was then converted to cDNA using the COPY-KIT (Invitrogen, San Diego, CA) with 2.5 μM oligonucleotide primer specific for rat TCR Cβ1 and 2, 5'CATAGAAtTcCACTTGGCAGCGGAAGTGGT3' (Genosys, The Woodlands, TX). Bases in small letters denote changes from the TCR Cβ sequence made to create an *Eco*R1 restriction endonuclease site. After incubation, the reaction mixture was heated to 95°C for 3 min to denature the DNA/RNA duplexes. The DNA was then amplified, using the polymerase chain reaction (22), in a 50-μl volume containing 1 μM Cβ oligonucleotide primer, 2 μM Vβ8-specific oligonucleotide, 200 μM dNTP each (Pharmacia Fine Chemicals, Piscataway, NJ), 0.5 U Taq DNA polymerase (Pharmacia) in 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin. The Vβ8-specific oligonucleotide, 5'GGGCCGCGGAACACATGGAAGCTGCAGTCAC3', is specific for rat Vβ8.2 as well as the three murine Vβ8 genes (18, 23), and contains a 5' *Sac*II restriction endonuclease site. This oligonucleotide will amplify all six rat Vβ8 genes (D. P. Gold, unpublished observations). Each sample was overlayed with mineral oil and subjected to 30 amplification cycles of 1 min at 92°C, 1.5 min at 55°C, and 2 min at 72°C in a thermocycler (Ericomp, San Diego, CA). After amplification, the samples were chloroform extracted to remove the mineral oil, ethanol precipitated and then digested with *Sac*II plus *Eco*R1 (New England Biolabs, Beverly, MA). The resulting DNA was separated on a 1.4% agarose gel, eluted onto NA45 paper (Schleicher & Schuell, Keene, NH), ethanol precipitated, and ligated into the *Sac*II/*Eco*R1 site of pBluescript II (Stratagene, La Jolla, CA). The ligation mixture was transformed into the bacterial strain XL1-blue and white colonies were selected for miniprep analysis from agar plates containing X-gal and isopropyl-β-D-thiogalactopyranoside. Miniprep DNA was sequenced on both strands by the dideoxy chain termination method (24) using the Sequenase sequencing system (U.S. Biochemical, Cleveland, OH).

TABLE I

LOU/M lymph node cell responses after immunization with Gp-BP/CFA[a]

Stimulant	Proliferation Response (cpm/1000)
Medium	7 ± 0
Con A	71 ± 8
Gp-BP	8 ± 1
Hu-BP	21 ± 2
Rt-BP	6 ± 1
Gp-38-67	7 ± 2
Gp-S49S	7 ± 2
S87-99	8 ± 1

[a] Rats were immunized with 100 μg Gp-BP in CFA s.c. for 15 days before collection of LNC. Cells were stimulated for 5 days with indicated antigens and evaluated for uptake of ^{3}H-Tdr. Underscored values indicate significant response.

RESULTS

Immunization of LOU/M rats with Gp-BP fails to induce EAE, but stimulates recognition of Hu-BP. Due to its potent ability to induce EAE in most strains of rats and mice studied thus far, Gp-BP has been the encephalitogen of choice over other sources of BP. However, LOU/M rats injected with Gp-BP/CFA (n = 12) or with two different peptides known to be encephalitogenic in Lewis rats (13–15), Gp-S49S/CFA (n = 10), and S87-99/CFA (n = 4), did not develop clinical or histologic EAE. Lymph node cells from Gp-BP/CFA-immunized LOU/M rats also failed to respond to whole Gp-BP, Gp-S49S, Gp-S87-99, and Gp-38-67, but responded strongly to both the T cell mitogen Con A (as expected) and Hu-BP (Table I). T cell lines could not be selected subsequently with Gp-BP (data not shown).

Selection of Hu-BP-specific T cell line identifies immunodominant and encephalitogenic epitope. Lymph node cells from Hu-BP/CFA-immunized LOU/M rats responded strongly to Hu-BP, but did not recognize Gp-BP, the Gp-S49S peptide that is immunodominant in Lewis rats, or Rt-BP (Table II). The LOU/M LNC also responded to the C terminal half of Hu-BP (P4, residues 90-171), and to Hu-S102-129 and Hu-S110-129 (Table II), but not to other synthetic peptides within P4 (data not shown). Subsequently, T cell lines were selected from the LNC, using either whole Hu-BP or Hu-S102-129 as the selecting Ag. Both of these T cell lines responded strongly to Hu-BP, Hu-S102-129, and Hu-S110-129, but did not rec-

ognize a shorter peptide within the 110-129 region (Hu-S110-123), Rt-BP, or any Gp-BP determinant (Table II). Both the Hu-BP and Hu-102-129-specific T cell lines transferred moderate to severe clinical and histologic EAE to naive LOU/M rats (Table III).

Antibody responses in LOU/M rats also favored determinants within the 102-129 region of Hu-BP. Rats immunized with Hu-BP or Hu-S110-129 responded best to the Hu-BP 110-129 epitope (Fig. 1). Moreover, rats immunized with Gp-BP responded better to Hu-BP than to Gp-BP, and to the 110-129 epitope within Hu-BP. It is noteworthy that LOU/M rats immunized with Hu-BP-produced antibody but not T cell responses to Gp-BP. In contrast, LOU/M rats immunized with the Lewis rat determinants Gp-S49S or Gp-S87-99 did not produce antibodies or T cell responses to any of the Hu- or Gp-BP determinants tested (data not shown).

Induction of active EAE requires immunomodulation in addition to CFA. Although T cells specific for Hu-BP and Hu-S102-129 could transfer EAE to naive LOU/M rats (Table III), immunization with Hu-BP/CFA or Hu-S110-129/CFA failed to induce clinical or histologic EAE (Table IV). To enhance susceptibility to EAE, LOU/M rats challenged with various Hu-BP preparations were further stimulated with *B. pertussis* toxin or cyclophosphamide. Although both of these immunomodulatory adjuncts were effective in enhancing EAE induction, the most severe EAE was induced by co-administration of pertussis toxin with Hu-BP, Hu-S102-129, and Hu-S110-129 (Table IV). The Hu-S110-129 sequence found to be encephalitogenic for LOU/M rats is distinct from other known encephalitogenic sequences in rats (Table V).

TCR Vβ usage in Hu-BP response. It has been shown previously that Vβ8.2 is the predominant TCR V region element expressed in the I-A-restricted response of Lewis rats to the major encephalitogenic peptide of Gp-BP, S72-89, as well as the I-E-restricted encephalitogenic response to the Rt-BP peptide, 87-99 (15, 17, 18). To determine whether Vβ8.2 is also the predominant Vβ used in the Lou/M response to Hu-BP, polymerase chain reaction analysis was performed on cDNA derived from a Hu-BP-specific T cell line using a Vβ oligonucleotide specific for the 6 rat Vβ8 genes (see *Materials and Methods*). Of 13 independent sequence determinations, 9 isolates contained the sequence encoding the rat Vβ8.5 gene, 2 isolates were rat Vβ8.6, 1 isolate was rat Vβ8.2, and 1 was rat Vβ8.3 (Fig. 2). Of the nine Vβ8.5 isolates, eight of these used Jβ2.3 although all isolates contained unique N and D region encoded amino acid residues (Fig. 3). Thus, unlike Gp-BP-specific T cells from other rat strains that preferentially use Vβ8.2, Hu-BP-specific T cells from the LOU/M rat preferentially utilize other Vβ8 subfamily genes, predominantly Vβ8.5. Inasmuch as the Vβ primer used in the PCR reaction will only amplify the 6 rat Vβ8 family members (D. P. Gold, unpublished observation), we cannot determine whether other Vβ genes contribute to the encephalitogenic responses against Hu-GP. Further studies with T cell clones will be necessary to answer this question.

TABLE II

Proliferation response of lymph node cells and T cell lines from Hu-BP challenged LOU/M rats[a]

Stimulant	Proliferation Response (cpm/1000)		
	LNC	Hu-BP T cell line	Hu-S102-129 T cell line
Medium	5 ± 1	15 ± 3	2 ± 1
Con A	50 ± 4	75 ± 2	128 ± 26
Gp-BP	5 ± 2	16 ± 3	2 ± 1
Gp-S72-89	9 ± 3	16 ± 1	ND
Rt-BP	5 ± 1	15 ± 1	2 ± 0
Hu-BP	40 ± 2	89 ± 2	143 ± 16
Hu-1-38	10 ± 1	ND	ND
Hu-45-89	10 ± 0	ND	ND
Hu-90-171	19 ± 0	ND	ND
Hu-S102-129	16 ± 2	78 ± 4	152 ± 5
Hu-S110-129	15 ± 1	50 ± 1	136 ± 17
Hu-S110-122	ND	13 ± 1	3 ± 1

[a] LOU/M rats were injected with Hu-BP/CFA, and LNC collected and cultured with the indicated Ag 17 days later. Some cells were expanded in IL-2 for 4 days and restimulated with either Hu-BP or Hu-S102-129 to make T cell lines.

DISCUSSION

The results of this study define the 110-129 peptide sequence of Hu-BP, by virtue of its immunodominance, as a major encephalitogenic epitope for the LOU/M strain (RT-1^{w}) of rat. This epitope induced both strong T cell and antibody responses comparable to whole Hu-BP, and T cells specific for this determinant transferred clinical and histologic EAE. Furthermore, active EAE could be induced with the Hu-S110-129 peptide in combination with CFA and a co-immunomodulator. These results illustrate that the identification of an immunodominant epitope recognized by BP-specific T cell lines can lead to a precise determination of an encephalitogenic sequence. By implication, one might also expect immunodominant epitopes recognized by BP-specific T cell lines in humans (25–29) to be encephalitogenic, at least under permissive conditions mimicked by the co-immunomodulators used in this study.

It was not expected that immunization with Gp-BP would induce strong T cell recognition of Hu- but not Gp-BP. Within the 110-129 sequence, Hu-BP differs from Gp-BP and Rt-BP by a single conservative amino acid substitution at position 123, where Hu-BP has arginine instead of lysine (19). This sequence difference accounts for the dramatic increase in T cell proliferation responses to Hu-BP and the Hu-S110-129 peptide that are absent to Gp-BP and Rt-BP (Tables I and II). A similar heteroclitic response by T cells has been reported in the cytochrome system (30). In contrast, immunization with either Hu-

TABLE III

Transfer of EAE to naive LOU/M rats with T cell lines[a]

Specificity of Donor T Cell Line	EAE in Recipient LOU/M Rats				
	Incidence	Onset	Maximum severity	Duration in days	Histology
Hu-BP	9/9	6 ± 1	2.5 ± 0.3	6 ± 1	2.5
Hu-S102-129	3/3	5 ± 0	2.8 ± 0.3	8 ± 0	2.5

[a] T cell lines characterized in Table III were activated with Hu-BP or Hu-S102-129 for 3 days before transfer of 9 million line cells into naive LOU/M rats.

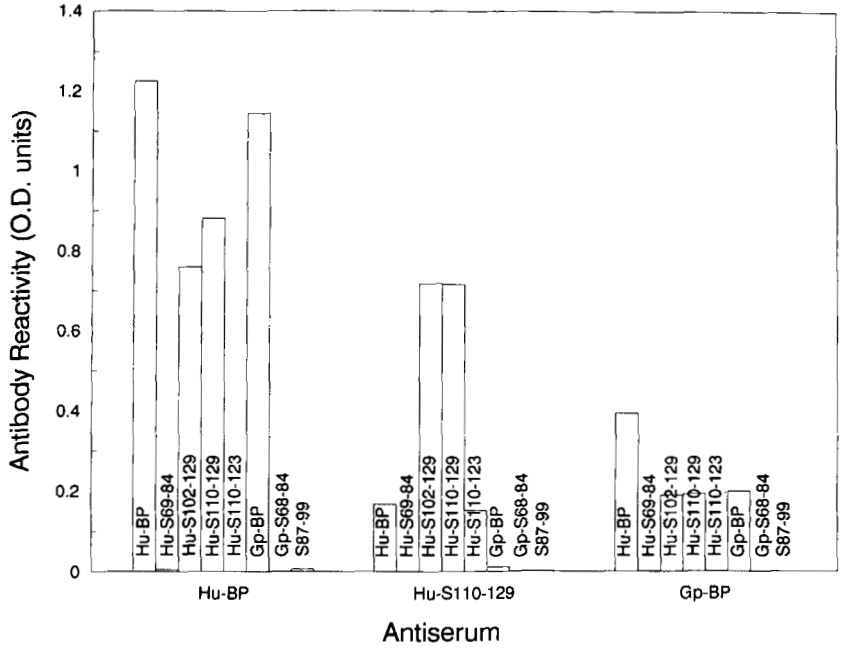

Figure 1. Serum antibody responses in LOU/M rats. Serum was collected 21 days after challenge with Hu-BP, Hu-S110-129, or Gp-BP in CFA. Antibody reactivity was measured by ELISA, using a 1/320 dilution of serum, and 25-ng plated Ag.

TABLE IV

Encephalitogenic activity of Hu-BP and peptides within 102-129 region of Hu-BP requires co-immunomodulation

Inoculum	Incidence	Onset	Maximum Severity	Duration in Days
Hu-BP/CFA[a]	0/4			
Hu-S110-129/CFA	0/4			
Hu-BP/CFA + PT[b]	5/5	11	2	8
Hu-BP/CFA + CY[c]	4/4	11	1.5	11
Hu-S102-129/CFA + PT	3/3	11	3	8
Hu-S110-129/CFA + PT	4/4	11	3	8

[a] Emulsified in IFA + 100 μg *M. butyricum* and given s.c.
[b] *B. pertussis* toxin (3.5 μg) given i.v. on days 1 and 3 after challenge.
[c] Cyclophosphamide, 8 and 5 mg i.p., on days 0 and 8 after challenge.

TABLE V

Encephalitogenic determinants in different rat strains

MHC Haplotype	Rat Strain	BP Epitope/ Restriction Molecule	Reference
RT-1[l]	LEW, F344	Gp-72-84/I-A; 87-99/I-E	(12, 15)
RT-1[n]	BN	Gp-43-70/I-A	(12)
RT-1[a]	ACI	Gp-39-54/I-A	(7)
RT-1[b]	BUF	87-99/I-A	(6)[a]
RT-1[w]	LOU/M	Hu-110-129	Current report

[a] R. E. Jones. Manuscript in preparation.

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        -1  1
            H  M  E  A  A  V  T  Q  S  P  R  N  K  V  T  V  T  G  K
1       AA CACATGGAAGCTGCAGTCACCCAAAGCCCAAGAAACAAGGTGACAGTTACAGGAAAA
            N  V  T  F  N  C  H  Q  T  D  N  H  N  Y  M  Y  W  Y  R  Q
60      AATGTGACGTTCAACTGTCACCAGACTGATAACCACAACTACATGTACTGGTATCGGCAG
            D  M  G  H  G  L  R  L  I  H  Y  S  Y  G  S  G  S  F  E  N
124     GACATGGGGCATGGTCTGAGGCTGATCCATTACTCATATGGTTCTGGCAGCTTTGAAAAT
            G  D  I  P  E  G  Y  K  V  S  R  P  N  Q  E  N  F  F  L  T
188     GGAGATATCCCTGAGGGGTACAAGGTCTCCAGACCAAACCAAGAAAATTTCTTCCTCACG
            L  E  S  A  S  P  S  Q  T  S  V  Y  F  C  A  S  S
252     CTGGAGTCGGCTTCCCCCTCTCAGACATCTGTGTACTTCTGTGCCAGCAGT
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Figure 2. Nucleotide and predicted amino acid sequence of LOU/M Vβ8.5. Underscored bases denote sequence of oligonucleotide used in the PCR reaction.

or Gp-BP induced comparable antibody responses to both Hu- and Gp-BP, although Hu-BP was clearly the better immunogen. In view of the requirement for T cell recognition of autologous BP, it is unclear why T cells that do not proliferate to the lysine-containing sequence (Table II) are encephalitogenic in LOU/M rats. It is conceivable that the lysine-containing Rt-BP epitope present in rat central nervous system is sufficient to activate the Hu-BP specific T cells to release lymphokines without inducing concomitant proliferation responses. That T cells induced by the lysine containing sequence (Gp-BP) provided help for antibody production (Fig. 1) without proliferating (Table II) supports this contention.

The results of this study show that the LOU/M rat is resistant to EAE induction by either Gp- or Hu-BP/CFA. However, the resistance mechanisms could be overcome by the use of co-immunomodulation (pertussis toxin, PT, or cyclophosphamide, CY, in addition to CFA) in a manner similar to a growing number of other rat and mouse strains. The effects of pertussis toxin are complex, but one activity may be to alter the permeability of the blood-brain barrier to allow passage of activated T cells (31). Cyclophosphamide, on the other hand, appears to reduce the effective level of suppression. The necessity for PT or CY for active EAE induction in LOU/M rats suggested that EAE resistance was not simply the inability to induce activated T cells. Indeed, immunization of LOU/M rats with Hu-BP/CFA without PT or CY did induce strong

Clone#	V_β	ND_BN	J_β	V_β-J_β
9	CysAlaSerSer	AspPheLeuThr	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	gattttctgact	acagacaag	
20	CysAlaSerSer	AspIleVal	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	gacattgtc	acagacaag	
32	CysAlaSerSer	AspGlnGluThr	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	gaccaggagact	acagacaag	
5	CysAlaSerSer	GluMetGlyArg	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	gagatggggagg	acagacaag	
33	CysAlaSerSer	GluLysPheThr	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	gagaagtttact	acagacaag	
10	CysAlaSerSer	ValGlyThrGly	AlaThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	atcgggacagga	gctacagacaag	
14	CysAlaSerSer	GlyGlnGluLeuGln	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagcagt	ggacaggaactacag	acagacaag	
12	CysAlaSer	GlnGlyAlaAsp	ThrAspLys	Vβ8.5-Jβ2.3
	tgtgccagc	caggggccgat	acagacaag	
24	CysAlaSerSer	ThrGlyThrGlyGly	AsnGluArg	Vβ8.5-Jβ1.4
	tgtgccagcagc	accgggacaggggg	aatgaaaga	
25	CysAlaSerSer	LeuThr	GluArg	Vβ8.6-Jβ1.4
	tgtgccagcagt	ctgacg	gaaaga	
4	CysAlaSerSer	AspArgGlnSer	ThrAspLys	Vβ8.6-Jβ2.3
	tgtgccagcagt	gatcgacagtct	acagacaag	
3	CysAlaSerSer	AspArgAla	AsnThrGly	Vβ8.2-Jβ2.2
	tgtgccagcagc	gacagggcg	aacaccggg	
21	CysAlaSerSer	GluAlaTyrGlyAsp	ThrGly	Vβ8.3-Jβ2.2
	tgtgccagcagt	gaggcgtacggggac	accggg	

Figure 3. Sequence analysis of 13 independent TCR Vβ8 isolates from PCR amplification of a LOU/M anti-Hu-BP T cell line.

responses to Hu-BP, but not clinical EAE. Resistance to active EAE would appear to involve additional mechanisms such as reduced levels of vasoactive substances (32, 33) or increased suppressor cell activity (34–39) that dampens the induction or maturation of encephalitogenic T cells. Once selected and activated, however, BP-specific T cells can apparently bypass the natural regulatory mechanisms, as is demonstrated by their ability to transfer paralytic EAE in the absence of PT or CY (Table III).

It was of interest to find, as in the case of the I-A^u-restricted murine response (40, 41), as well as the Lewis rat I-A- and I-E-restricted response to Gp-BP (15, 17, 18) (D. P. Gold, in preparation), that a member of the rat Vβ8 family was used in the LOU/M T cell response to Hu-BP. However, in the case of the LOU/M response, Vβ8.5 was used most frequently (see Fig. 2), in comparison with the murine and Lewis rat responses that predominantly used Vβ8.2. The homology between rat Vβ8.2 and Vβ8.5 is approximately 80% and is similar to the level of homology between the mouse Vβ8.2 and the rat Vβ8.2 or Vβ8.5. The finding that both mice and rats use the Vβ8 gene family in their response to multiple epitopes of BP is intriguing even though the mechanism that drives the TCR V gene selection process is unclear at present.

The 110-129 epitope of Hu-BP is distinct from encephalitogenic sequences described for other rat strains (see Table V), but is similar to the 114-123 peptide of bovine BP that is encephalitogenic for guinea pigs (42, 43). Of interest, the Hu-BP S110-129 sequence in association with HLA-DR molecules is also recognized by human BP-specific T cell clones (26). These data support the idea that the encephalitogenic property of BP is encompassed within relatively few (~10) discrete regions (25–29), a subset of which can form immunodominant epitopes in association with the available MHC class II molecules of a given strain. Our data indicate that one of these bioactive regions of BP is encompassed within the 110-129 sequence.

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