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IDENTIFICATION OF ENCEPHALITOGENIC Vβ-4-BEARING T CELLS IN SJL MICE

Further Evidence for the V Region Disease Hypothesis?[1]

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system mediated by T cells bearing TCR of restricted heterogeneity. Thus, in the murine PL strain, Vβ-8.2 is used by 80% of the encephalitogenic T cells. This observation has led to the successful prevention and reversal of EAE by the in vivo use of mAb directed to these restricted gene products. In SJL mice, the Vβ-17a gene product has been shown to be used by approximately 50% of encephalitogenic T cells subsequent to immunization with a myelin basic protein (MBP)-derived peptide. However, the other Vβ genes used by encephalitogenic T cells in SJL EAE have remained uncharacterized. We now report, for the first time, the β-chain-encoding DNA sequence of two encephalitogenic, MBP-reactive, SJL-derived T cell clones. These clones which are specific for H-2^S and the carboxyl-terminus (amino acid 92-103) of MBP, use TCR encoded by Vβ-4. In addition, we demonstrate that the transfer of EAE by a heterogenous SJL-derived encephalitogenic T cell line can be prevented using an anti-Vβ-4 antibody in vivo. Vβ-4 usage has been previously described in a H-2^u/MBP amino-terminus-reactive encephalitogenic T cell. The present findings may thus further support the "V region-disease" hypothesis.

EAE[3] is an autoimmune disease of the central nervous system inducible in a number of animal species by immunization with MBP or other neuroantigens and mediated by CD4$^+$ T cells. As such, EAE is a model for the human disease multiple sclerosis. With the significant progress made in our understanding of the TCR over the past few years, considerable insight into the cellular immune response in this disease has been gained. We and other investigators have demonstrated a restricted use of TCR genes in murine EAE (1–6). In the murine B10.PL and PL strains (H-2^u) it has been shown that approximately 80% of encephalitogenic T cell clones (specific for the amino-terminal encephalitogenic fragment of MBP) bear a Vβ-8.2$^+$ TCR (3–5). These observations have led to the successful prevention of EAE in these mouse strains by injection of a monoclonal anti-Vβ-8 antibody before immunization with MBP (4–6). In addition, in other experiments a reversal of paralysis occurred when these mAb were administered after the first signs of disease (4, 6).

EAE also is inducible in the SJL strain of mice (H-2^S) but there are a number of significant differences in the pathogenesis of EAE between the PL and SJL strains. In contrast to the encephalitogenic amino-terminus of MBP for the PL strains, in SJL the major encephalitogenic determinants are located at the carboxyl-terminus of the MBP molecule. Furthermore, the Vβ-8 gene segments are deleted from the genome in SJL mice (7). Steinman and colleagues (2) have reported that approximately 50% of encephalitogenic SJL T cell clones derived after sensitization to the MBP peptide p89-101 and specific for this peptide reacted with the anti-Vβ-17a antibody, KJ23a. However, to date, the TCR Vβ gene segment(s) used by the encephalitogenic SJL T cell clones that are Vβ-17a$^-$ have yet to be described.

Here we report, for the first time, the identification of a Vβ gene segment used by Vβ-17a$^-$ T cells involved in the pathogenesis of SJL EAE. We have derived encephalitogenic T cell clones from an SJL mouse that use Vβ-4. In addition, from a second SJL mouse, we have generated a T cell line that mediates EAE via Vβ-4-bearing T cells. This report represents not only the first identification of the "other" Vβ used by encephalitogenic SJL T cells, but also the first description of the Vβ sequence from an H-2^S/MBP-carboxyl-terminus-restricted encephalitogenic SJL T cell clone. The implications of the present results for the "V region-disease" hypothesis (8, 9) are discussed.

MATERIALS AND METHODS

Mice. Female SJL mice were obtained from The Jackson Laboratories, Bar Harbor, ME, and from the National Institute of Health, Frederick, MD.

Ag. PMBP was obtained as a generous gift from Eli Lilly Co., Indianapolis, IN. Guinea pig myelin basic protein and the pepsin-

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[3] *Abbreviations* used in this paper: EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; PMBP, porcine myelin basic protein.

digested fragments of guinea pig myelin basic protein were isolated as previously described (10). Murine myelin basic protein was isolated from mouse brains as previously described (11). The synthetic MBP peptides p92-119, p92-103, and p96-109 were synthesized as previously described (12). These peptides correspond exactly to the referenced pM87-114, pM87-98, and pM91-104 respectively, and have been renumbered here for the purpose of maintaining convention with the pepsin-digested carboxyl-terminal fragment (see Fig. 1).

T cell lines and clones. Clones MM.4 and MM.10 were derived by limiting dilution cultures from the heterogenous T cell line LNC8, which has been previously described (13). LNC8 is an IL-2-dependent, encephalitogenic T cell line derived from the lymph nodes of an SJL mouse immunized 10 days prior with PMBP and CFA. LNC8 has been maintained in culture for 3 yr with the twice weekly addition of 2 U/ml of human rIL-2 (Amgen, Inc., Thousand Oaks, CA) and with stimulation using irradiated SJL splenocytes and PMBP once every 2 wk. The heterogenous, encephalitogenic T cell line, LNC12, was derived from the 10-day PMBP-immunized lymph nodes of a second SJL mice and maintained in vitro in the same manner as LNC8.

To derive clones MM.4 and MM.10, LNC8 was plated at one cell per well and the resulting clones were fed as above with the exception that complete media (RPMI, 10% FCS, 5×10^{-5}M 2-ME) in the absence of IL-2 was used instead of 2 U/ml IL-2 for 1 wk of the feeding cycle, and IL-2 was only used at the time of the irradiated SJL splenocytes and PMBP feed and the following feed, 3 days later.

Proliferation assay. T cell clone proliferation assays were performed as previously described (1). Briefly, 5×10^4 T cells were plated in complete media (without IL-2), and 24 h later irradiated (2500 rad) SJL splenocytes (5×10^5/well) and various concentrations of antigens were added to the T cells. Then 48 h later, 2 μCi ^{3}H-thymidine (New England Nuclear, Boston, MA) was added to each well and 18 to 24 h later the cultures were harvested with the aid of a semiautomated cell harvester. Results are expressed as mean ± SEM of cpm of ^{3}H uptake in duplicate or triplicate wells.

Assay for encephalitogenicity. T cell lines and clones were injected i.p. or intracardiac into non-irradiated, naive SJL mice, four days after the T cells were last stimulated with irradiated splenocytes and PMBP, and 1 day after being fed with IL-2. Signs of EAE were assessed daily after transfer of the cells, and were graded as previously described (14): grade 1: tail weakness; grade 2: hind leg weakness; grade 3: hind leg paralysis; grade 4: hind and front leg paralysis; grade 5: death. For the in vivo inhibition of EAE, anti-Vβ-4 (protein G-purified monoclonal KT10-a rat Ig2b) or rat anti-rat T cell alloantibody, RT6.1 (clone DS4.23-a rat Ig2b monoclonal; not cross-reactive with mouse T cells; concentrated serum-free supernatant or protein G purified) were injected i.p. within 1 h of the intracardiac injection of LNC12. Purity of mAb was documented by SDS-PAGE stained with Coomassie blue. The anti-Vβ-4 mAb KT10 (15) was a gift of Dr. K. Tomonari (Clinical Research Center, Medical Research Council, Middlesex, UK) and the anti-rat T cell antibody RT6.1 (clone DS4.23) (16) was a gift of Dr. Dale Greiner (Department of Pathology, University of Connecticut Medical School, Farmington, CT).

RNA isolation and Northern blot analysis. Total cellular RNA was isolated from the T cell clones at least 10 days after Ag stimulation by guanidine thiocyanate/cesium chloride and quantitated by absorbance at 260 nm (17). A total of 20 μg of RNA was electrophoresed through a denaturing 1% agarose/0.66 M formaldehyde gel and transferred to GeneScreen Plus (NEN Research Products, Boston, MA). Filters were baked at 80°C for 2 h and prehybridized in 50% deionized formamide, 1% SDS, 1 M NaCl, and 10% dextran sulfate for 2 h at 42°C. Hybridization was performed in the prehybridization solution with the addition of denatured salmon sperm (final concentration of 200 μg/ml) and the ^{32}P-radiolabeled DNA at a final concentration of 1×10^6 cpm/ml for 16 h at 42°C. The DNA was labeled to a sp. act. of 0.5 to 1×10^9 cpm/μg by the random primer technique (18). Washes were performed at room temperature for 15 min × 2 with 2 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M trisodium citrate), 0.1% SDS followed by 15 min × 2 at 55°C with 0.2 × SSC, 0.1% SDS. Autoradiography was performed with intensifying screens at −70°C with exposure for up to 24 h. Blots were reprobed after boiling for 30 min in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 1% SDS.

Vβ gene segment probes. To determine which Vβ segments were being used by the T cell clones, Northern blot analysis was performed using a collection of murine Vβ segment probes representing the known Vβ subfamilies (a generous gift from Dr. Edward Palmer, National Jewish Hospital, Denver, CO).

Polymerase chain reaction. First-strand cDNA was synthesized from 10 μg of total cellular RNA with oligo(dT) as primer and Moloney murine leukemia virus reverse transcriptase in a total volume of 50 μl (19). Five μl of the cDNA synthesis reaction was then used as template for polymerase chain reaction amplification (20). The 5′ Vβ-4 primer was 5′-CCTCTAGATGGGCTCCATTTTCCT-3′. The 3′ constant region primer was 5′-CCAAGCTTCTGCTTTTGATGGCTC-3′. Amplification was performed in a volume of 100 μl for 30 cycles in a thermal controller (MJ Research, Watertown, MA) with primer annealing for 1 min at 54°C, extension for 2 min at 72°C, and denaturation for 1 min at 91°C. A 10-min extension at 72°C was performed at the completion of the cycling. The amplification buffer used included KCl 50 mM, Tris-HCl 20 mM, pH 8.4 (at 25°C), MgCl2 2.5 mM, BSA 0.1 mg/ml, and Taq DNA polymerase 2.5 U. Both primers were treated with kinase and used at a final concentration of 0.2 μM.

DNA sequence analysis. The amplified product was isolated by electrophoresis in low melt agarose and cloned into the *HincII* site of pBS (Stratagene, LaJolla, CA) for double-stranded sequencing by dideoxy chain termination (21).

Flow microfluorimetry analysis. LNC12, MM.4 and MM.10 were analyzed for Vβ expression 12 days after last being stimulated with irradiated splenocytes and PMBP. Cells were incubated either with supernatant from KJ23a (anti-Vβ-17a) a gift of Drs. John Kappler and Phillip Marrack, National Jewish Center, or with supernatant from KT10-4 (anti-Vβ-4), or with a control supernatant and subsequently stained with fluorescein-conjugated goat anti-mouse or anti-rat antibody as appropriate. Antibody binding was evaluated with a cytofluorograph.

RESULTS

Derivation of T cell clones. The SJL-derived, heterogeneous, MBP-reactive, encephalitogenic, IL-2-dependent T cell line LNC8 has been maintained in vitro culture for 3 yr and has been previously described (13). LNC8 is encephalitogenic after the intraperitoneal or intravenous adoptive transfer of approximately 10^7 cells to naive, nonirradiated SJL mice. As previously reported, LNC8 consists of approximately 40 to 50% KJ23a Vβ-17a$^+$ T cells as assessed by indirect immunofluorescence (13). We found, however, that the Vβ-17a-negative subpopulation of LNC8 was significantly more effective at adoptively transferring EAE than was the Vβ-17a$^+$ subpopulation (data not shown). To characterize the Vβ gene segments used by this Vβ-17a$^-$ population, T cell clones were derived from the LNC8 population by limiting dilution cultures. T cell clones were derived from 40% of limiting dilution culture wells in which LNC8 was plated at one cell per well. Two encephalitogenic clones, MM.4 and MM.10 were selected for further study.

Proliferative responses of T cell clones. T cell clones MM.4 and MM.10 both demonstrated proliferative responses to porcine, guinea pig, and murine MBP and were found to respond to the carboxyl-terminal fragment (amino acid 89-169) of the limited-pepsin-digested guinea pig MBP molecule, consistent with the known major encephalitogenic region of MBP in SJL mice (Table I). The fine specificity of clones MM.4 and MM.10 was further dissected using synthetic MBP peptides. Both clones responded to p92-119 and to p92-103, but did not respond to p96-109 (Table I). This suggests that MM.4 and MM.10 respond to an MBP epitope that probably includes amino acids 92-95 (Fig. 1).

TCR β-chain analysis. The encephalitogenic T cell clones MM.4 and MM.10 were first shown to be KJ23a$^-$ (Vβ-17a$^-$) by indirect immunoflourescense (Table II). Total cellular RNA was then isolated from the parent line LNC8, and from the clones MM.4 and MM.10, and Vβ segment usage was assessed by Northern blot analysis. As previously reported, the SJL strain of mouse has a significant deletion within its genome that eliminates the availability of the following Vβ gene segments: Vβ-5, Vβ-8, Vβ-9, Vβ-11, Vβ-12, and Vβ-13 (7). Therefore, sequen-

TABLE I

Proliferative responses of T cell clones MM.4 and MM.10[a]

Ag	[^{3}H]Thymidine Uptake[b] (cpm × 10^{-3})			
	Clone	MM.4	Clone	MM.10
Medium	0.4 ± 0.02	0.4 ± 0.04	0.5 ± 0.09	1.4 ± 0.3
PMBP[c]	153 ± 19	62 ± 4	278 ± 30	300 ± 38
MMBP[d]	—	49 ± 1.7	—	265 ± 38
GPMBP[e]	215 ± 29		273 ± 29	
Fx 1[f]	0.8 ± 0.09		0.4 ± 0.02	
Fx 2[g]	2.1 ± 0.3		1.3 ± 0.19	
Fx 4[h]	315 ± 7		248 ± 12	
MBP p92-119[i]		95 ± 2.9		255 ± 40
MBP p92-103[j]		596 ± 83		645 ± 35
MBP p96-109[k]		0.75 ± 0.06		1.2 ± 0.02

[a] The 5 × 10^4 T cell clones were stimulated with 5 × 10^5 irradiated (2500 rad) SJL splenocytes with the Ag listed. ^{3}H-thymidine (2 μCi/well) was added after 48 h and the cultures harvested and counted using β-scintillography 18 h later.
[b] Mean ± SEM of cpm ^{3}H-thymidine in duplicate or triplicate wells.
[c] Porcine myelin basic protein (15 μg/ml).
[d] Murine myelin basic protein (15 μg/ml).
[e] Guinea pig myelin basic protein (15 μg/ml).
[f] Fragment 1 of GPMBP (amino acid 47–86) (5 μg/ml).
[g] Fragment 2 of GPMBP (amino acid 1–46) (5 μg/ml).
[h] Fragment 4 of GPMBP (amino acid 89–169) (5 μg/ml).
[i] MBP p92–119 10 mM.
[j] MBP p92–103 10 mM.
[k] MBP p96–109 10 mM.

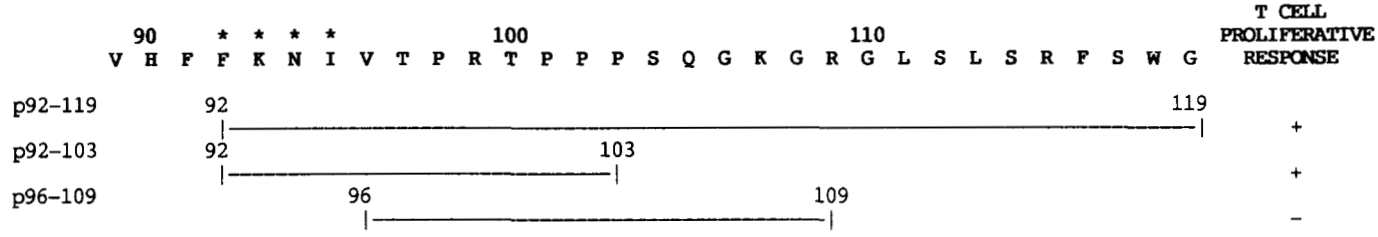

Figure 1. Synthetic peptides of MBP carboxyl-terminus. Numbering of amino acids according to Martenson (24). Results of proliferative response by MM.4 and MM.10 to peptides as shown. *Asterisks* indicate probable peptide epitope required for recognition by T cell clones, MM.4 and MM.10.

TABLE II

Percent binding with anti-Vβ antibodies[a]

	Percent Anti-Vβ-17a	Percent Anti-Vβ-4
MM.4	0	98
MM.10	0	98
LNC12	1	57

[a] T cell lines and clones were stained with anti-Vβ antibodies followed by an appropriate fluoresceinated second antibody as described in *Materials and Methods*. Results represent percent cells bound as determined by cytofluorography. Background percentages using the second antibody alone (<2.5%), have been subtracted from the final results.

tial hybridizations were performed with a series of DNA probes representing the known Vβ gene subfamilies relevant to the SJL mouse. These probes included Vβ-1, Vβ-2, Vβ-3, Vβ-4, Vβ-6, Vβ-7, Vβ-10, Vβ-14, Vβ-15, Vβ-16, and Vβ-17a. This investigation revealed that although the parent line LNC8 contained T cells that used Vβ-4, Vβ-10, Vβ-16, and Vβ-17a, both clones MM.4 and MM.10 used only Vβ-4. The hybridizing band for both clones had an appropriate electrophoretic mobility of 1.3 kb (data not shown). To ascertain the joining region sequence encoded in these two clones, cDNA was synthesized from the total RNA and the β-chain cDNA was selectively amplified using the polymerase chain reaction. After subcloning, the DNA was sequenced. As can be seen in Figure 2, MM.4 used Vβ-4, Dβ-2.1, and Jβ-2.3. The sequence of the β-chain cDNA from MM.10 was found to be identical to MM.4 throughout the J region.

Anti-Vβ-4 antibody binding. After ascertaining that the encephalitogenic clones MM.4 and MM.10 transcribed Vβ-4, we confirmed the surface expression of Vβ-4 with the anti-Vβ-4 mAb KT-10. As can be seen in Table II, both MM.4 and MM.10 express the Vβ-4 protein. To further explore the use of Vβ-4 in SJL EAE, we next assessed the Vβ-4 expression of another encephalitogenic T cell line, LNC12. As seen in Table II, 57% of the LNC12 cells express Vβ-4 whereas only 1% of the LNC12 cells express Vβ-17a.

Encephalitogenicity of T cell clones and lines. The clones MM.4 and MM.10 were both able to adoptively transfer EAE to naive, nonirradiated, SJL mice with approximately the same efficiency as the parent line, LNC8 (Table III). As few as 10^7 MM.4 injected intracardiac transferred severe EAE with the onset of signs within 5 days, whereas 1.7 × 10^7 MM.10 injected i.p. did the same. The heterogenous T cell line, LNC12, was also encephalitogenic. LNC12 mediated grade 5 EAE after intracardiac transfer of 1.2 to 1.6 × 10^7 cells with the onset of signs at day 6. However, when LNC12 was transferred intracardiac along with the simultaneous i.p. injection of antibody to Vβ-4, the mediation of EAE was significantly inhibited (Table III). This suggests that LNC12 mediates EAE largely, if not entirely, through T cells bearing Vβ-4.

DISCUSSION

Identification of a predominant Vβ gene segment usage by T cells mediating EAE has made possible novel therapeutic approaches. In the murine PL strain, in which 80% of encephalitogenic clones express Vβ-8$^+$ TCR, anti-Vβ-8 antibody can prevent or alleviate disease. The approach of using a mAb that is directed only to a disease-

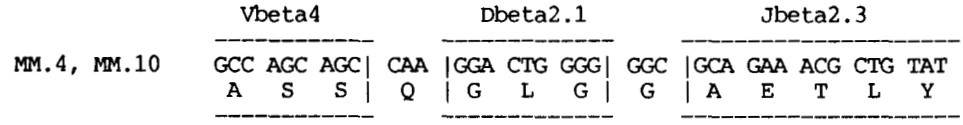

Figure 2. DNA sequence analysis of the J region of the cDNA encoding the β-chain of MM.4 and MM.10. β-chain specific cDNA from MM.4 and MM.10 was amplified by the polymerase chain reaction, subcloned, and sequenced by the dideoxy-chain termination method. Only the J region and immediately surrounding sequence is shown. Above the DNA sequence, the designations of the gene segments used are listed, and below the sequence, the single letter representations of the deduced inframe amino acid sequence are listed.

TABLE III

Encephalitogenicity of T cell lines and clones[a]

Line/Clone	No. of Cells Transferred	Onset of Signs	Maximum EAE-Grade
MM.4	1.4×10^7 i.p.	Day 5	4
MM.4	1.0×10^7 i.c.[b]	Day 5	3
MM.10	1.7×10^7 i.p.	Day 5	2.5
MM.10	1.7×10^7 i.p.	Day 7	2.5
Expt. 1			
LNC12 + control Ab[c]	1.2×10^7 i.c.	Day 6	5
LNC12 + Control Ab	1.2×10^7 i.c.	Day 6	5
LNC12 + anti-Vβ-4	1.2×10^7 i.c.	Day 7	1
LNC12 + anti-Vβ-4	1.2×10^7 i.c.		0
Expt. 2			
LNC12 + control Ab	1.6×10^7 i.c.	Day 6	5
LNC12 + control Ab	1.6×10^7 i.c.	Day 6	4
LNC12 + control Ab	1.6×10^7 i.c.	Day 6	3
LNC12 + anti-Vβ-4	1.6×10^7 i.c.		0
LNC12 + anti-Vβ-4	1.6×10^6 i.c.		0

[a] T cell lines or clones were stimulated with irradiated splenocytes and MBP 4 days previously, and stimulated with IL-2, 1 day before injection. Each horizontal row above represents one injected mouse.

[b] Intracardiac.

[c] The anti-Vβ-4 antibody (KT10), and the control antibody (RT6.1) were injected i.p. within 1 h after the i.c. injection of LNC12 cells. In experiment 1, the control Ab was 400 μg/mouse of concentrated serum-free supernatant of antibody RT6.1; in experiment 2, the control antibody was 200 μg/mouse of protein-G-purified RT6.1. In both experiments, 200 μg/mouse was used of protein-G-purified anti-Vβ-4 antibody (KT10).

related subpopulation of T cells (whereas apparently leaving the rest of the immune system intact), has obvious appeal in the treatment of a number of human diseases. Several investigators have attempted to identify a limited heterogeneity of the TCR used in multiple sclerosis and rheumatoid arthritis in hopes of applying a similar therapeutic strategy (22, 23). It is likely, however, that in these human autoimmune diseases there will be more than one predominant Vβ gene segment involved in the T cell-mediated pathogenesis. Therefore, one would need to use a mix of specific anti-Vβ antibodies to significantly affect the natural history of these diseases.

In the SJL model of EAE, whereas Vβ-17a$^+$ T cells have clearly been shown to play a part in mediating the disease, antibody directed to this particular segment does not successfully protect SJL mice from EAE after sensitization to MBP (2). This was shown by inducing EAE after Vβ-17a$^+$ cells were depleted in vivo. Such Vβ-17a-depleted mice developed disease that was just as severe as control mice. Furthermore, in experiments in which Vβ-17a$^+$ and Vβ-17a$^-$ lines were derived, both populations were able to adoptively transfer disease. Administration of the anti-Vβ-17a antibody in vivo suppressed the disease induced after transfer of the Vβ-17a$^+$ cells but not after transfer of the Vβ-17a$^-$ cells. Thus, in its use of multiple Vβ segments in the mediation of this autoimmune process, the SJL mouse may be more similar to the human than is the PL mouse.

In this communication we have identified for the first time a second Vβ gene segment usage in SJL EAE. We also report for the first time the derived primary amino acid structure of the TCR β-chain from encephalitogenic, H-2^s/MBP-carboxyl-terminus-specific T cell clones derived from an SJL mouse. It may be of special significance that this second Vβ identified is Vβ-4. Acha-Orbea et al. (4) demonstrated that of two MBP-specific T cell clones generated from (PL × SJL)F$_1$ mice, one used Vβ-8 whereas the other clone used Vβ-4 (4). These clones, however, were both specific for H-2^u and the MBP amino terminus, peptide 1-9. Our results may, therefore, represent a second example of a Vβ-correlation with EAE that crosses MHC/antigenic-restriction barriers, as in the case of mouse (H-2^u) and rat EAE. Heber-Katz and co-workers (8) have reported that, as in murine (H-2^u) EAE, there is a predominant Vβ-8 (homologous to murine Vβ-8.2) usage in rat EAE. This was true despite the fact that the encephalitogenic rat T cells are specific for a different MHC and MBP-epitope than the encephalitogenic murine T cells (8). These investigators have postulated a "V region-disease" hypothesis in which the TCR V regions used are correlated with EAE through mechanisms other than MHC/Ag-specificity, e.g., acting as homing receptors (9). Our present findings of Vβ-4 usage in H-2^s/carboxyl-terminus MBP-restricted, SJL-derived, encephalitogenic T cells, taken together with the reported Vβ-4 usage in H-2^u/amino-terminus MBP-restricted, encephalitogenic T cells derived from (PL × SJL)F$_1$ mice, may further support this hypothesis. As with Vβ-8, Vβ-4 may also have a significant function in the mediation of EAE over and above its role in MHC Ag-specific activation.

Although it clearly has been demonstrated that the majority of encephalitogenic determinants for SJL mice reside in the carboxyl-terminal fragment of MBP (amino acid 89-169), fine specificity mapping with synthetic peptides has led to additional interesting results. In contrast to PL (H-2^u) EAE where there is one major encephalitogenic epitope, it appears that in SJL there may be at least three major encephalitogenic epitopes. Sakai et al. (2), have reported the association of encephalitogenicity, Vβ-17a usage, and reactivity to MBP amino acid residues p89-101, whereas encephalitogenicity with reactivity to the nested epitope of p89-100 correlated with the lack of usage of Vβ-17a (2). Kono et al. (12), have described encephalitogenic SJL-derived T cell clones (with uncharacterized Vβ usage) that are reactive to at least two epitopes within p92-109. Further analysis suggested one epitope localized within p92-103 and another localized within p96-109. In the present report, MM.4 and MM.10 have been shown to react to p92-119 and 92-

103 as well as the carboxyl-terminal peptide fragment p89-169 but not p96-109. These findings suggest that these T cell clones respond to an epitope that requires amino acids 92 through 95. At the present time, we have not determined whether these clones require amino acids 101 (in addition to 92 through 95) as described for Vβ-17a$^-$ clones (2).

This report documents for the first time that Vβ-4 can be used by encephalitogenic T cells in the SJL model of EAE. Future studies will continue the characterization of the receptors used by T cells involved in the mediation of EAE in SJL mice, and the role of these receptors in the pathogenesis of the disease.

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