Analysis of T Cell Receptor β Chains in Lewis Rats with Experimental Allergic Encephalomyelitis: Conserved Complementarity Determining Region 3

By Daniel P. Gold,* Halina Offner,‡§ Deming Sun,‖ Sandra Wiley,* Arthur A. Vandenbark,‡§¶ and Darcy B. Wilson*

From the *La Jolla Institute for Experimental Medicine, La Jolla, California 92037; ‡Neuroimmunology Research, Veterans Affairs Medical Center, Portland, Oregon 97201; the §Department of Neurology, Oregon Health Sciences University, Portland, Oregon 97201; the ‖Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101; and the ¶Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon 97201

Summary

This study explores the usage of T cell antigen receptor (TCR) β chain elements in Lewis rats with experimentally induced allergic encephalomyelitis (EAE). TCRs from 15 different T cell clones and hybridomas derived from animals immunized with myelin basic protein (MBP), and all having specificity for the 21-mer encephalitogenic fragment MBP 68–88, utilized Vβ8.2. In addition, there was a marked conservation of the first two amino acid residues of the junctional complementarity determining region 3 (CDR3) associated with the Vβ8.2 receptors. 12 of 15 contained an aspartic acid followed by serine regardless of the associated Jβ element. At the nucleotide level, this conservation of AspSer residues was accomplished with few or no nongermline-encoded nucleotide (N) additions. A similar pattern of AspSer usage and N region nucleotide additions was observed in a number of Vβ8.2 isolates derived from MBP-immunized lymph nodes. In contrast, Vβ8.2 polymerase chain reaction amplified isolates from Lewis T cells activated with concanavalin A or from lymph nodes of complete Freund's adjuvant–immunized animals showed no AspSer utilization (0/31) in the CDR3, and four to nine N region nucleotide additions. We conclude from this finding that AspSer residues in the CDR3, limited N region nucleotide additions, along with Vβ8.2 sequences, contribute to TCR specificity for MBP 68–88. This raises the possibility that encephalitogenic, disease-causing T cells either represent a population that derives from late fetal life or alternatively, that they are rare cells with this particular TCR phenotype contributed to the T cell pool throughout adulthood and are selected by antigen. In either case, the CDR3 AspSer sequences as well as Vβ8.2 sequences are candidates for the receptor target structures recognized by regulator T cells in recovery from and resistance to active EAE. In this respect, a preliminary analysis of TCR utilization in three T cell clones specific for MBP 68–88 isolated from animals recovered from active EAE indicates that while all three use Vβ8.2, only one contains AspSer in the CDR3.

N early all T cell immune responses involve the recognition by α/β heterodimeric TCR of antigen that has been processed and represented on the surface of APC as peptides bound to MHC molecules (1). TCR α and β chains are each derived from multiple germline-encoded elements which undergo somatic recombination during T cell development. In the mouse, the repertoire of α chain germline genes is thought to number approximately 100 different variable (Vα) segments and also 100 different joining (Jα) elements. TCR β chains are derived from a repertoire of gene segments which has been estimated to consist of 25 different Vβ elements, 12 Jβ genes, and two diversity (Dβ) elements.

Random joining of these various intra α and β chain elements plus combinatory associations of different α and β chains permit a considerable degree of TCR diversity. In addition, during the somatic process of V/J recombination (V/D/J for β chains), further diversity is created in the junctional regions through the addition of nongermline encoded nucleotides (N region). Given the number of different germline elements, the number of possible junctional region sequences, and the combinatory associations of the two chains, it has been estimated that the potential TCR repertoire is of the order 10^{15}–10^{22} specificities (2, 3).

Theoretical modeling of the interaction between TCR mol-

ecules and peptide/MHC complexes has suggested that certain regions encoded within Vα and Vβ, corresponding to the CDR1 and CDR2 of Ig, interact with α helices of the MHC molecule, and recognition of the MHC-associated peptide occurs primarily via the (N)Jα and (N)Dβ(N)Jβ elements comprising the CDR3 of the two chains (2, 4).

Despite the very large number of possible TCR specificities, a number of studies have shown that the major portion of the T cell response to a number of different protein antigens is directed to a few immunodominant epitopes within the protein molecule. In addition, TCR utilization in these responses, even to a single immunodominant epitope, is often quite limited (5–10). An example of such a pauciclonal response, dominated by a single or a few TCR α/β chain combinations, was first demonstrated to be the case for pigeon cytochrome c in H-2^a mice (5).

Another example of limited TCR utilization occurs in the immune response of rats immunized with guinea pig myelin basic protein (MBP)[1] to induce experimental allergic encephalomyelitis (EAE), a paralytic, T cell–mediated demyelinating autoimmune disease that is widely studied as an animal model for multiple sclerosis in humans (11). Here, the T cell response of Lewis rats is directed primarily to an immunodominant epitope contained within the encephalitogenic amino acid 68–88 fragment of the MBP molecule (MBP 68–88). This T cell response is also highly restricted, dominated by T cells that use Vβ8 almost exclusively and Vα2 frequently (12).

This finding of conserved Vα and Vβ utilization in EAE raises the possibility that CDR3 junctional regions might also be utilized in a limited way since these are believed to be involved in recognition of the peptide in the MHC groove. Here we report TCR β chain sequences of numerous T cell clones and hybridomas reactive to MBP 68–88. All utilized the Vβ8.2 segment. But in addition, these sequence comparisons reveal a marked dominance (12/15) of AspSer in the first two amino acid positions associated with multiple Jβ elements, and few or no N region nucleotide additions in the CDR3 region. Receptor utilization in lymph nodes taken directly from MBP-immunized rats showed a similar pattern not seen in adjuvant-immunized control animals. CDR3 sequences of Vβ8.2$^+$ control populations activated with Con A or derived from CFA-immune donors showed no such utilization of AspSer sequences and many more N region nucleotide additions.

Materials and Methods

Animals. 8–12-wk-old female Lewis (LEW) rats were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN).

Isolation of T Cell Clones. The origins of all T cell clones used in this study have been previously described (13, 14). Briefly, cells were obtained from the draining lymph nodes of animals undergoing active EAE as a consequence of immunization 2 wk earlier

with 50 μg of guinea pig MBP emulsified in CFA containing 400 μg *Mycobacterium tuberculosis* strain H37Ra (Difco Laboratories, Inc., Detroit, MI). Lymphocyte suspensions were cultured briefly with antigen and T cell clones were derived in soft agar (13). Lymphocytes were also obtained from the primary draining lymph nodes of animals immediately after recovery from active disease and T cell clones were established by limiting dilution (14). All clones derived from animals undergoing active disease, and two of the three clones from recovered animals assessed in this study have been shown previously to be encephalitogenic in adoptive transfer experiments (13, 14).

Isolation of T Cell Hybridomas. T cell hybridomas were produced by fusion of lymphoblasts from MBP-specific T cell lines to the murine TCR α/β^- BW1100.129.237 cell line (15). Wells positive for cell growth in HAT medium were tested for IL-2 production (10^5/well) after stimulation with MBP (20 μg/ml) in the presence of irradiated LEW spleen cells (5 × 10^5/well) and then subcloned at limiting dilution. All hybrids used in this study secreted IL-2 when stimulated with whole MBP or with the synthetic peptide MBP68–88.

Isolation of CD8$^-$ Con A Blasts. Vβ8.2$^+$ MBP 68–88–reactive T cells are almost exclusively CD4$^+$; thus, for control purposes it was necessary to prepare a population of Con A–activated T cells enriched in CD4 from which a Vβ8.2 cDNA library could be generated and sequenced. A panning method (16) was used to deplete lymph node cells of the CD8$^+$ subpopulation. Cells (2–3 × 10^7) were incubated on ice for 1 h in saturating amounts of supernatants of post-log phase growth cultures of the OX8 hybridoma (anti-rat CD8) (17). The cells were washed three times in HBSS (Fisher Scientific Co. Allied Corp., Pittsburgh, PA), resuspended in 3 ml HBSS containing 2% FCS (J.R. Scientific, Woodland, CA), and then placed on petri dishes which had been coated overnight at 4°C with 10 ml goat anti–mouse Ig (10 μg/ml; Fisher Scientific Co. Allied Corp.). Dishes were returned to 4°C for 1 h and nonadherent cells were removed by gentle pipetting. These nonadherent cells were panned a second time on anti-Ig coated dishes. Nonadherent cells recovered with this protocol contained <5% contaminating cells of the CD8$^+$ subset. Recovered cells were cultured at 2 × 10^5/ml in RPMI 1640 containing 10% FCS, 5 × 10^{-5} M 2-ME, 2 mM glutamine, 100 μg/ml penicillin/streptomycin, and 5 μg/ml Con A. T cell blasts were harvested after 48 h.

Determination of TCR Vβ Utilization. TCR Vβ utilization was determined by PCR amplification of cDNA as described previously (18). RNA was isolated from various cell populations after lysis in guanidinium isothiocyanate and phenol extraction (19) or centrifugation through a cesium chloride cushion (20). cDNA was prepared from 5–10 μg total RNA in Taq polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH8.3, 2.5 mM MgCl$_2$, 0.01% gelatin) in a 50 μl reaction volume containing 40 mM 2-ME, 0.5 mM dNTPs (Pharmacia Fine Chemicals, Piscataway, NJ), 20 U RNA guard (Pharmacia Fine Chemicals), 15 U AMV reverse transcriptase (Pharmacia Fine Chemicals), and 1 μM Cβ-specific oligonucleotide primer. This primer, 5'CATAGAAtTcCACTTGGCAGCGGAAGT-GGT3' (Genosys, The Woodlands, TX), anneals to both rat TCR Cβ1 and Cβ2 mRNA. Bases in small letters denote changes in the TCR Cβ sequence made to create an EcoRI restriction endonuclease site. The cDNA was then amplified in 1.5 mM MgCl$_2$ Taq buffer in the presence of 1 μM Cβ oligonucleotide, 1 μM of a 5' consensus Vβ8 oligonucleotide primer (Vβ8-5'), 5'GGGCCGC-GGAACACATGGAAGCTGCAGTCAC3' (containing a 5'-SacII restriction endonuclease site), 200 μM each dNTP (Pharmacia Fine Chemicals), and 2 U Taq DNA polymerase (Pharmacia Fine Chemicals). Samples were overlaid with mineral oil, heated for 5 min

---

at 94°C to denature DNA/RNA duplexes, and then subjected to 30 amplification cycles of 1 min at 94°C, 1.5 min at 55°C, and 2 min at 72°C in a thermal cycler (Perkin-Elmer Corp., Norwalk, CT). After amplification, the samples were extracted with chloroform to remove mineral oil, precipitated with ethanol, and digested with SacII plus EcoRI (New England Biolabs, Beverly, MA). The resulting DNA was separated on a 1.4% agarose gel. The appropriate size product was isolated using Prep-A-Gene (Bio-Rad Laboratories, Richmond, CA) and ligated into the ScaII/EcoRI site of pBluescript II (Stratagene Inc., La Jolla, CA). The ligation mixture was transformed into the bacterial strain DH5. Multiple ampicillin-resistant colonies were selected and miniprep DNA was prepared by standard methods (21). The plasmid DNA was then sequenced directly by the dideoxy chain termination method (22) using the Sequenase sequencing method (U.S. Biochemical, Cleveland, OH).

Results

Vβ Utilization in EAE T Cells. cDNA samples from 15 different MBP 68–88–reactive T cell clones and hybridomas derived from MBP/CFA immunized LEW rats were amplified using a Vβ8 consensus primer (Vβ8-5'). Sequence analyses of the PCR products indicated that all of the MBP-reactive T cells used the Vβ8.2 gene segment.

CDR3 Sequences in EAE T Cells. Table 1 summarizes the details of our comparison of CDR3 sequences of LEW Vβ8.2⁺ TCR T cell clones and hybridomas (Table 2), along with several independent Vβ8.2⁺ PCR isolates from MBP/CFA-immune lymph nodes (Table 3), with the CDR3 sequences from LEW Vβ8.2⁺ TCR from CD8⁻ Con A blasts (Table 4) and CFA-immunized lymph nodes (Table 5). CD8⁻ blasts were used as control material because Vβ8.2⁺ T cells reactive to MBP 68–88 are exclusively CD4⁺. The clones and hybridomas were derived from rats immunized with intact MBP, and all were shown to be reactive in culture to the encephalitogenic peptide MBP 68–88. Lymph node isolates were derived from MBP-immunized animals, but their reactivity with respect to MBP 68–88 is not known.

There are two main findings with respect to this comparison. First, of isolates from 15 different MBP peptide-reactive T cell clones and hybridomas, most (12) use AspSer and some (2) use ThrSer in the (N)D(N) region of CDR3. Also, of 25 different MBP-immune lymph node isolates, nine use AspSer. These numbers are markedly different for Vβ8.2⁺ TCR derived from Con A blasts or CFA-immune lymph nodes. As reported by Burns et al. (12) and Chluba et al. (23), we define the COOH terminus of the Vβ8.2 segment as ending at amino acids 91–94 (CysAlaSerSer).

The second finding of interest in this comparison of Vβ8.2-associated CDR3 sequences concerns the number of N region nucleotide additions in CDR3. Fig. 1 shows a marked difference between TCR reactive to MBP 68–88 and TCR from control cells. A majority of the former (8/15) use only one nucleotide addition, and in the latter, most (25/31) of the Vβ8.2⁺ TCR use four to nine nucleotide additions.

Utilization of different Jβ elements proved to be only somewhat limited among the MBP 68–88–reactive T cell clones and hybridomas (Table 1). There appears to be a disproportionate usage of Jβ2.7. The remaining use Jβ1.1, 1.3, 1.4, and 2.5. In comparison, Jβ utilization among control populations of Vβ8.2⁺ Con A blasts and CFA immune lymph nodes is more extensive, although here too, Jβ2.7 appears to be used disproportionately with Vβ8.2.

Post EAE T Cells. Finally, we sequenced the TCR from three different MBP 68–88–reactive T cell clones derived from LEW rats that had recovered from paralytic EAE. These three clones also utilized Vβ8.2⁺, but only one of them uses AspSer in CDR3 (Table 6). The two AspSer⁻ clones, BB12 and A2, were found to be encephalitogenic in adoptive transfer protocols (14).

Table 1. *Summary of CDR3 Sequences of LEW Vβ8.2 TCR*

PCR-amplified isolates	CDR3 sequences starting:			Jβ utilization											
	AspSer	ThrSer	Other	1.1	1.2	1.3	1.4	1.5	1.6	2.1	2.2	2.3	2.4	2.5	2.7
MBP 68–88 rx															
Clones and	12/15			1/12		3/12	2/12							1/12	5/12
hybridomas		2/15				1/2	1/2								
			1/15				1/1								
MBP-immune	9/25												3/9		6/9
lymph node		16/25		1/16			2/16				1/16		3/16	3/16	6/16
Controls															
Con A blasts		15/15				1/15		1/15		2/15	2/15	1/15	1/15	1/15	6/15
CFA-immune LN		16/16		1/16			2/16	2/16		2/16	2/16		1/16	1/16	5/16

Table 2. *Junctional Elements Associated with Vβ8.2 in EAE*

Cells	V	(N)D*(N)	J	
		9 1		
Clone				
6‡	Vβ8.2—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r S e r** g a t t c a t c t	A s n T h r G l u V a l a a t a c a g a a g t t	Jβ1.1
4	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p** g a t	S e r G l y A s n V a l t c t g g a a a t g t g	Jβ1.3
12	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p** g a t	S e r G l y A s n V a l t c t g g a a a t g t g	
5	—C y s A l a S e r S e r —t g t g c c a g c a g t	T h r **S e r** a c t t c c	A s n G l u A r g L e u a a t g a a a g a t t g	Jβ1.4
15	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r S e r** g a c a g t t c g	G l u A r g L e u g a a a g a t t g	
16	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r S e r** g a c a g t t c g	G l u A r g L e u g a a a g a t t g	
3	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r** g a c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	Jβ2.7
6‡	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r** g a c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	
14	—C y s A l a A l a S e r —t g t g c c a g c a g t	**A s p S e r** g a c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	
13	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r G l y I l e** g a t t c a g g g a t t	G l u G l n g a g c a g	
Hybridomas				
S1B1B10	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p** g a t	S e r G l y A s n V a l t c t g g a a a t g t g	Jβ1.3
S23F4F4	—C y s A l a S e r S e r —t g t g c c a g c a g t	**T h r** a c g	S e r G l y A s n V a l t c t g g a a a t g t g	
S1C2A6	—C y s A l a S e r S e r —t g t g c c a g c a g t	S e r G l y A l a t c g g g g g c a	A s n G l u A r g L e u a a t g a a a g a t t g	Jβ1.4
S22C2	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r L e u** g a c a g t c t t	G l n G l u T h r G l n c a a g a g a c c c a g	Jβ2.5
S23B1E11	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r** g a c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	Jβ2.7

* Dβ1 = gggacaggggg; Dβ2 = gggactgggggggggc (38).
‡ Clone 6 displays two different Jβ sequences and thus must be considered to be a mixed clone.

Table 3. *Junctional Region Sequences of Vβ8.2⁺, AspSer⁺ TCR from MBP-immune Lymph Nodes*

Isolate*	V	(N)D(N)	J	
	9 1			
2‡	Vβ8.2−C y s A l a S e r S e r	AspSerGly	A s n T h r L e u P h e	Jβ2.4
	− t g t g c c a g c a g t	g a t a g t g g g	a a c a c c t t g t t c	
8	−C y s A l a S e r S e r	AspSerGly	A s n T h r L e u P h e	
	− t g t g c c a g c a g t	g a t t c c g g a	a a c a c c t t g t t c	
9‡	−C y s A l a S e r S e r	AspSerGly	A s n T h r L e u P h e	
	− t g t g c c a g c a g t	g a t a g t g g g	a a c a c c t t g t t c	
1	−C y s A l a S e r S e r	AspSer	S e r T y r G l u G l n	Jβ2.7
	− t g t g c c a g c a g t	g a c a g c	t c c t a t g a g c a g	
3	−C y s A l a S e r S e r	AspSer	S e r T y r G l u G l n	
	− t g t g c c a g c a g t	g a c a g t	t c c t a t g a g c a g	
4	−C y s A l a S e r S e r	AspSer	S e r T y r G l u G l n	
	− t g t g c c a g c a g t	g a c a g c	t c c t a t g a g c a g	
5	−C y s A l a S e r S e r	AspSer	S e r T y r G l u G l n	
	− t g t g c c a g c a g t	g a c a g c	t c c t a t g a g c a g	
6	−C y s A l a S e r S e r	AspSer	S e r T y r G l u G l n	
	− t g t g c c a g c a g t	g a c a g c	t c c t a t g a g c a g	
7	−C y s A l a S e r S e r	AspSerLeu	S e r T y r G l u G l n	
	− t g t g c c a g c a g t	g a c a g t c t c	t c c t a t g l u g l n	

* 25 Vβ8.2 isolates from lymph nodes of two MBP-immune rats were assessed; these nine contained AspSer.
‡ These two isolates were derived from different animals.

Discussion

Several studies have focused attention on one interesting feature of EAE in experimental animal models: the limited usage of TCR Vα and Vβ segments in immune responses to encephalitogenic fragments of myelin basic protein (7, 8, 12, 23–25). The T cell response of B10.PL/J mice to MBP 1–9 is dominated by Vβ2Vβ8.2 and Vα4Vβ8.2 (7) but PL/J mice use Vα4Vβ8.2 (8). SJL mice use Vβ4 and Vβ17 predominantly in response to MBP 81–98 (24, 25). Despite the fact that it is a different species, uses different MHC molecules for presenting antigen, and responds to a different encephalitogenic fragment (MBP 68–88), the T cell response of LEW rats in EAE, like that of B10.PL and PL/J mice, is also heavily dominated by Vα2 and Vβ8.2 (12, 23, this report). The implications of this finding are not clear.

We report here that the CDR3 of Vβ8.2⁺ MBP-immune T cells is also highly conserved in LEW rats with respect to some elements and shows somewhat limited usage with respect to others. Of 15 different PCR isolates from Vβ8.2⁺ T cell clones and hybridomas, all with demonstrable reactivity to MBP 68–88, 12 use AspSer in the first two amino acid positions of CDR3 and two others use ThrSer (Table

2). Many (5/15) of these use Jβ2.7 (Table 2) and a majority (8/15) use only a single N nucleotide addition (Fig. 1). This contrasts with Vβ8.2⁺ control T cells from Con A blasts (Table 4) and CFA-immune lymph nodes (Table 5), where none of 31 CDR3 sequences use AspSer or ThrSer. Jβ utilization is more extensive in this control material, although here too, Jβ2.7 seems to have a disproportionate association (11/31) with Vβ8.2. N region nucleotide addition among control Vβ8.2⁺ T cells is also more extensive, only 1 of 31 with a single nucleotide addition and the majority showing four to nine N region additions (Fig. 1). Dominant presence of AspSer in the CDR3 region is also seen in material from MBP-immune lymph nodes. Here, 9/25 Vβ8.2⁺ isolates use AspSer, but none of 16 Vβ8.2⁺ isolates from CFA-immune nodes use AspSer (Table 5, Fig. 1). It should be noted that three previously reported CDR3 sequences associated with Vβ8.2 in EAE were AspSer⁺ (12, 23).

It may be important to point out in these considerations that even the limited number of N region additions we have indicated for MBP 68–88–reactive T cells (Fig. 1) for two reasons may be an overestimate. First, in the absence of known

Table 4. *CDR3 Sequences of Vβ8.2⁺ TCR from CD8⁻ LEW Con A Lymphoblasts*

Isolate number	V	(N) D(N)	J	
		9 1		
1	Vβ8.2 – C y s A l a S e r	A r g A r g G l y T h r G l u G l n A l a	T y r A s p T y r T h r	Jβ1.2
	– t g t g c c a g c a g	g a g a g g g a c a g g g g a g g c c	t a t g a c t a c a c c	
2	– C y s A l a S e r S e r	P h e G l n G l u T r p	G l n A l a	Jβ1.5
	– t g t g c c a g c a g t	t t t c a g g a g t g g	c a g g c c	
3	– C y s A a S e r S e r	G l u G l y A s n	A l a G l u	Jβ2.1
	– t g t g c c a g c a g t	g a g g g g a a t	g c t g a a	
4	– C y s A l a S e r S e r	G l y T h r G l y	A l a G l u	
	– t g t g c c a g c a g t	g g c a c g g g g	g c t g a a	
5	– C y s A l a S e r	A r g A s p A r g A r g	A s n T h r G l y G l u	Jβ2.2
	– t g t g c c a g c a g	a g a c a g g a g a	a a c a c c g g g c a g	
6	– C y s A l a S e r S e r	A l a G l n G l y A l a	A s n T h r G l y G l u	
	– t g t g c c a g c a g t	g c c c a g g g g g c a	a a c a c c g g g c a g	
7	– C y s A l a S e r S e r	G l u A s n	T h r A s p L y s	Jβ2.3
	– t g t g c c a g c a g t	g a g a a t	a c a g a c a a g	
8	– C y s A l a S e r S e r	A s p V a l M e t G l y A s p	T h r L e u	Jβ2.4
	– t g t g c c a g c a g t	g a t g t g a t g g g t g a c	a c c t t g	
9	– C y s A l a S e r	T r p G l y A r g	A s n G l n G l u T h r	Jβ2.5
	– t g t g c c a g c	t g g g g a c g g	a a c c a a g a g a c c	
10	– C y s A l a S e r S e r	S e r M e t A s p L e u M e t	G l u G l n	Jβ2.7
	– t g t g c c a g c a g t	a g t a t g g a t c t t a t g	g a g c a g	
11	– C y s A l a S e r S e r	A s p G l y G l y G l y	G l u G l n	
	– t g t g c c a g c a g t	g a c g g t g g g g g a	g a g c a g	
12	– C y s A l a S e r	T h r P r o T h r G l y A l a	G l u G l n	
	– t g t g c c a g c a g	a c c g a c t g g g g c t	g a g c a g	
13	– C y s A l a S e r S e r	A l a G l y P r o	T y r G l u G l n	
	– t g t g c c a g c a g t	g c g g g t c c c	t a t g a g c a g	
14	– C y s A l a S e r S e r	G l u A l a A s p T r p G l y G l y	T y r G l u G l n	
	– t g t g c c a g c a g t	g a g g c t g a c t g g g g g g g c	t a t g a g c a g	
15	– C y s A l a S e r	A r g P r o G l y T h r P r o	T y r G l u G l n	
	– t g t g c c a g c a g	a c c c g g g a c t c c c	t a t g a g c a g	

germline sequences for rat Vβ8.2 and the Jβ elements, we cannot definitively determine the contribution of P nucleotides (26) in our analysis. Second, while we have provisionally assigned a 91–94 (CysAlaSerSer) as the COOH terminus of Vβ8.2 based on two previous studies of Vβ utilization in the rat EAE model (12, 23), and on the sequence data of Vβ8.2 usage in various cell populations in this paper, it must be recalled that the three murine Vβ8 sequences terminate with CysAlaSerSerAsp (Vβ8.1 and 8.3) or CysAlaSerGlyAsp (Vβ8.2) (27). Thus, the possibility exists that the Asp residue we have assigned to the D region is, in fact, the COOH terminus of rat Vβ8.2. Were this to be the case, the receptor

sequences indicated for the CDR3 of two MBP 68–88–reactive clones (nos. 4 and 12) and one hybridoma (S1B1B10) (Table 2) would be devoid of a D region, the result of a direct V to J recombination event. This would not violate the one-turn to two-turn joining rule (28).
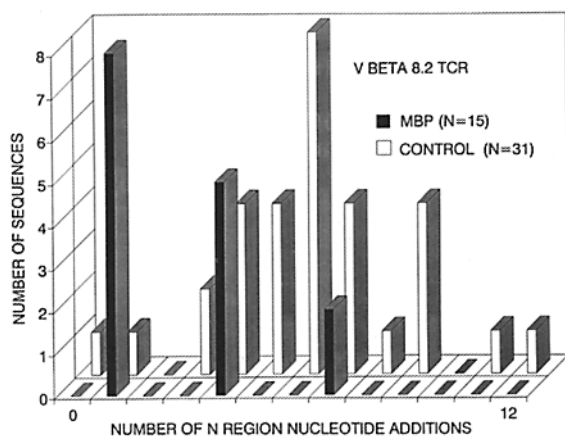
While lack of N region addition in TCRs has been reported previously in the fetal development of γ/δ-bearing T cells, the significance of this is not known. Since N region addition correlates with the existence of terminal deoxytransferase (TdT) activity (30), it is possible that α/β-expressing T cells developing before expression of high levels of TdT in the thymus may show no or only limited N region additions.

Table 5. *Junctional Region Sequences of Vβ8.2⁺ TCR from CFA-immune Lymph Nodes*

Isolate number	V	(N) D(N)	J	
	9 1			
1	Vβ8.2−C y s A l a S e r − t g t g c c a g c	A l a T y r A r g G l y A r g g c c t a c a g g g g g g g g a	A s n T h r G l u V a l a a c a c a g a a g t t	Jβ1.1
2	−C y s A l a S e r S e r − t g t g c c a g c a g t	A l a G l y A r g T h r g c a g g g a g g a c c	A s n G l u A r g L e u a a t g a a a g a t t g	Jβ1.4
3	−C y s A l a S e r − t g t g c c a g c	P r o c c t	G l u A r g L e u g a a a g a t t g	
4	−C y s A l a − t g t g c c	C y s A s p A r g A r g t g c g a c a g g c g g	A s n A s n G l u A l a a a c a a c c a g g c c	Jβ1.5
5	−C y s A l a S e r S e r − t g t g c c a g c a g t	G l y g g g	A s n G l u A l a a a c c a g g c c	
6	−C y s A l a S e r S e r − t g t g c c a g c a g t	G l u T h r G l y g a g a c t g g c	A l a G l u g c t g a a	Jβ2.1
7	−C y s A l a S e r S e r − t g t g c c a g c a g t	G l u G l y V a l H i s g a g g g g g t g c a t	S e r T y r A l a G l u t c c t a t g c t g a a	
8	−C y s A l a S e r − t g t g c c a g c	T h r G l u A s p a c c g a g g a c	T h r G l y G l n a c c g g g c a g	Jβ2.2
9	−C y s A l a S e r S e r − t g t g c c a g c a g t	T y r A r g G l y A r g t a c a g g g g c c g c	T h r G l y G l n a c c g g g c a g	
10	−C y s A l a S e r S e r − t g t g c c a g c a g t	L y s P r o G l y L e u L y s a a a c c g g g a c t a a a a	A s n T h r a a t a c c	Jβ2.4
11	−C y s A l a S e r S e r − t g t g c c a g c a g t	G l y A s p S e r A l a G l y g g g g a c t c c g c g g g a	G l u T h r g a g a c c	Jβ2.5
12	−C y s A l a S e r S e r − t g t g c c a g c a g t	A l a V a l S e r g c g g t c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	Jβ2.7
13	−C y s A l a − t g t t g c	L y s G l y L e u L e u G l u a a g g g a c t t t t g g a g	G l u G l n g a g c a g	
14	−C y s A l a S e r − t g t g c c a g c	T h r A s p T h r V a l a c g g a c a c c g t a	G l u G l n g a c c a g	
15	−C y s A l a S e r S e r − t g t g c c a g c a g t	P r o G l y G l y c c c g g g g g g	T y r G l u G l n t a t g a c c a g	
16	−C y s A l a S e r S e r − t g t g c c a g c a g t	A r g A r g L e u G l y G l y A s p a g g c g a c t g g g g g g g g a c	S e r T y r G l u G l n t t c t a t g a g c a g	

This would mean that MBP 68–88–reactive T cells in adult rats represent a pool of long-lived T cells that originated from a late fetal period. Alternatively, T cell specificity for MBP 68–88 may require AspSer expression in CDR3 encoded primarily from germline sequences and limited N region addition. By this explanation, antigen selection leads to an expanded population of otherwise rare T cells having few N region additions. In this context, we found 1/31 such ex-

Figure 1. Comparison of the numbers of N region additions in the CDR3 of Vβ8.2⁺ β chains of PCR isolates of MBP 68–88–reactive T cell clones and hybridomas, and with the numbers of N region additions in Vβ8.2⁺ TCR from Con A lymphoblasts and CFA-immune LEW lymph nodes.

ther Asn or Asp in the first position of CDR3β. In this case this amino acid is associated with two different Jβ elements and a considerable degree of diversity in the (N)Dβ(N) region (6). Similar findings have been reported for TCR responses to λ repressor protein (9).

A second interesting feature of EAE, particularly in the LEW rat, is that this is usually a monophasic, self-limiting autoimmune disease. Rats become paralyzed 12–16 d after immunization with MBP, and then recover. Recovered rats are refractory to subsequent attempts to induce active EAE, and this resistance appears to be mediated by regulatory T cells with antireceptor specificity for Vβ8.2⁺ disease-causing T cells. Whether recovery from paralytic EAE and subsequent resistance to further disease induction is a reflection of limited TCR utilization in MBP responses is not yet clear, but it is a critical issue in considerations of immunotherapeutic approaches to at least some forms of autoimmune disease.

Considerable support exists for an antireceptor regulatory T cell population in recovery from and induced resistance to EAE in LEW rats. First, Vβ8.2⁺ disease-causing T cells reactive to MBP 68–88 can be used in attenuated form or in subclinical numbers as vaccines to induce resistance (31); T cells from recovered animals can transfer resistance to secondary recipients (32); and T cells from resistant animals have been shown to have lytic specificity for disease-causing T cell populations (33). In this respect the EAE resistance model in rats resembles closely a model of specifically induced resistance to GVH in F₁ rats after immunization with disease-causing T cells of parental strain origin (34). Second, TCR peptides encompassing part of the CDR2 region of Vβ8.2 are effective vaccines for inducing resistance to EAE (35), and they are also therapeutically effective in reducing the severity of ongoing disease (36). This demonstrates that an immune response directed to a particular portion of TCR molecules highly conserved in a particular disease-causing T cell population can inhibit the function of cells bearing these receptors.

If regulatory T cells with antireceptor specificity are a significant factor in recovery and resistance to autoimmune disease in the EAE model, an important question to be considered concerns the identity of the idiotypic target structure. Logic dictates that this target structure be conserved, as it is common to most if not all of the disease-causing T cells. Some portion of the Vβ8.2 sequence, and, from the

ample of D region expression without N region addition in the control population of Con A blasts and CFA-immune lymph nodes (Tables 4 and 5).

Conserved β chain junctional region sequences have also been described previously in murine T cell responses to antigen, but each of these studies indicates an interesting difference with the rat model described here, where Vβ8.2 chains use AspSer in the first positions of CDR3, a minimal number of N region additions, and extensive representation of Jβ elements.

(a) In the B10.PL mouse, EAE model Vβ8.2⁺ T cells reactive to the encephalitogenic sequence MBP 1–9 express distinct amino acid sequences in the β chain junctional region (7, 8), but these conserved Dβ regions are always associated with a particular Jβ element, whereas in the rat, AspSer sequences are common to the CDR3 region of the β chain regardless of the Jβ element used.

(b) The response of I-E^d-restricted T cell clones to the 110–121 fragment of sperm whale myoglobin is highly homogeneous, being dominated by Vβ8.2⁺ T cells (10), but here the whole β chain CDR3 junctional region, consisting of Dβ2.1 and Jβ2.6, is also conserved.

(c) In B10.A mice, specificity for cytochrome c requires ei-

Table 6. *Junctional Region Sequences of Vβ8.2⁺ TCR from LEW Rats after Recovery from EAE*

Clone	V	(N) D(N)	J	
	9 1			
BB12	Vβ8.2—C y s A l a S e r S e r —t g t g c c a g c a g t	G l y T h r G l y g g g a c a g g g	T h r A s p L y s a c a g a t a a g	Jβ2.3
A2	—C y s A l a S e r —t g t g c c a g c	A r g A r g G l y G l y G l y S e r a g g a g a g g g g g g g g g t c a	G l u T h r g a g a c c c	Jβ2.5
E	—C y s A l a S e r S e r —t g t g c c a g c a g t	**A s p S e r** g a c a g c	S e r T y r G l u G l n t c c t a t g a g c a g	Jβ2.7

findings in this paper, a portion of the CDR3 expressing AspSer sequences, are obvious candidates. In this context, it is of interest that T cells reactive to MBP 68–88 are difficult to find in recovered animals (14), and AspSer sequences in the CDR3, which almost completely dominate Vβ8.2 T cells present during active disease (12/15; Table 1), appear in only one of three Vβ8.2-positive, MBP 68–88–reactive T cell clones obtained from animals that have recovered from EAE (Table 5). This finding is consistent with the possibility that recovery from disease depends upon immune elimination or inactivation of Vβ8.2$^+$ AspSer$^+$ T cells. It is also of interest that of the two clones from recovered animals lacking AspSer in CDR3, one of these (BB12) contained a CDR3 region devoid of N region nucleotide additions.

Previous findings that a Vβ8.2 CDR2 region peptide (35), as well as a CDR3 peptide (37), of TCR used in the MBP response are effective vaccines for EAE raise the possibility that a natural target idiotype may be encompassed in these TCR regions. However, definitive proof for this would require the demonstration that T cells obtained from animals recovering from EAE are reactive to these TCR peptides and that these peptide-reactive T cells confer disease protection in secondary recipients. Finally, it should be noted that this issue may be more complex. Previously one of us (D. Sun) has noted that clonotypic regulation of the encephalitogenic response may also exist (33).

References

1. Allen, P.M., B.P. Babbitt, and E.R. Unanue. 1987. T-cell recognition of lysozyme: the biochemical basis of presentation. *Immunol. Rev.* 98:171.
2. Davis, M.M., and P.J, Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature (Lond.).* 334:395.
3. Hunkapillar, T., and L. Hood. 1989. Diversity of the immunoglobulin gene superfamily. *Adv. Immunology.* 44:1.
4. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of the T-cell $\alpha\beta$ receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3745.
5. Fink, P.J., L.A. Matis, D.L. McElligott, M. Bookman, and S.M. Hedrick. 1986. Correlations between T-cell specificity and the structure of the antigen receptor. *Nature (Lond.).* 321:219.
6. Hedrick, S.M., I. Engel, D.L. McElligott, P.J. Fink, M.-L. Hsu, D. Hansburg, and L.A. Matis. 1988. Selection of amino acid sequences in the beta chain of the T cell antigen receptor. *Science (Wash. DC).* 239:1541.
7. Acha-Orbea, H., D.J. Mitchell, L. Timmermann, D.C. Wraith, G.S. Tausch, M.K. Waldor, S.S. Zamvil, H.O. McDevitt, and L. Steinman. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell.* 54:263.
8. Urban, J.L., V. Kumar, D.H. Kono, C. Gomez, S.J. Horvath, J. Clayton, D.G. Audo, E. Sercarz, and L. Hood. 1988. Restricted use of T Cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell.* 54:577.
9. Lai, M.-Z., Y.-J. Jang, L.-K. Chen, and M.L. Gefter. 1990.

Restricted V-(D)-J junctional regions in the T cell response to λ-repressor. Identification of residues critical for antigen recognition. *J. Immunol.* 144:4851.
10. Danska, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. *J. Exp. Med.* 172:27.
11. Alvord, E.C., Jr. 1984. Species-restricted encephalitogenic determinants. *In* Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. E.C. Alvord, Jr., M.W. Kies, and A.J. Suckling, editors. Alan R. Liss, Inc., New York. 523–537.
12. Burns, F.R., X. Li, N. Shen, H. Offner, Y.K. Chou, A.A. Vandenbark, and E. Heber-Katz. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. *J. Exp. Med.* 169:27.
13. Chou, Y.K., A.A. Vandenbark, R.E. Jones, G. Hashim, and H. Offner. 1989. Selection of encephalitogenic rat T-lymphocyte clones recognizing an immunodominant epitope on myelin basic protein. *J. Neurosci. Res.* 22:181.
14. Vainiene, M., H. Offner, W.J. Morrison, M. Wilkenson, and A.A. Vandenbark. 1991. Clonal diversity of basic protein specific T cells in Lewis rats recovered from experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 33:207.
15. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D.P.

Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. *J. Immunol.* 143:1822.

16. Wysocki, L.J., and V.L. Sato. 1978. Panning of lymphocytes: A method for cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844.

17. Mason, D.W., R.P. Arthur, M.J. Dallman, J.R. Green, G.P. Spikett, and M.L. Thomas. 1983. Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. *Immunol. Rev.* 74:57.

18. Offner, H., M. Vainiene, D.P. Gold, W.J. Morrison, R.-Y., Wang, G.A. Hashim, and A.A. Vandenbark. 1991. Protection against experimental encephalomyelitis: idiotype autoregulation induced by a non-encephalitogenic T cell clone expressing a cross-reactive T cell receptor V gene. *J. Immunol.* 146:4165.

19. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.

20. Chirgwin, J.M., E. Przybyla, R.J. MacDonald, and W.J. Rudder. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294.

21. Maniatas, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

22. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 78:5453.

23. Chluba, J., C. Steeg, A. Becker, H. Wekerle, and J.T. Epplen. 1989. T cell receptor β chain usage in myelin basic protein-specific rat T lymphocytes. *Eur. J. Immunol.* 19:279.

24. Padula, S.J., E.G. Lingenheld, P.R. Stabach, C.-H.J. Chou, D.H. Kono, and R.B. Clark. 1991. Identification of encephalitogenic Vβ-4-bearing T cells in SJL mice. *J. Immunol.* 146:879.

25. Sakai, K., A.A. Sinha, D.J. Mitchell, S.S. Zamvil, J.B. Rothbard, H.O. McDevitt, and L. Steinman. 1988. Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to nested epitopes of myelin basic protein. *Proc. Natl. Acad. Sci. USA.* 85:8608.

26. Lafille, J.J., A. DeCloux, M. Bonneville, Y. Takagaki, and S. Tonegawa. 1989. Junctional sequences of T cell receptor γδ genes: implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining. *Cell.* 59:859.

27. Chou, H.S., S.J. Anderson, M.C. Louie, S.A. Godambe, M.R. Pozzi, M.A. Behlke, K. Huppi, and D.Y. Loh. 1987. Tandem linkage and unusual RNA splicing of the T cell receptor β-chain variable-region genes. *Proc. Natl. Acad. Sci. USA.* 84:1992.

28. Kronenberg, M., G. Siu, L.E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Annu. Rev. Immunol.* 4:529.

29. Havran, W.L., and J.P. Allison. 1988. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature (Lond.).* 335:443.

30. Desiderio, S.V., G.D. Yancopoulos, M. Paskind, E. Thomas, M.A. Bass, N. Landau, F.W. Alt, and D. Baltimore. 1984. *Nature (Lond.).* 311:752.

31. Ben-Nun, A., H. Wekerle, and I.R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis with a T-lymphocyte line reactive against myelin basic protein. *Nature (Lond.).* 292:60.

32. Welch, A.M., J.H. Holda, and R.H. Swanborg. 1980. Regulation of experimental allergic encephalomyelitis. II. Appearance of suppressor cells during the remission phase of the disease. *J. Immunol.* 125:186.

33. Sun, D., Y. Qin, J. Chulba, J.T. Epplen, and H. Wekerle. 1988. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. *Nature (Lond.).* 332:843.

34. Wilson, D.B. Idiotypic regulation of T cells in graft-versus-host disease and autoimmunity. 1989. *Immunol. Rev.* 107:159.

35. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region protein peptide protects against experimental autoimmune encephalomyelitis. *Nature (Lond.).* 341:541.

36. Offner, H., G.A. Hashim, and A.A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. *Science (Wash. DC).* 251:430.

37. Howell, M.D., S.T. Winters, T. Olee, H.C. Powell, D.J. Carlo, and S.W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. *Science (Wash. DC).* 246:668.

38. Williams, C.B., E.P. Blankenhorn, K.E. Byrd, G. Levinson, and G.A. Gutman. 1991. Organization and nucleotide sequence of the rat T cell receptor β-chain complex. *J. Immunol.* 146:4406.