PREDOMINANT EXPRESSION OF A T CELL RECEPTOR V_β GENE SUBFAMILY IN AUTOIMMUNE ENCEPHALOMYELITIS

By SCOTT S. ZAMVIL,* DENNIS J. MITCHELL,* NADINE E. LEE,‡ ANNE C. MOORE,* MATTHEW K. WALDOR,* KOICHIRO SAKAI,* JONATHAN B. ROTHBARD,§ HUGH O. McDEVITT,‡ LAWRENCE STEINMAN,* AND HANS ACHA-ORBEA‡

*From the Departments of *Neurology, *Genetics, and ‡Medical Microbiology, Stanford University, Stanford, California 94305; and the §Imperial Cancer Research Fund, London, United Kingdom WC2A3PX*

Immunization with the autoantigen myelin basic protein (MBP)[1] causes experimental allergic encephalomyelitis (EAE) (1), an autoimmune disease mediated by antigen-specific class II–restricted T lymphocytes (1, 2). Recent studies have shown that MBP-specific T cell clones mediate EAE, causing relapsing paralysis and demyelination within the central nervous system (2, 3), features associated with the human disease multiple sclerosis (MS) (4). By in vitro analysis of these clones and the in vivo induction of EAE with MBP peptides, we have observed that the encephalitogenic T cell response to the NH_2 terminus of MBP in PL/J and (PLSJ)F_1 mice is limited to a discrete population of T lymphocytes that share the same phenotypic class II ($I\text{-}A^u$) restriction and similar NH_2-terminal (1–9) MBP specificity (5).

In this report, we have examined TCR β chain expression of individual NH_2-terminal MBP-specific T cell clones. These clones were analyzed with mAbs that recognize TCR β chains of the variable (V) $V_{\beta 8}$ TCR subfamily (6, 7). 14 of 18 (78%) PL/J T cell clones examined express a member of the $V_{\beta 8}$ subfamily. By Southern analysis with V_β- and J_β-specific gene probes, we examined four $V_{\beta 8}{}^+$ PL/J clones, demonstrating that each uses the same TCR V_β gene, $V_{\beta 8.2}$. Two distinct $V_{\beta 8.2}$-$J_{\beta 2}$ combinations were identified with two clones showing one type of rearrangement and the other two sharing a separate $V_{\beta 8.2}$-$J_{\beta 2}$ combination. These results represent the first molecular analysis of the TCR of T cell clones that mediate autoimmune disease. Our results indicate that there is a restricted use of TCR V_β genes in the autoimmune T cell response to the dominant encephalitogenic NH_2-terminal nonapeptide of MBP. We have also investigated whether in vivo administration of an antibody to the antigen-specific TCR could

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[1] *Abbreviations used in this paper:* EAE, experimental allergic encephelomyelitis; MBP, myelin basic protein, MS, multiple sclerosis; V, variable.

inhibit T cell–mediated autoimmune disease. Our results demonstrate that an mAb specific for the TCR $V_{\beta 8}$ subfamily is effective in preventing autoimmune encephalomyelitis.

Material and Methods

Mice. PL/J and (PL/J × SJL/J)F_1[PLSJ]F_1) female mice were purchased from the Jackson Laboratory, Bar Harbor, ME, and housed in our animal facilities in the Departments of Genetics and Medical Microbiology, Stanford University, Stanford, CA.

Antigens. NH_2-terminal MBP peptides were synthesized according to the sequences for rat and bovine MBP by solid phase techniques as described previously (5). These peptides contained >90% of the desired product as determined by high-pressure liquid phase column (Merck & Co., Inc., Rahway, NJ) and amino acid analysis.

T Cell Clones. NH_2-terminal MBP-specific T cell clones were isolated as described previously, using intact MBP and MBP peptides (2, 3, 5). These clones were isolated from homozygous PL/J mice, except clones F_1-12 and F_1-21, which were isolated from a (PLSJ)F_1 mouse (3). All clones described share the same class II ($I\text{-}A^u$) restriction and NH_2-terminal MBP (1–9) specificity. Clones isolated from the same T cell line differed in their TCR β chain rearrangement or their reactivity to TCR $V_{\beta 8}$-specific mAbs (Table I). Thus, none of the clones discussed are duplicate "sister" clones. T cell clones PJR-25, PJB-20, PJpR-6.2, PJpR-6.4, PJpBR-3.2, and F_1-12 are encephalitogenic as described (2, 3, 5). T cell clones PJB-18, PJH-1.5, and F_1-21 are nonencephalitogenic (8). Other NH_2-terminal MBP-specific T cell clones have not been tested in vivo.

Proliferation Assay. The proliferative responses were determined as described previously (2). Either 10^4 cloned T cells (Fig. 1) or 4×10^4 lymph node cells were cultures with 5×10^5 γ-irradiated (3,000 rad) PL/J splenic APC in 0.2 ml culture media in 96-well flat-bottomed microtitre plates (model 3072; Falcon Labware, Oxnard, CA). At 48 h of incubation, each well was pulsed with 1 μCi[^{3}H]thymidine and harvested 16 h later. The mean cpm thymidine incorporation was calculated for triplicate cultures. Standard deviations from replicate cultures were within 10% mean value.

FACS Analysis. Each clone was stained with KJ16 (6) and anti-L3T4 (GK1.5) (9) as described previously (10). 5×10^5 T cells were incubated with 1 μg biotin-conjugated KJ16 (KJ16.133[6]) and 1 μg fluorescein-conjugated GK1.5, followed by Texas red-avidin. KJ16 (red) fluorescence is shown on the vertical axis and L3T4 (green) fluorescence on the horizontal axis. Immunofluorescence analysis was performed on a dual laser FACS IV (Becton Dickinson Immunocytometry, Fullerton, CA) equipped with logarithmic amplifiers. Two-color staining data are presented as "contour plots", in which the levels of green and red fluorescence per cell define the location on a two-dimensional surface. The elevation at each location represents a 7% frequency of cells with a given fluorescence intensity.

Southern Analysis. TCR probe $V_{\beta C5.1}$ is a 280-bp Pst-Pvu II subclone of $V_{\beta 8.1}(V_{\beta C5.1})$ (reference 11). DNA probe $5'V_{\beta C5.2}$, a 0.5-kb Pvu II fragment (11a), located 2 kb 5′ of $V_{\beta 8.2}$. $J_{\beta 2}$-specific probe is a 1.2-kb genomic probe encompassing $J_{\beta 2.1-2.7}$ (12). Liver and T cell DNA were isolated with standard methods (13). Each sample, containing 10μg DNA, was digested to completion with either Xba I or Hind III, separated on 0.8% agarose gels, and transferred to nitrocellulose filters. The filters were prehybridized at 42°C for 4 h in 50% formamide, 5X SSPE, 100 μg/ml salmon sperm DNA, 1X Denhardt's solution, 0.1% SDS, and 10mM Tris (pH 8.0). Hybridizations were performed in the same buffer containing 5×10^6 cpm/ml of hexamer-primed probe for 20 h at 42°C. The filters were washed in 2X SSC, 0.1% SDS at room temperature. They were washed two more times for 1 h each at 60°C in 0.2X SSC, 0.1% SDS.

Induction of EAE. Induction of EAE with encephalitogenic MBP-specific T cell clone PJR-25 has been described previously (2, 3). 5×10^6 ficoll-separated cells of clone PJR-25 were injected intravenously into recipient (PLSJ)F_1 mice 6 h after intraperitoneal injection of mAb. EAE was graded as described previously (2, 3): 0, no sign of EAE; 1, decreased tail tone only; 2, mild paraparesis; 3, moderately severe papaparesis; 4, complete paraplegia; 5, moribund.

Results

TCR β Chain Expression of NH_2-terminal MBP-specific T Cells. 20 MBP-specific T cell clones ($L3T4^+$, $Lyt2^-$) were examined in this study. 18 of these clones were isolated from 14 individual homozygous PL/J mice, and two clones were isolated from a single $(PLSJ)F_1$ mouse. These clones were isolated after immunization with different forms of MBP and MBP peptides (2, 3, 5). All 20 clones share the same NH_2-terminal nonapeptide specificity and class II ($I\text{-}A^u$) restriction (2, 3, 5). They are not alloreactive for foreign MHC class II molecules (8). The NH_2-terminal MBP-specificity of six representative clones is shown in Fig. 1. TCR β chain gene expression of these clones has been examined with mAbs specific for the TCR $V_{\beta 8}$ gene subfamily. The TCR-specific mAb KJ16 (6) recognizes a determinant associated with expression of two members of this TCR V_β gene subfamily (7) (also known as $V_{\beta C5}$ [11]), $V_{\beta 8.1}$ ($V_{\beta C5.1}$), and $V_{\beta 8.2}$ ($V_{\beta C5.2}$). The KJ16 phenotype is expressed by 16% of PL/J T lymphocytes. In contrast, SJL/J mice do not express the $V_{\beta 8}$ (KJ16) subfamily (6). Fig. 2 shows the FACS-staining pattern with KJ16 for six representative T cell clones, four of which are $KJ16^+$ and two of which are $KJ16^-$. Of the 18 PL/J clones, 14 (78%) are $KJ16^+$ (Table I). This high percentage of $KJ16^+$ ($V_{\beta 8}{}^+$) clones is not an artifact from examining duplicate "sister" clones isolated from the same T cell line. For those lines where two clones are shown (Table I), the clones differed either in their antibody reactivity or their TCR β chain gene rearrangement. Thus 78% $KJ16^+$ clones is a minimum. When additional NH_2-terminal MBP-specific sister clones

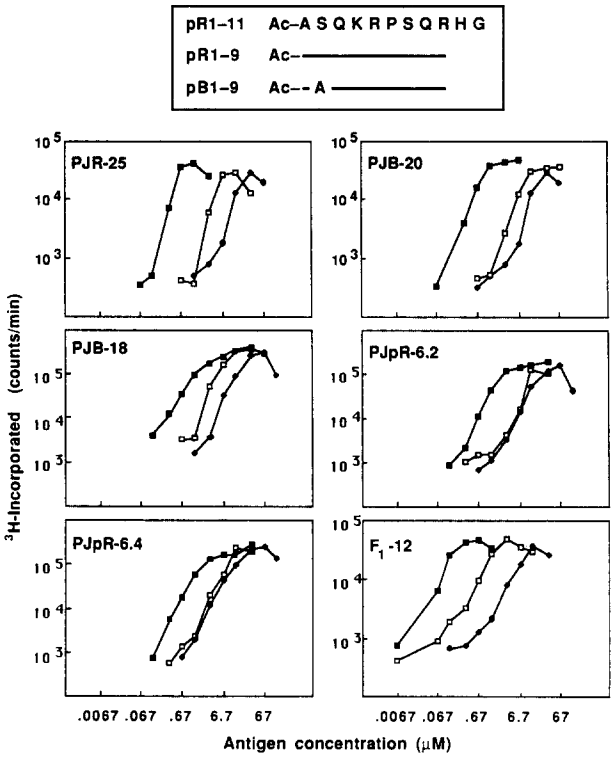

FIGURE 1. Encephalitogenic T cell clones recognize the NH_2 terminus of MBP. Synthetic MBP peptides pR(rat) 1–11 (-■-), pR1–9 (-□-) and pB(bovine) 1–9 (-♦-) were tested for their ability to stimulate encephalitogenic proliferative T cell clones. NH_2-terminal MBP peptides were synthesized as described previously using the sequences for rat and bovine MBP (30). Encephalitogenic clones PJR-25 and F_1-12 were isolated for individual homozygous and $(PLSJ)F_1$ mice, respectively, after immunization with intact rat MBP (5). Encephalitogenic clone PJB-20 and nonencephalitogenic clone PJB-18 were isolated from individual homozygous PL/J mice immunized with bovine MBP (5). Encephalitogenic clones PJpR-6.2 and PJpR-6.4 were isolated from a homozygous PL/J mouse immunized with MBP peptide pR1-11. Proliferation assays were performed as described in Materials and Methods.

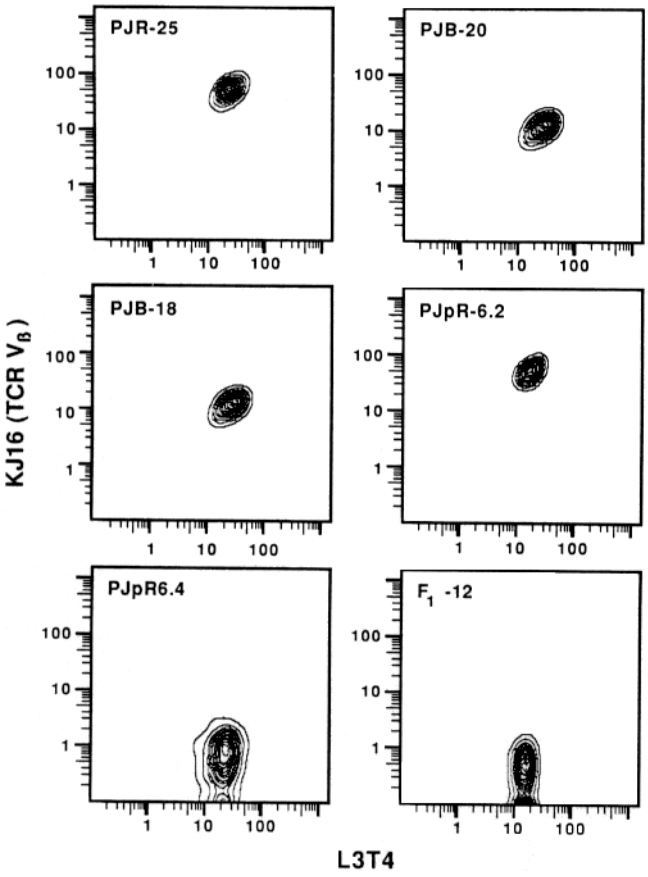

FIGURE 2. NH_2-terminal MBP-specific T cell clones were examined by FACS with TCR $V_{\beta 8}$-specific mAb KJ16. These are the same clones examined in Fig. 1. Each clone was stained with KJ16 (6) and L3T4 (GK1.5) (reference 9) using two-color immunofluorescence analysis. The staining procedure was performed as described previously (10). T cells were incubated with biotin-conjugated KJ16 and fluorescein (green)-conjugated GK1.5, followed by Texas red–avidin. Propidium iodide was added to stain dead cells. KJ16 (red) fluorescence is shown on the *y*-axis and L3T4 (green) fluorescence on the *x*-axis. Immunofluorescence analysis was performed on a dual-laser FACS equipped with logarithmic amplifiers. Two-color staining data are presented as "contour plots" in which the levels of green and red fluorescence per cell define the location on a two-dimensional surface. The elevation at each location represent a 7% frequency of cells with a given fluorscence intensity.

were examined, we observed that 85% are KJ16$^+$($V_{\beta 8}^+$). Furthermore, the KJ16$^-$ clones are not stained with another mAb, F23.1 (reference 14), which recognizes a TCR determinant associated with the expression of all three members of the $V_{\beta 8}$ subfamily, $V_{\beta 8.1}$, $V_{\beta 8.2}$, and $V_{\beta 8.3}$ (7).

The predominant use of the TCR $V_{\beta 8}$ gene subfamily is not an in vitro cloning artifact. We have examined the proliferative T cell response from MBP 1-11-primed PL/J mice after FACS sorting L3T4$^+$ lymph node cells with F23.1 into L3T4$^+$/$V_{\beta 8}^+$ and L3T4$^+$/$V_{\beta 8}^-$ subpopulations. When cultured with APC and MBP 1-11, we have observed that >90% of the proliferative T cell response to MBP 1-11 occurs in the $V_{\beta 8}^+$ subpopulation (Acha-Orbea, H., manuscript in preparation). Although the $V_{\beta 8}^-$ subpopulation of MBP 1-11-primed lymph node cells does not respond to MBP 1-11, this subpopulation does respond to PPD, a positive control. Thus, by examining both in vitro isolated T cell clones and primary cultures, we have shown that there is predominant usage of the TCR $V_{\beta 8}$ subfamily in the autoimmune T lymphocyte response to the NH_2 terminus of MBP.

Genetic Analysis of TCR β-Chains of NH_2-terminal MBP-specific T Cell Clones. Southern blot analysis has been used to determine whether NH_2-ter-

TABLE I

Examination of NH_2-terminal MBP-specific T Cell Clones with TCR $V_{\beta 8}$-specific mAbs

Clone*	Antigen‡ for selection	Class II restriction	MBP specificity	KJ16	F23.1
PJR-25	Rat MBP	I-A^u	1-9	+	+
PJB-18	Bovine MBP	I-A^u	1-9	+	+
PJB-20	Bovine MBP	I-A^u	1-9	+	+
PJH-1.5	Human MBP	I-A^u	1-9	+	+
PJH-2.4	Human MBP	I-A^u	1-9	+	+
PJH-3.2	Human MBP	I-A^u	1-9	+	+
PHJ-4.4	Human MBP	I-A^u	1-9	+	+
PJpR-2.2	pR1-11	I-A^u	1-9	+	+
PJpR-5.2	pR1-11	I-A^u	1-9	+	+
PJpR-6.2	pR1-11	I-A^u	1-9	+	+
PJpR-7.5	pR1-11	I-A^u	1-9	+	+
PJpBR-2.5	pBR1-11	I-A^u	1-9	+	+
PJpBR-3.2	pBR1-11	I-A^u	1-9	+	+
PJpBR-5.2	pBR1-11	I-A^u	1-9	+	+
PJpR-5.3	pR1-11	I-A^u	1-9	−	−
PJpR-6.4	pR1-11	I-A^u	1-9	−	−
PJpBR-3.1	pBR1-11	I-A^u	1-9	−	−
PJpBR-4.1	pBR1-11	I-A^u	1-9	−	−
F_1-21	Rat MBP	I-A^u	1-9	+	+
F_1-12	Rat MBP	I-A^u	1-9	−	−

T cell clones were stained with KJ16 (6) and F23.1 (14) as described in Materials and Methods.

* Intact MBP and MBP peptides were used for selection as described previously (2, 3, 5). Peptide pR1-11 is Ac-ASQKRPSQRHG and pBR1-11 is Ac-AAQKRPSQRHG (5).

‡ All clones were isolated from homozygous PL/J mice except F_1-12 and F_1-21, which were isolated from a (PLSJ)F_1 mouse (3). Clones PJB-18 and PJB-20 were isolated from the same T cell line. These two clones differ in their β chain gene rearrangement (Fig. 3). Clones PJpR-5.2 and PJpR-5.3, PJpR-6.2 and PJpR-6.4, and PJpBR-3.1 and PJpBR-3.2 were isolated from lines established from three separate mice. For each pair, one member differed from the other in the reactivity to $V_{\beta 8}$-specific antibodies. Therefore, none of the reported clones are duplicate "sister" clones. T cell clones PJR-25, PJB-20, PJpR-6.2, PJpR-6.4, PJpBR-3.2, and F_1-12 are encephalitogenic, as described (2, 3, 5). T cell clones PJB-18, PJH-1.5, and F_1-21 are nonencephalitogenic (8). Other NH_2-terminal MBP-specific T cell clones have not been tested in vivo.

minal MBP-specific KJ16$^+$ T cell clones use the same β chain V-D-J combination and to identify which member(s) of the $V_{\beta 8}$ subfamily is used. A blot of Xba I digested genomic DNA isolated from several of these clones was screened with a probe, designated $V_{\beta C5.1}$ (11), which is specific for the $V_{\beta 8}(V_{\beta C5})$ subfamily. This probe hybridizes to all three $V_{\beta 8}$ genes, $V_{\beta 8.1}$ being 92 and 85% homologous to $V_{\beta 8.2}$ and $V_{\beta 8.3}$, respectively (15). On germline DNA digested with Xba I, $V_{\beta 8.1}$, $V_{\beta 8.2}$, and $V_{\beta 8.3}$, genes are located on separate 10.0-, 5.5- and 3.9-kb fragments, respectively. In Fig. 3 *a*, it can be seen that there are rearrangements of a $V_{\beta 8}$ subfamily member of KJ16$^+$ clones PJR-25, PJB-18, and PJpR-6.2. A germline configuration for all $V_{\beta 8}$ members is observed for the KJ16$^-$ clone PJpR-6.4. The sizes of these rearranged bands are consistent with all the KJ16$^+$ clones using $V_{\beta 8.2}$. To confirm this possibility, a blot of Xba I digested DNA was also

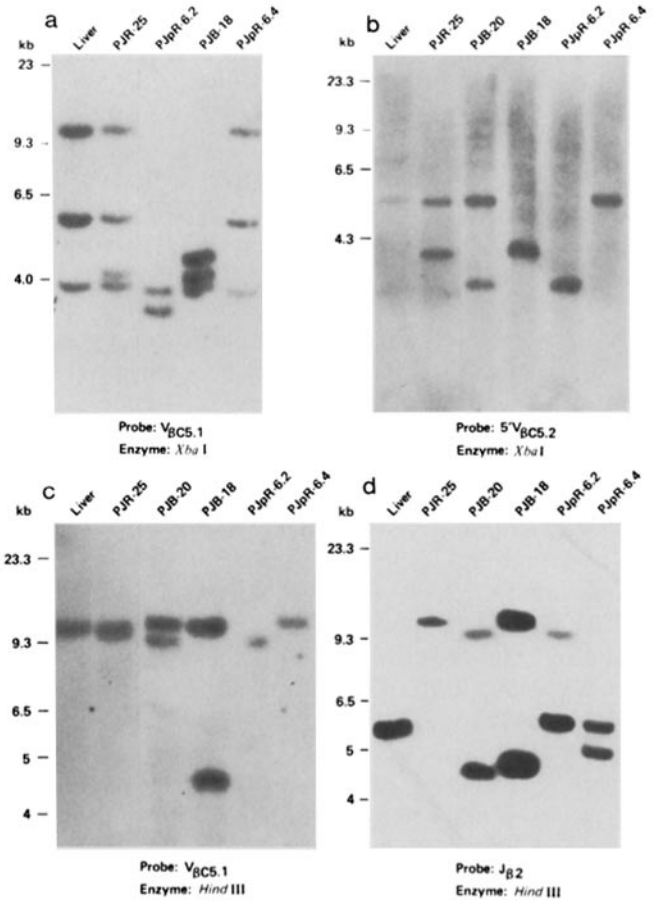

FIGURE 3. Examination of TCR β chains by Southern analysis. Above is a genomic restriction enzyme diagram for the region encoding the TCR $V_{\beta 8}$ (11a, 27) genes and the region encoding TCR D_β, J_β, and C_β genes (28). Individual DNA probes used are indicated below. All clones are KJ16$^+$ except PJpR-6.4. (*a*) DNA was digested with restriction enzyme Xba I and hybridized with probe $V_{\beta C5.1}$, a 280-bp Pst-Pvu II subclone of $V_{\beta 8.1}$ ($V_{\beta C5.1}$[reference 11]). (*b*) Each DNA sample was digested with Xba I and hybridized with DNA probe $5'V_{\beta C5.2}$, a 0.5-kb Pvu II fragment, located 2 kb 5′ of $V_{\beta 8.2}$ (see restriction map). (*c*) DNA was digested with Hind III and hybridized with $V_{\beta C5.1}$. (*d*) DNA samples were digested with Hind III and hybridized with a 1.2-kb $J_{\beta 2}$ genomic probe (12) encompassing $J_{\beta 2.1-2.7}$. $V_{\beta 5.1}$, which is not shown, is located between $V_{\beta 8.3}$ and $V_{\beta 8.2}$ (27).

probed with a 0.5-kb Pvu II fragment (11a) located 2 kb 5′ to $V_{\beta 8.2}$ (see restriction map, Fig. 3). A rearrangement detected using this 0.5-kb probe is specific for the use of $V_{\beta 8.2}$. Rearrangement of the $V_{\beta 8.2}$ gene is observed for all four KJ16$^+$ clones examined by hybridization with this 0.5-kb probe (Fig. 3 *b*). Another KJ16$^+$ clone, encephalitogenic clone PJpBR-3.2 (Table I), which has been examined in the same manner (not shown), also uses TCR $V_{\beta 8.2}$.

The sizes of the fragments seen on DNA digested with Xba I are consistent with the use of members of the $J_{\beta 2}$ locus. To confirm this, blots were made from DNA digested with Hind III. $V_{\beta 8.2}$ and $V_{\beta 8.3}$ are both located on the same 9.6-kb Hind III fragment. $V_{\beta 8.1}$ is located on a 1.2-kb fragment. Using $V_{\beta 8.2}$, a rearrangement involving $J_{\beta 1}$ produces a Hind III fragment of 13–14 kb, whereas rearrangement involving $J_{\beta 2}$ produces a Hind III fragment of 8–10 kb. Blots made from DNA digested with Hind III were hybridized with the probe $V_{\beta C5.1}$($V_{\beta 8.1}$) (Fig. 3 *c*) and a $J_{\beta 2}$-specific probe (12) (Fig. 3 *d*). The 1.2-kb fragment containing $V_{\beta 8.1}$ has migrated off these gels. The rearranged bands seen by hybridization with both $J_{\beta 2}$ and $V_{\beta C5.1}$($V_{\beta 8.1}$) probes are 8.5–9.5 kb, indicating that each rearrangement of $V_{\beta 8.2}$ is a $V_{\beta 8.2}DJ_{\beta 2}$ rearrangement. Two patterns of

rearrangements were seen with the V_β and J_β probes indicating that two different J_β genes at the second J_β cluster must be used. Results of an Eco RV blot (not shown) also indicate the use of two separate J_β genes. The rearrangement of clone PJR-25 is like that of PJB-18 and the rearrangement of clone PJB-20 is the same as PJpR-6.2.

T cell clone PJB-18 has TCR β chain rearrangements involving the $V_{\beta 8}$ locus on both homologous chromosomes. One rearrangement uses $V_{\beta 8.2}$ (Fig. 3, *a* and *b*) and a separate rearrangement is consistent with the use of $V_{\beta 8.3}$ (Fig. 3 *a*). The two V-D-J rearrangements have resulted in the deletion of both germline $V_{\beta 8.1}$ and $V_{\beta 8.2}$ bands. T cell clone PJpR-6.2 has deleted all of the members of the $V_{\beta 8}$ gene subfamily on one chromosome and has a rearrangement to $V_{\beta 8.2}$ on the other, leaving one germline copy of the $V_{\beta 8.3}$ gene on that chromosome (Fig. 3 *a*). With a $J_{\beta 1}$-specific probe, it was shown that this clone uses a $J_{\beta 1}$ gene for the V-D-J rearrangement on the other chromosome (results not shown). Both chromosomes can rearrange TCR genes, though in accordance with allelic exclusion (16), only one is productive. Of the two rearrangements for PJB-18 and PJpR-6.2, the $V_{\beta 8.2}$-containing rearrangement must be the functional one because both clones react with KJ16, which recognizes $V_{\beta 8.1}$ and $V_{\beta 8.2}$, but not $V_{\beta 8.3}$.

In Vivo Administration of an mAb Specific for the TCR $V_{\beta 8}$ Subfamily Prevents Induction of T Cell–mediated Autoimmune Encephalomyelitis. In previous studies, antibodies specific for class II molecules (17–19) and antibodies specific for the T_H subset marker L3T4 (20) have been shown to be effective in preventing EAE as well as other autoimmune diseases (21, 22). Although antigen-specific T cells participate in the pathogenesis of each of these diseases, antibodies specific for the antigen-specific TCR have not been tested for their potential role in therapy of autoimmune disease. We have examined whether $V_{\beta 8}$-specific mAb F23.1 can inhibit EAE. The results in Fig. 4 demonstrate that mAb F23.1 inhibits in vitro proliferation of a representative $V_{\beta 8}^+$ T cell clone, but not a representative $V_{\beta 8}^-$ clone. TCR $V_{\beta 8}$-specific mAb F23.1 has been tested in vivo. In two separate experiments, none of six and none of ten mice injected with F23.1 developed EAE after injection of encephalitogenic clone PJR-25 (Table II). However, 100% of mice in these experiments that were given PJR-25 without F23.1 (or injected with an isotype-matched control mouse mAb) developed chronic paralysis in the same manner as we have described previously (2, 3). In contrast, administration of F23.1 into SJL/J mice (not shown), which lack the $V_{\beta 8}$ subfamily, does not prevent EAE.

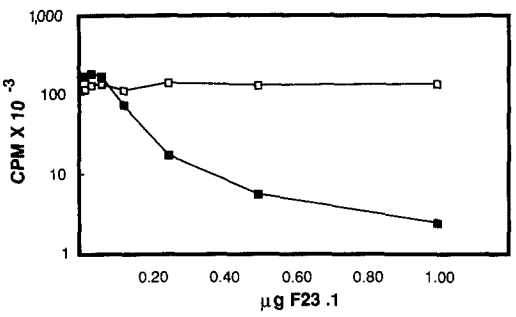

FIGURE 4. TCR $V_{\beta 8}$-specific mAb F23.1 inhibits proliferation of $V_{\beta 8}^+$ T cell clone PJR-25. 10^4 $V_{\beta 8}^+$ MBP 1–11-specific clone PJR-25 (■) and $V_{\beta 8}^-$ MBP35-47-specific clone F3.3 (□) were cultured with 6.7-μM antigen (either MBP 1-11 or MBP35-47) in the presence of various concentrations of $V_{\beta 8}$-specific mAb F23.1. (PLSJ)F_1 splenic antigen-presenting cells were used for each incubation. Antigen and mAb F23.1 were added at the beginning of the culture period. Proliferative responses were determined as described in Materials and Methods.

TABLE II

In Vivo Administration of an mAb Specific for the Antigen-specific TCR Prevents Induction of EAE

Exp.	mAb	Incidence	Severity	Day of onset
1	F23.1	0/6	—	—
	—	6/6	3.5	42
2	F23.1	0/5	—	—
	F23.1	0/5*	—	—
	S5.2	5/5	4.0	27

Induction of EAE by encephalitogenic T cell clone PJR-25 has been described previously (2, 3). Recipient (PLSJ)F_1 mice were injected intraperitonealy with 500 μg DEAE column–purified mAb F23.1 or 500 μg of an isotype-matched (γ2a) control mouse mab S5.2 (anti-Leu-5b [Becton-Dickinson Immunocytometry, Fullerton, CA]). Control mice (in experiment 1) were injected intraperitonealy with 1.0 ml PBS. 6 h later, recipient mice were injected with 5×10^6 cells of encephalitogenic clone PJR-25. Mice were examined daily by two independent observers. Severity of EAE was graded as follows: 0, no sign of EAE; 1, decreased tail tone only; 2, mild paraparesis; 3, moderately severe paraparesis; 4, complete paraplegia; 5, moribund.

* Injected with 500 μg mAb F23.1 14 d after injection of PJR-25.

Discussion

EAE is a model for T cell-mediated autoimmune disease. In previous studies, we have identified MBP-specific T lymphocyte clones that mediate EAE, causing relapsing paralysis and demyelination within the central nervous system (2, 3). We have shown that these clones recognize the NH_2-terminal-nine residue peptide of MBP, and this peptide is the dominant encephalitogenic T cell epitope for both PL/J and (PLSJ)F_1 mice (5). T lymphocyte recognition of this epitope is restricted by I-A^u class II molecules (5, 8). In this report, we have investigated TCR β chain expression of individual T cell clones that express this phenotypic self antigen specificity and MHC class II (I-A^u) restriction. Both examination of TCR expression with TCR β chain–specific antibodies and molecular analysis with TCR V_β chain gene probes have shown that the encephalitogenic T cell response is not genetically clonal, as both $V_{\beta 8}^+$ and $V_{\beta 8}^-$ T cell clones recognize MBP1-9. Our results indicate, however, there is a restricted TCR β chain expression in the autoimmune response to the encephalitogenic NH_2 terminus of MBP. There is a predominant expression of the TCR $V_{\beta 8}$ subfamily, in particular $V_{\beta 8.2}$. In another study, predominant usage of a V_α gene was observed for T cell clones that share the same fine specificity the COOH-terminal epitope of cytochrome *c* (23). Whether the predominant use of a particular V_β or V_α subfamily reflects a unique Ag/MHC specificity or MHC class II restriction alone is not yet clear (24). We are currently examining TCR V_β expression of T cells that recognize other epitopes of MBP and have different class II restriction patterns (3). It is possible, however, that predominant expression of an individual TCR V_β (or V_α) may be relevant to diagnosis and treatment of T cell–mediated

autoimmune disease. In this report, we have investigated whether an antibody to the antigen-specific TCR may have application to therapy of autoimmune disease. Our results demonstrate that in vivo administration of a TCR $V_{\beta 8}$-specific mAb can prevent T cell–mediated autoimmune encephalomyelitis. Thus, in clinical situations where autoaggressive T cells can be identified, antibodies to the Ag/MHC TCR may provide a more specific form of therapy.

The results in this report demonstrate the importance of examining both phenotypic Ag/MHC specificity as well as TCR gene rearrangement to assess heterogeneity in T cell–mediated diseases. In recent studies, T lymphocytes (without known antigen specificity) have been isolated from the brain and cerebrospinal fluid of MS patients, cloned in vitro, and examined by Southern analysis with J_β and C_β TCR gene probes. Those T cells clones did not rearrange their TCR β chain genes in a strictly clonal manner (25, 26). However, in adoptive transfer studies of EAE (a model for MS), it has been shown that only a small percentage of T cells within inflammatory CNS lesions are encephalitogenic (29). Thus, identifying the relevant population of T cells may be extremely difficult. Furthermore, observing separate TCR β chain gene rearrangements does not necessarily imply phenotypic differences in Ag/MHC specificity. It is clear from our results that T lymphocyte clones that mediate autoimmune encephalomyelitis express the same phenotypic self-antigen specificity and MHC restriction, yet rearrange their DNA in a different manner, many using $V_{\beta 8.2}$-D_β-$J_{\beta 2}$ combinations, and a minority of clones using a separate V_β gene. Because most of these T cells use a common V_β gene, we have been able to test a novel therapeutic approach, demonstrating for the first time that an antibody to the antigen-specific TCR may have a role in the therapy of autoimmune disease.

Summary

TCR β chain gene expression of individual T cell clones that share the same MHC class II restriction and similar fine specificity for the encephalitogenic NH_2 terminus of the autoantigen myelin basic protein (MBP) has been examined. TCR V_β expression was examined by FACS analysis with mAbs specific for the $V_{\beta 8}$ subfamily of TCR β chain genes. 14 of 18 (78%) NH_2-terminal MBP-specific clones examined express a member of the TCR $V_{\beta 8}$ subfamily. Southern analysis was used to identify which member(s) of the TCR $V_{\beta 8}$ subfamily is expressed by these clones. Each of four clones examined uses $V_{\beta 8.2}$, though two different $V_{\beta 8.2}$-$J_{\beta 2}$ combinations were identified. Our findings indicate that there is restricted TCR V_β usage in the autoimmune T cell response to the dominant encephalitogenic NH_2-terminal epitope of the MBP. The use of an mAb to the antigen-specific TCR in the prevention of T cell–mediated autoimmune disease has been investigated. Our results demonstrate that in vivo administration of a TCR $V_{\beta 8}$-specific mAb prevents induction of autoimmune encephalomyelitis.

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