

PREVENTION AND TREATMENT OF MURINE
EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS
WITH T CELL RECEPTOR $V\beta$ -SPECIFIC ANTIBODIES

BY DENNIS M. ZALLER, GAMAL OSMAN,
OSAMI KANAGAWA,* AND LEROY HOOD

*From the Division of Biology, California Institute of Technology, Pasadena, California 91125;
and the *Department of Pathology, Washington University School of Medicine,
St. Louis, Missouri 63110*

Experimental allergic encephalomyelitis (EAE)¹ is an autoimmune disease of the central nervous system (CNS) that is induced by immunization of susceptible animal strains with myelin basic protein (MBP). The disease is characterized by lymphocyte infiltration leading to CNS lesions, demyelination, and chronic relapsing paralysis, which are symptoms similar to those seen for multiple sclerosis (MS) in humans (1–6). Prevention of EAE by treatment with CD4-specific antibodies (7, 8) and adoptive transfer of disease symptoms with MBP-specific T helper (Th) cells (9–16) have implicated CD4⁺ Th cells as the primary disease-inducing component of EAE.

The dominant T cell response to MBP in mice of particular MHC haplotypes is directed towards distinct MBP determinants (16–20). For example, B10.PL and PL/J mice (H-2^u) respond primarily to an acetylated NH₂-terminal epitope, while SJL/J mice (H-2^s) respond primarily to a more COOH-terminal epitope. Recent studies have indicated that in H-2^u mice the T cell response to the dominant NH₂-terminal epitope of MBP is of limited heterogeneity (21–23). Molecular characterization of the TCRs used by B10.PL-derived Th cells revealed the use of only two distinct $V\beta$ gene segments, $V\beta 8.2$ and $V\beta 13$, and two distinct $V\alpha$ gene segments, $V\alpha 2.3$ and $V\alpha 4.3$ (21). The gene products of either $V\beta$ segment can pair with the gene products of either $V\alpha$ segment to yield a total of four discrete types of Th cells. The majority of Th cells examined expressed the $V\beta 8.2$ gene segment (84%) while the remainder expressed the $V\beta 13$ gene segment (16%). The distribution of $V\alpha$ gene segments was less skewed among these MBP-specific Th cells with 60% expressing $V\alpha 2.3$ and 40% expressing $V\alpha 4.3$.

The limited use of TCR V region genes in the response to MBP allows the application of unique strategies of specific immune intervention. Since the majority of MBP NH₂-terminal reactive Th cells in H-2^u mice use $V\beta 8.2$, mAbs to $V\beta 8$ (F23.1,

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Address correspondence to Dr. Leroy Hood, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125.

¹ *Abbreviations used in this paper:* CNS, central nervous system; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; MS, multiple sclerosis.

KJ16) were administered *in vivo* in attempts to both prevent and cure EAE (21, 22). Treatment with $V\beta 8$ -specific antibodies led to significant reductions of disease incidence when administered before immunization with an MBP NH_2 -terminal peptide (93–57% in B10.PL mice; 45–5% in (PL/J \times SJL) F_1 mice), and to a reversal of disease symptoms in 12 of 19 (PL/J \times SJL) F_1 mice when these antibodies were administered after the first signs of MBP-induced paralysis. Mice that developed EAE despite anti- $V\beta 8$ antibody treatment developed disease symptoms that were just as severe as those seen in untreated animals.

There remained several possible explanations for the failure to abolish EAE completely in mice treated with anti- $V\beta 8$ antibodies: (a) In addition to the NH_2 -terminal epitope, there are several other T cell encephalitogenic epitopes present on the MBP molecule. T cell responses to these alternative epitopes could have been responsible for the EAE observed in anti- $V\beta 8$ -treated animals. (b) mAb treatment may not have led to the complete elimination of all $V\beta 8^+$ T cells. Residual MBP-specific $V\beta 8^+$ T cells may have led to the observed incidents of autoimmunity. (c) EAE may have resulted from MBP NH_2 -terminal-specific T cells that express $V\beta$ regions other than $V\beta 8$. For example, in B10.PL mice, $V\beta 13^+$ T cells reactive to the MBP NH_2 -terminal would not have been eliminated by anti- $V\beta 8$ treatment, and these T cells could have been responsible for the failure of anti- $V\beta 8$ to completely protect against EAE.

In an attempt to resolve this issue and to improve anti-TCR mAb treatment of EAE, we initiated experiments in which a combination of $V\beta 8.2$ - and $V\beta 13$ -specific antibodies were used to block the response to MBP in B10.PL mice. We report here that treatment with this combination of antibodies led to a significant reduction in MBP responsiveness, a near-complete protection against EAE induction and a dramatic reversal of paralysis in afflicted animals.

Materials and Methods

Mice. B10.PL mice (6–10 wk old) were purchased from The Jackson Laboratories (Bar Harbor, ME) or bred at the California Institute of Technology animal facility.

Myelin Basic Protein. MBP was prepared from frozen rat or mouse brains purchased from Pel-Freeze Biologicals (Rogers, AR) according to a previously published protocol (24).

Immunization. MBP was emulsified with an equal volume of CFA supplemented with 4 mg/ml H37Ra (Difco Laboratories, Inc., Detroit, MI). Mice were immunized in the hind footpads with 150 μg of MBP. For induction of EAE, mice were also given an intravenous dose of 75 ng of purified pertussis toxin (List Biological Laboratories, Inc., Campbell, CA) 24 and 72 h after MBP immunization.

Proliferation Assays. Cells were isolated from draining popliteal and inguinal lymph nodes 10 d after MBP immunization (except where noted). Lymph node cells were resuspended in Ventrex HL-1 medium and plated at 4×10^5 cells per well in 96-well plates. MBP and protein A-purified antibodies were added to the cultures as indicated in the text. All samples were run in triplicate. 4 d later, 1 μCi [^3H]Thymidine was added to each well and cells were collected after an additional 16 h of culture using a PHD cell harvester (Cambridge Technology, Inc., Cambridge, MA). Counts per minute were determined by liquid scintillation counting.

Flow Cytometry. Anti- $V\beta 8.2$ (F23.2) was a gift from Michael Bevan. Anti- $V\beta 13$ (MR12-4) was provided by Osami Kanagawa. The antibodies were purified by protein A chromatography and then biotinylated with NHS-LC-Biotin (Pierce Chemical Co., Rockford, IL) according to the manufacturer's recommendations. Biotinylated antibodies were diluted in PBS containing 5% FCS and 1 mM Hepes. 10^6 cells were stained with 20 $\mu\text{g}/\text{ml}$ of antibody at

37°C for 20 min. Cells were washed with PBS and then resuspended in 50 μ l of a 1:50 dilution of FITC-conjugated streptavidin (Pierce Chemical Co.). After 20 min at 20°C, cells were washed twice with PBS and analyzed using a 50H Cytofluorograph (Ortho Diagnostic Systems Inc., Westwood, MA; 535 nm green band pass filter with an argon laser excitation of 488 nm).

Results

Antibodies Specific for V β 8.2 and V β 13 Inhibit MBP-specific Lymph Node Proliferation Responses in B10.PL Mice. Our previous analysis of 33 independent MBP-specific B10.PL-derived T hybridomas had detected the use of only V β 8.2 and V β 13 gene segments (21). To measure the contribution of T cells expressing these two V-region genes in the response to MBP, specific antibodies were used to block in vitro proliferation responses after MBP immunization. The monoclonal antibodies employed were F23.2 (V β 8.2-specific) (25), and MR12-4 (V β 13-specific) (Kanagawa, O., manuscript in preparation). Constant region isotype analysis of these mAbs classified them both as IgG1/ κ . Lymph node cells from B10PL mice were primed in vivo with MBP and restimulated in vitro with various concentrations of MBP in the presence of anti-V β antibodies or an IgG1 control antibody (MOPC-31C). Proliferative responses to MBP were measured by [3 H]thymidine-incorporation and the results are shown in Fig. 1. Both anti-V β 8.2 and anti-V β 13 partially blocked the response to MBP. At the highest concentration of MBP used for stimulation (3 μ M), the percent inhibitions were 59% for anti-V β 8.2 and 34% for anti-V β 13. Simultaneous addition of both antibodies resulted in an 85% inhibition of the response. We conclude that the bulk

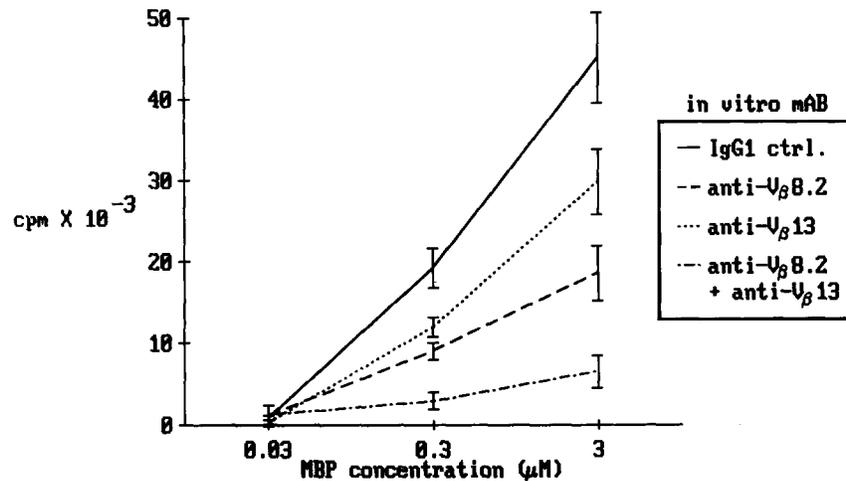


FIGURE 1. Inhibition of MBP response with anti-TCR mAbs. 10 d after the MBP immunization of B10.PL mice (five animals), draining lymph node cells were removed, pooled, and restimulated in vitro with various concentrations of MBP in the presence of the indicated antibodies. After 4 d of culture, cells were pulsed with [3 H]thymidine for 16 h. Each point represents mean cpm from triplicate measurements with background values (no antigen) between $1-4 \times 10^3$ subtracted. The antibodies were purified by protein A affinity chromatography and added to these cultures at a final concentration of 10 μ g/ml. Similar results were obtained with a final antibody concentration of 1 μ g/ml. The experiment was repeated once with similar results.

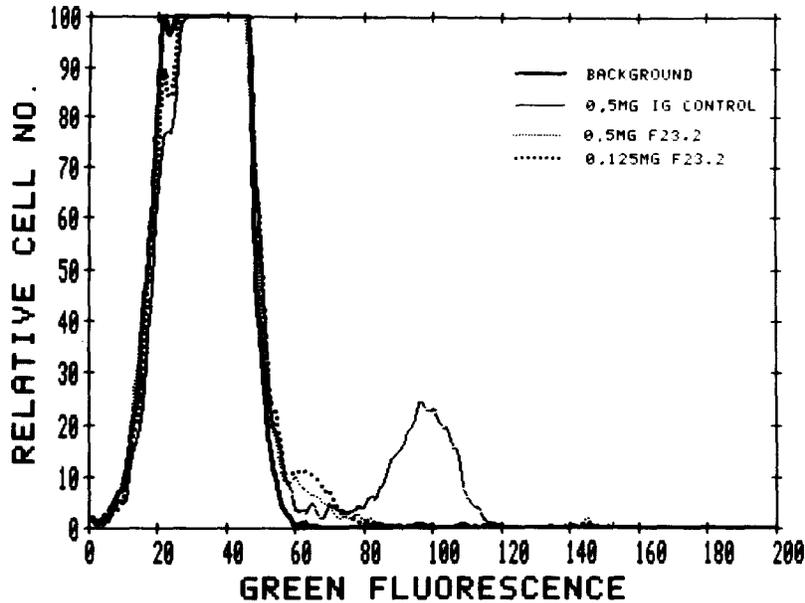


FIGURE 2. Down-modulation of $V\beta 8.2$ T cells following in vivo antibody injections. Groups of three B10.PL mice were injected intraperitoneally with the indicated amounts of either F23.2 ($V\beta 8.2$ -specific) or IgG1 control antibody. 3 d later, lymph node cells were collected, pooled, purified over nylon wool, and subjected to fluorescence flow cytometry. Cells were stained with biotinylated F23.2 followed by FITC-conjugated streptavidin. 2×10^4 cells were counted for each histogram. Similar results were obtained with nylon wool-purified T cells isolated from the spleens of the antibody-treated mice.

of the response to MBP in B10.PL mice is composed of $V\beta 8.2$ - and $V\beta 13$ -expressing T cells.

In Vivo Injection of Anti- $V\beta$ Antibodies Leads to a Long-Term Depletion of Corresponding $V\beta$ -expressing T Cells. Anti-TCR V region antibodies can be used to deplete specific T cells. To optimize the conditions necessary for T cell depletion, various concentrations of anti- $V\beta 8.2$ and anti- $V\beta 13$ were injected into the peritoneal cavity of B10.PL mice. Antibody concentrations in unpurified ascites were determined by comparison to a IgG1 standard in an ELISA. The antibodies were then diluted and injected without further purification in a total volume of 0.1 ml per mouse. 72 h later, peripheral T cells were purified over nylon wool and analyzed by fluorescence flow cytometry. A representative histogram is shown in Fig. 2 and the data are summarized in Table I. At all concentrations tested, injection of both anti- $V\beta 8.2$ and anti- $V\beta 13$ resulted in a virtually complete elimination of corresponding T cells expressing high levels of surface receptors.

A small population of T cells expressing lower levels of surface TCRs persisted following in vivo antibody injection. In fact, the percentage of T cells present in this dull staining population appeared to increase after antibody treatment. This increase was especially noticeable when the lowest concentrations of antibody (0.125 mg) were injected. If some of the injected anti- $V\beta$ antibodies remained on the T cell surface, a residual dull staining population of T cells could result from subsatu-

TABLE I
*Depletion of V β 8.2 and V β 13 T cells
 Following In Vitro Antibody Treatment*

Antibody	Amount	Percent V β 8.2 ^{low}	Percent V β 8.2 ^{high}	Percent V β 13 ^{low}	Percent V β 13 ^{high}
	<i>mg</i>				
IgG1 control	0.500	1.6	8.5	0.1	2.0
Anti-V β 8.2	0.500	2.1	0.2	ND	ND
Anti-V β 8.2	0.250	2.1	0.1	0.3	1.9
Anti-V β 8.2	0.125	2.7	0.0	ND	ND
Anti-V β 13	0.250	1.5	8.3	0.5	0.0
Anti-V β 13	0.125	ND	ND	0.9	0.1

Various concentrations of either F23.2 (V β 8.2-specific) or MR12-4 (V β 13-specific) antibody were injected into groups of three B10.PL mice. 3 d later, lymph node cells were purified over nylon wool and analyzed by quantitative fluorescence flow cytometry. Cells were stained with either biotinylated F23.2 or biotinylated MR12-4 followed by FITC-conjugated streptavidin.

rated binding of the biotinylated anti-V region antibodies used in the fluorescence analysis due to competitive binding of the remaining injected antibodies. However, no increase over background was seen after cell surface staining with a fluorescein-conjugated anti-mouse Ig reagent (data not shown). This indicates that after 72 h, all of the injected antibodies were cleared from the T cell surface. The observed elevation in the percentage of dull staining T cells following antibody treatment could be due to either the expansion of pre-existing dull staining populations or the antibody-induced down-modulation of TCR levels on formerly bright-staining T cells.

Mice examined at 4 and 8 wk after antibody injections showed identical staining patterns to the mice examined after 72 h (data not shown). 12-16 wk after antibody injection the depleted T cells began to reemerge. Thus, the elimination of specific T cells following a single anti-V region antibody treatment appears to be a relatively long-term effect.

Responsiveness to MBP Is Diminished Following In Vivo Administration of Anti-V β Antibodies. Since in vivo depletion of V β 8.2 and V β 13 T cells was possible, we evaluated the effect of this depletion on the response to MBP. B10.PL mice were treated with anti-V β 8.2 and anti-V β 13, alone and in combination, or with an IgG1 control antibody, and then primed in vivo with MBP. Draining lymph node cells were isolated 10 d later and restimulated in vitro with MBP in the presence of various blocking antibodies (Fig. 3).

Injection of the control antibody had no effect on reactivity to MBP. There was a vigorous proliferation response that was inhibitable by the in vitro addition of both anti-V β 8.2 and anti-V β 13. Anti-V β 8.2 injection reduced, but did not eliminate, reactivity to MBP. The remaining response was inhibited by anti-V β 13 but not significantly inhibited by addition of anti-V β 8.2. Anti-V β 13 injection, by itself, did not lead to a significant reduction in MBP reactivity. However, the response was no longer significantly inhibited by anti-V β 13, while it remained susceptible to inhibition by anti-V β 8.2. Simultaneous injection of both anti-V β 8.2 and anti-V β 13 resulted in the most extreme reduction in MBP reactivity. However, a minor proliferative response

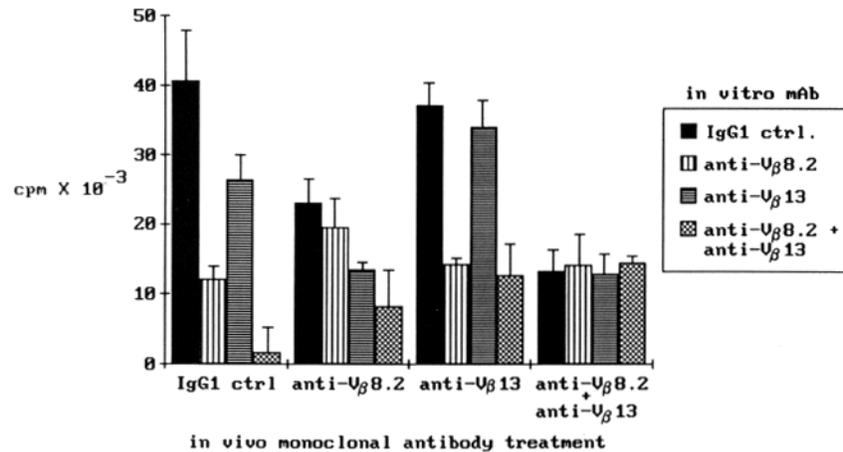


FIGURE 3. Effect of in vivo mAb treatment on the response to MBP. Groups of three B10.PL mice were treated in vivo with 250 μ g of the indicated antibodies and then restimulated in vitro with a final concentration of 3 μ M MBP. Various blocking antibodies were added to the in vitro cultures at a final concentration of 10 μ g/ml. Cells were cultured for 4 d and then pulsed with [3 H]thymidine. Each data point represents the mean cpm from triplicate cultures minus background. The experiment was repeated once with similar results.

to MBP remained, that was not significantly inhibited by either anti-V β 8.2 or anti-V β 13. This residual response could be the consequence of subdominant MBP-specific T cells that might expand after the in vivo elimination of the dominant V β 8.2 and V β 13 MBP-specific T cells. The reactivity of this T cell population is directed towards the MBP NH₂-terminal epitope (data not shown).

Anti-V β Antibody Treatment Is an Effective Therapy for the Prevention of EAE in B10.PL Mice. The considerable reduction in responsiveness to MBP brought on by injection of anti-V β antibodies suggested that this treatment might be effective in preventing the induction of EAE. To assess the efficacy of this treatment, B10.PL mice were either left untreated or injected with various mAbs, and then immunized with MBP in attempts to induce EAE. Results from these experiments are summarized in Table II. 14 of 17 mice (82.4%) that were either untreated or had been injected with an IgG1 control antibody developed EAE. The average disease severity index of the animals in this group was 2.2 (see legend to Table II for a description of the severity index scale).

9 of 10 mice (90%) pretreated with anti-V β 13 developed EAE. Disease symptoms in the afflicted mice were just as severe as those seen in the control mice. Therefore, anti-V β 13 pretreatment, by itself, is not an effective therapy for EAE, nor did it even have a noticeable effect. This is consistent with the in vitro proliferation data presented in Fig. 3 which demonstrated that anti-V β 13 when injected by itself did not reduce the overall responsiveness to MBP. 5 of 20 mice treated with anti-V β 8.2 developed EAE. The average severity index of the animals in this group was 0.7. Therefore, anti-V β 8.2 treatment alone can lead to a very significant protection against EAE ($p < 0.005$). This is consistent with previous results that demonstrated a reduction in EAE incidence in H-2^d mice pretreated with mAbs that recognize members of

TABLE II
Anti-TCR mAb Treatment Protects Against EAE

Treatment	EAE incidence	Percent incidence	Avg. severity		Avg. day of onset
			All animals	Diseased animals	
None	11/13	82.4	2.2 ± 1.4	2.7 ± 1.1	9.4 ± 0.6
IgG1 control	3/4				
Anti-V β 8.2	5/20	25.0	0.7 ± 1.2*	2.6 ± 0.5	11.4 ± 0.5
Anti-V β 13	9/10	90.0	2.7 ± 1.4	3.0 ± 1.2	9.3 ± 0.7
Anti-V β 8.2 + anti-V β 13	1/20	5.0	0.1 ± 0.2†	1	19

Groups of B10.PL mice were treated with the indicated antibodies and then immediately immunized in the hind footpads with 150 μ g of MBP. 75 ng of purified pertussis toxin was injected intravenously at 24 and 48 h after immunization. Mice were then observed daily for signs of EAE. Disease severity was graded on a 5-point scale: 0, normal; 1, loss of tail tone; 2, hind limb weakness, difficulty walking; 3, hind limb paralysis, difficulty turning over; 4, severe whole body paralysis; 5, death. The average severity for each group was calculated in two different ways: (a) by averaging the maximum severity of all of the animals in the group, and (b) by averaging the maximum severity of only the diseased animals in the group. The arithmetic means and standard deviations are indicated in the table. p values were calculated using the Student's t -test.

* $p < 0.005$ when compared with the control group.

† $p < 0.001$ when compared with the control group and $p < 0.025$ when compared with the anti-V β 8.2 treated group.

the V β 8 family (22, 26). The symptoms in the five diseased animals in this group were just as severe as those seen in the control animals but were slightly delayed in onset. Pretreatment with a combination of anti-V β 8.2 and V β 13 proved to be extremely effective in protecting B10.PL mice from EAE. Only 1 of 20 mice (5%) that had been injected with this combination of antibodies developed signs of paralysis. The average severity index of the animals in this group was 0.1. This is a very significant protection against EAE when compared with control mice ($p < 0.001$) and a significant protection when compared with anti-V β 8.2-treated mice ($p < 0.025$).

The paralysis in the one mouse that exhibited disease symptoms despite double antibody treatment was mild (a loss of tail tone) and extremely delayed in onset compared with control mice (19 d vs. an average of 9.4 d after MBP immunization). The T cells responsible for the development of EAE in this single exception could be either V β 8.2 or V β 13 T cells that were not eliminated by antibody treatment or subdominant T cells that express as yet uncharacterized V β genes. To address this issue, MBP-induced proliferative responses of lymph node cells isolated from this animal were compared with those of control animals with EAE and double antibody-treated mice without EAE. Draining lymph node cells used in this experiment were isolated 21 d after MBP immunization. A proliferative response was measured in the control animals with EAE that was inhibitable by both anti-V β 8.2 and anti-V β 13 (Fig. 4). No response to MBP was detected in mice that had received double antibody treatment and were symptom free. This is in contrast to the slight proliferative response that was detected in lymph node cells removed from double antibody-treated animals 10 d after MBP immunization (Fig. 3). This difference in re-

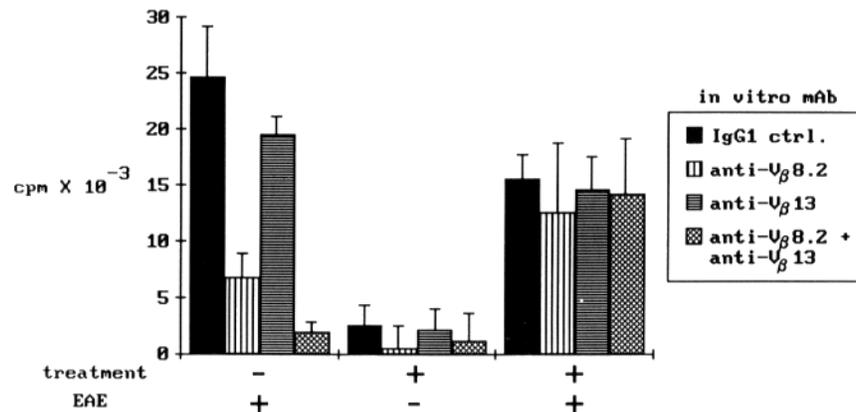


FIGURE 4. Comparisons of the response to MBP in mAb-treated mice with and without EAE. B10.PL mice were either untreated or treated with injections of both anti-V β 8.2 and anti-V β 13. Mice were then immunized with MBP and followed for signs of EAE. 3 wk after immunization, animals with and without EAE were killed. Draining lymph node cells were collected and assayed for MBP reactivity as described in the legend to Fig. 3.

sponsiveness might be due to the delay in removing lymph node cells for analysis. Indeed, proliferative responses were lower in cells from control animals isolated at day 21 compared with those of cells isolated at day 10. A response to MBP was seen in lymph node cells isolated from the one double antibody-treated mouse that showed signs of EAE. This response was not inhibitable by either anti-V β 8.2 or anti-V β 13. This implies that EAE in this mouse may have been caused by MBP-specific T cells that do not express either V β 8.2 or V β 13 TCRs.

Anti-V β Antibody Treatment Can Reverse Paralysis in B10.PL Mice With EAE. We next asked whether the symptoms of EAE could be reversed with double antibody treatment. Groups of mice were treated with an IgG1 control antibody or with a combination of anti-V β 8.2 and anti-V β 13 3 d after the first signs of MBP-induced paralysis. The animals were then observed on a daily basis and graded for the severity of paralysis (Fig. 5). Disease symptoms remained the same or worsened in all five of the control antibody-treated animals during the first 2 wk of observation. In contrast, dramatic improvements were observed in anti-V β treated animals within 2–7 d after antibody injection. Reversal from severe hind leg paralysis (grade 3) to a completely normal phenotype was seen in three of the five anti-V β -treated mice. In another anti-V β -treated mouse, a reversal from a near complete whole body paralysis (grade 4) to a limited tail paralysis (grade 1) was observed. One anti-V β -treated mouse that began with whole body paralysis died 3 d after treatment. We conclude that treatment with the combination of anti-V β 8.2 and anti-V β 13 is an effective therapy for the reversal of paralysis in B10.PL mice.

Discussion

MBP-induced encephalomyelitis is considered to be a paradigm for T cell-mediated autoimmune disease. Striking similarities in the pathology of EAE and MS in humans make EAE an especially important model system for study. The dominant T cell response to MBP in H-2^d mice is directed towards a single NH₂-

T cell development. A previous study demonstrated that the maturation of T cells expressing particular V regions could be blocked by neonatal injections of a mAb specific for these V regions (27). Alternatively, injection of V β -specific antibodies may stimulate some type of regulatory network in which the development of T cells expressing particular V β regions is suppressed.

Several immunological approaches have been used successfully to block EAE, including injection of peptide analogue to an MBP encephalitogenic determinant (26, 28), passive immunization by injection of antibodies specific for CD4 (7, 8) or class II MHC gene products (29, 30), and active immunization by injection of attenuated MBP-specific T cells (31) or synthetic peptides corresponding to TCR determinants (32, 33). Active immunization approaches have advantages since a single vaccination can lead to immunological memory and perhaps a permanent protection; however, such treatment would be ineffective for individuals that are nonresponsive to the vaccinating antigens.

The ability to suppress the response to MBP by injections of V β -specific antibodies permits analysis of antibody treatment as a strategy to block EAE. This treatment eliminates all V β 8.2-expressing cells, irrespective of the V α chain they may be associated with. It is possible that the *in vivo* elimination of T cell subsets that express particular V region subfamily members could lead to reduced immune responsiveness and consequential increase in susceptibility to environmental pathogens. However, this seems unlikely in light of the fact that inbred mouse strains that lack 50% of the known V β genes (SJL, C57L, C57BR, and SWR) do not exhibit obvious immunodeficiencies (34).

In one series of experiments, antibodies were administered before MBP immunization to test the efficacy of this treatment in preventing EAE (Table II). Injection of anti-V β 8.2 (F23.2) by itself resulted in a significant reduction in EAE incidence (82.4 to 25%). This finding is consistent with previous reports of partial protection against EAE after treatment with a V β 8-specific antibody (F23.1) (21) or a V β 8.2, V β 8.3-specific antibody (KJ16) (22). The use of F23.2 for therapy is preferential to the use of either F23.1 or KJ16, since the later reagents have broader specificities that would lead to the elimination of larger percentages of non-MBP-specific T cells.

Injection of both anti-V β 13 and anti-V β 8.2 resulted in an enhanced reduction in EAE incidence when compared with injection of V β 8.2 alone (25% to 5%). This result suggests that the failure of anti-V β 8.2 to protect animals completely from EAE was due, at least in part, to the continued presence of MBP-reactive V β 13⁺ T cells. One animal in our study with double antibody treatment developed a delayed outbreak of tail paralysis. It is likely that rare V β 8.2⁻, V β 13⁻, MBP-specific T cells occasionally expand and can lead an autoimmune attack. This explanation is consistent with the observation that draining lymph node cells isolated from this mouse could respond *in vitro* to MBP and the response could not be blocked by the addition of either anti-V β 8.2 or anti-V β 13 (Fig. 4). Characterization of a panel of MBP-specific hybridoma cells produced from T cells isolated from this mouse is now underway and should lead to the identification of the TCRs responsible for these residual MBP responses. Preliminary analysis indicates that a large majority of these hybridomas are specific for the MBP NH₂-terminal epitope and that none of these hybridomas express either V β 8.2 or V β 13 TCRs.

We have also demonstrated that anti-V β treatment can rapidly reverse the paralytic symptoms of EAE (Fig. 5). This observation confirms previous results demonstrating reversal of EAE after treatment with KJ16 (22). This suggests that the maintenance of an EAE disease state must require the constant presence of MBP-reactive T cells. Neurological damage associated with EAE may be readily reversed once autoaggressive T cells are removed. Relapses have not been in any of the double antibody-treated animals during the first 10 wk of post-treatment observations.

Therapeutic strategies using antibodies reactive to TCR idiotypic determinants would have the advantage of deleting only very specific subsets of T cells. In fact, a clonotypic antibody raised against an MBP-specific rat T cell hybridoma has been shown to protect rats from EAE (35). In practice, it may prove difficult to raise anti-idiotypic antibodies that can crossreact with encephalitogenic T cells from different individuals. Accordingly, V β and/or V α -specific antibodies will constitute a more useful set of general reagents for autoimmune T cell elimination. In addition, since the deletions of all T cells with particular V regions (e.g., V β 8.2 or V β 13) do not appear to immunologically compromise the animal, the generation of antibodies to the 22 different murine V β regions may generate a specific set of reagents that will permit the deletion of any particular subset of T cells. Humans encode somewhere between 50 and 75 functional V β gene segments.

Summary

Experimental allergic encephalomyelitis (EAE) is a model system for T cell-mediated autoimmune disease. Symptoms of EAE are similar to those of multiple sclerosis (MS) in humans. EAE is induced in susceptible animal strains by immunization with myelin basic protein (MBP) and potent adjuvant. The major T cell response to MBP in B10.PL mice is directed towards an NH₂-terminal epitope and involves T cells expressing either V β 8.2 or V β 13 gene segments. Animals treated with a TCR V β 8-specific mAb have a reduced incidence of EAE. We report here that the *in vivo* administration of a combination of anti-V β 8.2 and anti-V β 13 mAbs results in a long-term elimination of T cells involved in the response to MBP. When given before MBP immunization, anti-TCR antibody treatment leads to nearly complete protection against EAE. Antibody treatment also results in a dramatic reversal of paralysis in diseased animals. Thus, treatment with a combination of V β -specific antibodies is a very effective therapy for the prevention and treatment of EAE. It is hoped that the future characterization of TCR V gene usage in human autoimmune diseases may lead to similar strategies of immune intervention.

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