PREVENTION AND TREATMENT OF MURINE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITH T CELL RECEPTOR Vβ-SPECIFIC ANTIBODIES

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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 MPB in mice of particular MHC haplotypes is directed towards distinct MBP determinants (16–20). For example, B10.PL and PL/J mice ($H\text{-}2^u$) respond primarily to an acetylated NH_2-terminal epitope, while SJL/J mice ($H\text{-}2^s$) respond primarily to a more COOH-terminal epitope. Recent studies have indicated that in $H\text{-}2^u$ mice the T cell response to the dominant NH_2-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 Vβ gene segments, Vβ8.2 and Vβ13, and two distinct Vα gene segments, Vα2.3 and Vα4.3 (21). The gene products of either Vβ segment can pair with the gene products of either Vα segment to yield a total of four discrete types of Th cells. The majority of Th cells examined expressed the Vβ8.2 gene segment (84%) while the remainder expressed the Vβ13 gene segment (16%). The distribution of Vα gene segments was less skewed among these MBP-specific Th cells with 60% expressing Vα2.3 and 40% expressing Vα4.3.

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 NH_2-terminal reactive Th cells in $H\text{-}2^u$ mice use Vβ8.2, mAbs to Vβ8 (F23.1,

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[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 Vβ8-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 × SJL)F_1 mice), and to a reversal of disease symptoms in 12 of 19 (PL/J × SJL)F_1 mice when these antibodies were administered after the first signs of MBP-induced paralysis. Mice that developed EAE despite anti-Vβ8 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-Vβ8 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-Vβ8-treated animals. (*b*) mAb treatment may not have led to the complete elimination of all $V\beta8^+$ T cells. Residual MBP-specific $V\beta8^+$ 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 Vβ regions other than Vβ8. For example, in B10.PL mice, $V\beta13^+$ T cells reactive to the MBP NH_2-terminal would not have been eliminated by anti-Vβ8 treatment, and these T cells could have been responsible for the failure of anti-Vβ8 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 Vβ8.2- and Vβ13-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 [^{3}H]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-Vβ8.2 (F23.2) was a gift from Michael Bevan. Anti-Vβ13 (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 μg/ml of antibody at

37°C for 20 min. Cells were washed with PBS and then resuspended in 50 μl 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 Vβ8.2 and Vβ13 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 Vβ8.2 and Vβ13 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 (Vβ8.2-specific) (25), and MR12-4 (Vβ13-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-Vβ 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-Vβ8.2 and anti-Vβ13 partially blocked the response to MBP. At the highest concentration of MBP used for stimulation (3 μM), the percent inhibitions were 59% for anti-Vβ8.2 and 34% for anti-Vβ13. Simultaneous addition of both antibodies resulted in an 85% inhibition of the response. We conclude that the bulk

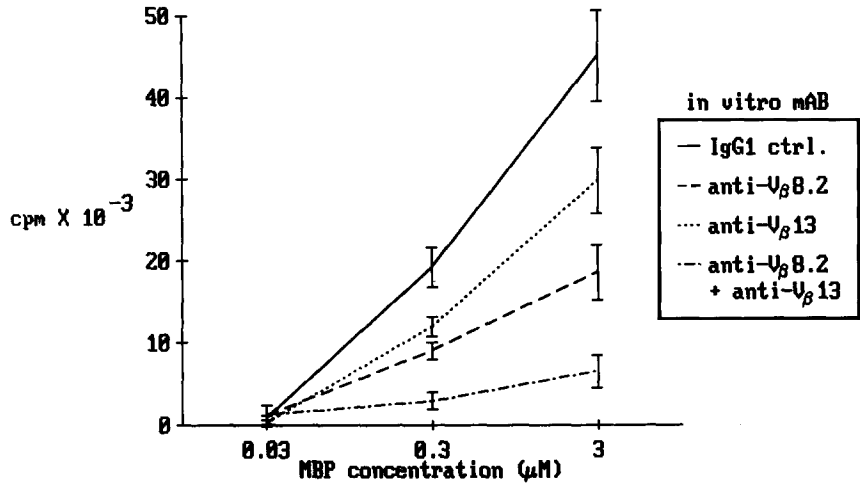

FIGURE 1. Inhibition of MBP response with anti-TCR mAbs. 10 d after the MBP immunization of B10.PL mice (five animals), draining lymph node cells were removed, pooled, and restimulated in vitro with various concentrations of MBP in the presence of the indicated antibodies. After 4 d of culture, cells 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 × 10^3 subtracted. The antibodies were purified by protein A affinity chromatography and added to these cultures at a final concentration of 10 μg/ml. Similar results were obtained with a final antibody concentration of 1 μg/ml. The experiment was repeated once with similar results.

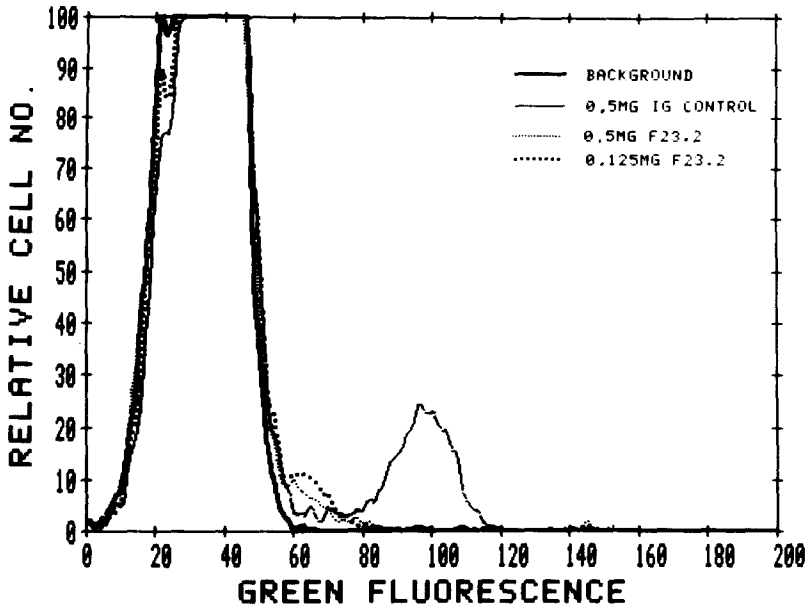

FIGURE 2. Down-modulation of Vβ8.2 T cells following in vivo antibody injections. Groups of three B10.PL mice were injected intraperitoneally with the indicated amounts of either F23.2 (Vβ8.2-specific) or IgG1 control antibody. 3 d later, lymph node cells were collected, pooled, purified over nylon wool, 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 Vβ8.2- and Vβ13-expressing T cells.

In Vivo Injection of Anti-Vβ Antibodies Leads to a Long-Term Depletion of Corresponding Vβ-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-Vβ8.2 and anti-Vβ13 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-Vβ8.2 and anti-Vβ13 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-Vβ antibodies remained on the T cell surface, a residual dull staining population of T cells could result from subsatu-

TABLE I
Depletion of Vβ8.2 and Vβ13 T cells Following In Vitro Antibody Treatment

Antibody	Amount	Percent Vβ8.2^{low}	Percent Vβ8.2^{high}	Percent Vβ13^{low}	Percent Vβ13^{high}
	mg				
IgG1 control	0.500	1.6	8.5	0.1	2.0
Anti-Vβ8.2	0.500	2.1	0.2	ND	ND
Anti-Vβ8.2	0.250	2.1	0.1	0.3	1.9
Anti-Vβ8.2	0.125	2.7	0.0	ND	ND
Anti-Vβ13	0.250	1.5	8.3	0.5	0.0
Anti-Vβ13	0.125	ND	ND	0.9	0.1

Various concentrations of either F23.2 (Vβ8.2-specific) or MR12-4 (Vβ13-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-Vβ Antibodies. Since in vivo depletion of Vβ8.2 and Vβ13 T cells was possible, we evaluated the effect of this depletion on the response to MBP. B10.PL mice were treated with anti-Vβ8.2 and anti-Vβ13, 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-Vβ8.2 and anti-Vβ13. Anti-Vβ8.2 injection reduced, but did not eliminate, reactivity to MBP. The remaining response was inhibited by anti-Vβ13 but not significantly inhibited by addition of anti-Vβ8.2. Anti-Vβ13 injection, by itself, did not lead to a significant reduction in MBP reactivity. However, the response was no longer significantly inhibited by anti-Vβ13, while it remained susceptible to inhibition by anti-Vβ8.2. Simultaneous injection of both anti-Vβ8.2 and anti-Vβ13 resulted in the most extreme reduction in MBP reactivity. However, a minor proliferative response

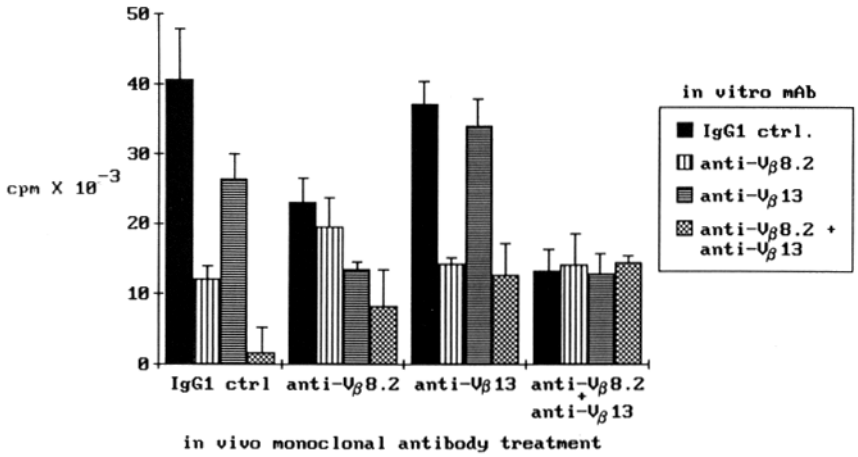

FIGURE 3. Effect of in vivo mAb treatment on the response to MBP. Groups of three B10.PL mice were treated in vivo with 250 μg of the indicated antibodies and then restimulated in vitro with a final concentration of 3 μM MBP. Various blocking antibodies were added to the in vitro cultures at a final concentration of 10 μg/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-Vβ8.2 or anti-Vβ13. This residual response could be the consequence of subdominant MBP-specific T cells that might expand after the in vivo elimination of the dominant Vβ8.2 and Vβ13 MBP-specific T cells. The reactivity of this T cell population is directed towards the MBP NH_2-terminal epitope (data not shown).

Anti-Vβ 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-Vβ 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-Vβ13 developed EAE. Disease symptoms in the afflicted mice were just as severe as those seen in the control mice. Therefore, anti-Vβ13 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-Vβ13 when injected by itself did not reduce the overall responsiveness to MBP. 5 of 20 mice treated with anti-Vβ8.2 developed EAE. The average severity index of the animals in this group was 0.7. Therefore, anti-Vβ8.2 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^u mice pretreated with mAbs that recognize members of

TABLE II

Anti-TCR mAb Treatment Protects Against EAE

Treatment	EAE incidence	Percent incidence	Avg. severity: All animals	Avg. severity: Diseased animals	Avg. day of onset
None IgG1 control	11/13 3/4	82.4	2.2 ± 1.4	2.7 ± 1.1	9.4 ± 0.6
Anti-Vβ8.2	5/20	25.0	0.7 ± 1.2*	2.6 ± 0.5	11.4 ± 0.5
Anti-Vβ13	9/10	90.0	2.7 ± 1.4	3.0 ± 1.2	9.3 ± 0.7
Anti-Vβ8.2 + anti-Vβ13	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 μg of MBP. 75 ng of purified pertussis toxin was injected imtravenously 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-Vβ8.2 treated group.

the Vβ8 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-Vβ8.2 and Vβ13 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-Vβ8.2–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 Vβ8.2 or Vβ13 T cells that were not eliminated by antibody treatment or subdominant T cells that express as yet uncharacterized Vβ 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-Vβ8.2 and anti-Vβ13 (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-

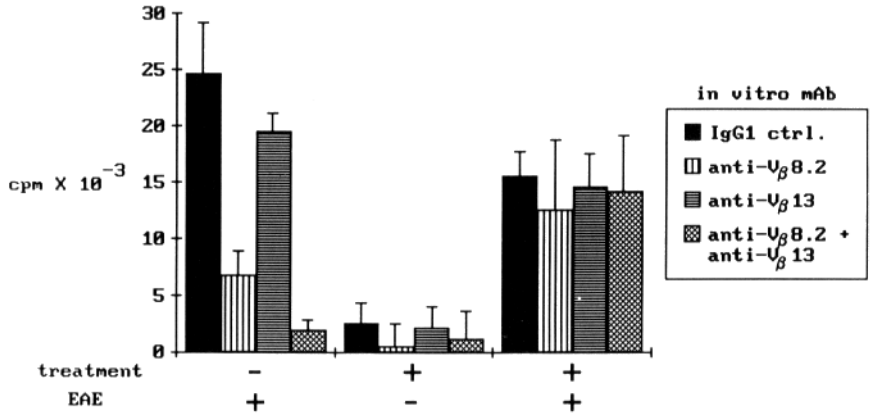

FIGURE 4. Comparisons of the response to MBP in mAb-treated mice with and without EAE. B10.PL mice were either untreated or treated with injections of both anti-Vβ8.2 and anti-Vβ13. Mice were then immunized with MBP and followed for signs of EAE. 3 wk after immunization, animals with and without EAE 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-Vβ8.2 or anti-Vβ13. This implies that EAE in this mouse may have been caused by MBP-specific T cells that do not express either Vβ8.2 or Vβ13 TCRs.

Anti-Vβ 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-Vβ8.2 and anti-Vβ13 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-Vβ 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-Vβ-treated mice. In another anti-Vβ-treated mouse, a reversal from a near complete whole body paralysis (grade 4) to a limited tail paralysis (grade 1) was observed. One anti-Vβ-treated mouse that began with whole body paralysis died 3 d after treatment. We conclude that treatment with the combination of anti-Vβ8.2 and anti-Vβ13 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^u mice is directed towards a single NH_2-

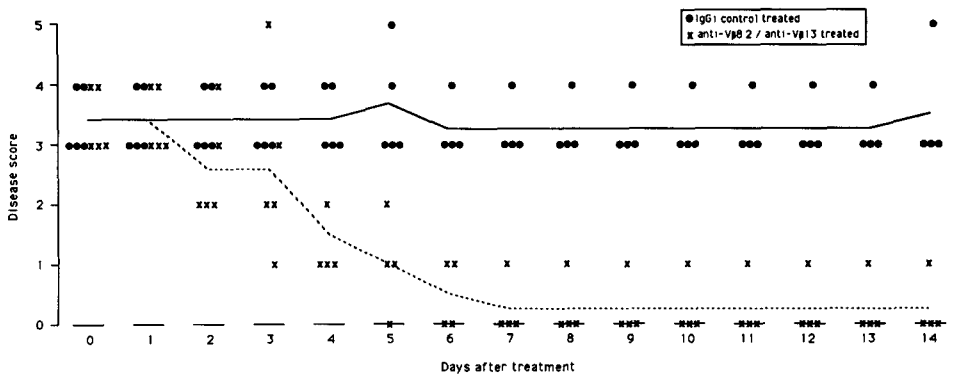

FIGURE 5. Treatment with anti-Vβ mAbs results in reversal of MBP-induced paralysis. Groups of five B10.PL mice matched for severity of EAE symptoms were treated with either an IgG1 control antibody or a combination of anti-Vβ8.2 and anti-Vβ13 antibodies. Mice were then scored daily for disease severity. Each symbol represents the disease score for an individual mouse on a given day. The lines correspond to the average disease severity of surviving mice in each group on that day. The solid line represents the control antibody-treated group; the broken line represents the anti-Vβ treated group.

terminal determinant and involves T cells that express a limited number of TCR V-regions. In this report, we demonstrate that in vivo T cell depletion by a combination of mAbs specific for the TCR Vβ regions used in the response to MBP is an effective therapy for both the prevention and treatment of EAE.

Two approaches were used to characterize the T cells involved in the response to MBP. In the first approach, B10.PL mice were immunized with MBP and the response was once again recalled in vitro in the presence of blocking antibodies (Fig. 3). Depletion of Vβ8.2-expressing T cells before immunization led to a reduction in MBP responsiveness, while depletion of Vβ13-expressing T cells did not lead to a significant reduction. This implies that the presence of $V\beta8.2^+$ T cells is necessary and sufficient for efficient in vivo priming to MBP. Simultaneous depletion of both Vβ8.2- and Vβ13-expressing T cells led to the most dramatic loss in MBP responsiveness, however, a small proliferative response was still observed. This response was directed towards the MBP NH_2-terminal epitope. This suggests that in the absence of Vβ8.2- and Vβ13-expressing T cells, subdominant T cells that use alternative V regions might expand in response to MBP immunization. We are now in the process of further characterizing these T cells.

A single intraperitoneal injection of anti-Vβ antibodies led to a depletion of T cells expressing corresponding Vβ gene products that lasted ~12 wk. T cell elimination could have resulted from antibody-mediated cytotoxicity, as the mAbs were a complement fixing isotype, IgG1. However, the emergence of a small population of dull staining T cells following antibody treatment (Fig. 2) suggests that down-modulation of cell surface TCR levels may also play a role. The persistence of T cell depletion long after the expected clearance of injected Vβ-specific antibodies is puzzling. Presumably, continued TCR gene rearrangements should lead to a rapid replenishment of the depleted T cell populations. Perhaps, small amounts of the injected antibodies remained sequestered in locations where they interfered with

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 Vβ-specific antibodies may stimulate some type of regulatory network in which the development of T cells expressing particular Vβ 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 Vβ-specific antibodies permits analysis of antibody treatment as a strategy to block EAE. This treatment eliminates all Vβ8.2-expressing cells, irrespective of the Vα 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 Vβ 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-Vβ8.2 (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 Vβ8-specific antibody (F23.1) (21) or a Vβ8.2, Vβ8.3-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-Vβ13 and anti-Vβ8.2 resulted in an enhanced reduction in EAE incidence when compared with injection of Vβ8.2 alone (25% to 5%). This result suggests that the failure of anti-Vβ8.2 to protect animals completely from EAE was due, at least in part, to the continued presence of MBP-reactive $V\beta13^+$ T cells. One animal in our study with double antibody treatment developed a delayed outbreak of tail paralysis. It is likely that rare $V\beta8.2^-$, $V\beta13^-$, 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-Vβ8.2 or anti-Vβ13 (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 NH_2-terminal epitope and that none of these hybridomas express either Vβ8.2 or Vβ13 TCRs.

We have also demonstrated that anti-Vβ 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, Vβ and/or Vα-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., Vβ8.2 or Vβ13) do not appear to immunologically compromise the animal, the generation of antibodies to the 22 different murine Vβ 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 Vβ 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 NH_2-terminal epitope and involves T cells expressing either Vβ8.2 or Vβ13 gene segments. Animals treated with a TCR Vβ8-specific mAb have a reduced incidence of EAE. We report here that the in vivo administration of a combination of anti-Vβ8.2 and anti-Vβ13 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 Vβ-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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