The Involvement of T Cell Receptor Peptide-specific Regulatory CD4+ T Cells in Recovery from Antigen-induced Autoimmune Disease

By Vipin Kumar and Eli E. Sercarz

From the Department of Microbiology and Molecular Genetics, University of California, Los Angeles, California 90024

Summary

Experimental allergic encephalomyelitis (EAE) is a prototype for CD4+ T cell–mediated autoimmune diseases. Immunization with myelin basic protein (MBP) in B10.PL mice results in EAE, and a majority of animals recover permanently from the disease. Most MBP-reactive encephalitogenic T cells recognize an immunodominant NH_2-terminal peptide, Ac1-9, and predominantly use the T cell receptor (TCR) $V\beta8.2$ gene segment. Here we report that in mice recovering from MBP-induced EAE, peripheral T cells proliferate in response to a single immunodominant TCR peptide from the $V\beta8.2$ chain (amino acids 76–101), indicating natural priming during the course of the disease. Cloned T cells, specific for this TCR peptide, specifically downregulate proliferative responses to Ac1-9 in vivo and also protect mice from MBP-induced EAE. These regulatory T cells express CD4 molecules and recognize a dominant peptide from the TCR variable framework region of $V\beta8.2$, in the context of the major histocompatibility complex class II molecule, $I-A^u$, and predominantly use the TCR $V\beta14$ gene segment. This is the first demonstration of the physiological induction of TCR peptide–specific CD4+ T cells that result from MBP immunization and that are revealed only during the recovery from disease. The downregulation of disease-causing T cells by TCR peptide-specific T cells offers a mechanism for antigen-specific, network-induced recovery from autoimmune disease.

Experimental allergic encephalomyelitis (EAE)[1] is a T cell–mediated autoimmune demyelinating disease of the central nervous system that can be induced after immunization with myelin basic protein (MBP) or its peptide fragments. TCRs of MBP-reactive T cells in several strains of mice (1–3) and rats (4) have been shown to be encoded by a limited set of V region gene segments. After immunization with MBP, most CD4+ T cells in B10.PL or PL/J mice recognize the immunodominant NH_2-terminal fragment Ac1-9 and a majority of CD4+ T cells use the TCR $V\beta8.2$ gene segment (2, 3). The limited use of TCR V genes in response to MBP has allowed the use of mAbs to $V\beta8$ in vivo to successfully prevent EAE (2, 3).

It has been shown that vaccination with disease-causing MBP-reactive CD4+ T cells protects rats from EAE (5, 6). This treatment is thought to depend on regulatory responses raised to the V region of the TCR. More recently, it has been shown that rats immunized with TCR peptides from the CDR II region (amino acids [aa] 39–59) of the TCR β or the CDR III region of the TCR α chain of encephalito-

genic T cells are protected from the disease (7–9). However, it remains to be established whether the protection from EAE by the TCR peptide can be mediated by regulatory T cells that are physiologically induced after MBP immunization (10). On the other hand, it has been noted that TCR peptide immunization may lead to the exacerbation of EAE in rats (11). The characterization of TCR peptide–reactive T cells at the clonal level and their physiological role during the course of EAE has not been clearly established. Moreover, the involvement of anti-TCR responses in the murine model of EAE has not been shown.

We have studied the dynamics of the spontaneous physiological induction of anti-TCR peptide responses during EAE in B10.PL mice. Antigen-induced disease in B10.PL mice is acute but transitory, in that a majority of mice recover permanently from EAE. We reasoned that if TCR peptide-specific responses were involved in the regulation of MBP-reactive effector T cells, TCR-reactive T cells might well become primed during recovery from MBP-induced EAE without the necessity for external challenge by TCR peptides. In fact, we demonstrate here that the peripheral T cells from mice recovered from MBP-induced EAE proliferate to the dominant TCR peptide B5 (aa 76–101) from framework region III of the $V\beta8.2$ chain. We have isolated $V\beta14$-expressing

T cell clones specific for B5. These T cells are CD4$^+$, CD8$^-$, and are MHC class II restricted. In adoptive transfer experiments, the B5-reactive T cell clones specifically inhibit proliferative responses to Ac1-9 and protect mice from MBP-induced EAE. Thus, our study clearly demonstrates the antigen-dependent, in vivo priming of regulatory T cells with specificity for a dominant TCR peptide determinant in animals recovering from EAE.

Materials and Methods

Mice. B10.PL mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred under specific pathogen-free conditions in our own colony. Female mice were used at 8–14 wk of age.

Antibodies. For staining TCR peptide–specific T cells, the following mAbs were used: anti-CD4-PE and anti-CD8-FITC (GK1.5 and 53-6, respectively, from Becton Dickinson & Co., Mountain View, CA), anti-Vβ14 (14-2, rat IgM; 12), and anti-Vβ3 (KJ-25; 13). All of the other tested anti-TCR-Vβ-specific antibodies, anti-TCR Vβs 2 (B20.6), 5 (MR9-4), 6 (RR4-7), 7 (TR310), 9 (MR10-2), 10 (B21.5), 11 (RR3-15), 13 (MR12-3), and Vβ17 (KJ-23), were from Pharmingen (San Diego, CA). MHC restriction of TCR-specific T cells was determined using anti-I-A^u (10-2-16), anti-I-E^u (14.4.4S), anti-CD4 (GK1.5), and anti-CD8 (53.6.72) antibodies. Purified anti-CD3 (145.2C11) (14) and anti-Vβ8.2 (F23.2) (15) antibodies were used for TCR crosslinking experiments. The hybridomas producing anti-Vβ8.2 and anti-Vβ14 antibodies were generously provided by Drs. Michael Bevan (University of Washington, Seattle, WA) and David Raulet (University of California, Berkeley, CA), respectively.

TCR Peptides. TCR peptides were synthesized by S. Horvath (Caltech, Pasadena, CA) using a solid phase technique on a peptide synthesizer (430A; Applied Biosystems, Inc., Foster City, CA) and were purified on a reversed phase column by HPLC (16). TCR Vβ8.2 chain peptides correspond to the sequence predominantly used in the MBP-specific response in B10.PL mice (2) and are as follows (single-letter amino acid code): B1, EAAVTQSPRNKVAVTGGK-VTLSCNQTNNHNL (1–30,L); B2, LSCNQTNNHNNMYWY-RQDTGHGLRLIHYSY (21–50); B3, HGLRLIHYSYGAGST-EKGDIPDGYKASRPS (41–70); B4, PDGYKASRPSQENFSLIL-ELATPSQTSVYF (61–90); B5, LILELATPSQTSVYFCASGDA-GGGYE (76–101).

Lymph Node and Splenic Proliferation Assay. Lymph nodes or spleens of mice were removed 10 or 30 d after immunization and a single cell suspension was prepared. Lymph node cells (4 × 10^5 cells per well) and splenocytes (8 × 10^5 cells per well) were cultured in 96-well microtiter plates in 200 μl of serum-free medium (HL-1; Ventrex, Portland, ME) supplemented with 2 mM glutamine; peptides were added at concentrations ranging from 0.1 to 7 μM final concentration. Proliferation was assayed by addition of 1 μCi [^{3}H]thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 h of a 5-d culture, and incorporation of label was measured by liquid scintillation counting.

Antibody-dependent Proliferation. 96-well plates were coated with 50 μl of a solution of goat anti–hamster or goat anti–mouse antibody (10 μg/ml; Southern Biotechnology Associates, Birmingham, AL) in PBS at 37°C for 1 h. After washing the wells two to three times with PBS, various concentrations of purified anti-CD3 (145.2.C11; 14) or anti-Vβ8.2 (F23.2; 15) antibodies were added to the wells in a total volume of 50 μl. Plates were incubated at 37°C for 90 min and washed twice with PBS. Lymph node T cells

(2 × 10^5) were added to wells in a final volume of 200 μl of serum-free medium (HL-1) in triplicate. 2 d later, 1 μCi of [^{3}H]thymidine was added to each well. Plates were harvested 18 h later and proliferation was determined by scintillation counting.

Establishment of T Cell Lines and Clones. TCR peptide–specific T cell lines were established from B10.PL mice after peptide immunization as well as from mice that had recovered from EAE. Mice were immunized in the hind foot pads with 7 nmol of peptide in saline, in a 1:1 emulsion with CFA. After 10 d, the draining lymph nodes were removed and single cell suspensions were cultured at a concentration of 4 × 10^5 cells/ml in serum-free HL-1 medium. After 7–10 d of culture, the cells were washed and resuspended with antigen (0.3 μM) and syngeneic irradiated spleen cells in DMEM (supplemented with 1 mM glutamine, 1 mM pyruvate, 0.05 mM 2-ME, and 10% FCS). They were maintained by periodic restimulation with TCR peptides every 10–15 d. T cell lines, selected from mice recovered from EAE, were only reactive to TCR peptide B5.

T cell clones were isolated from TCR peptide–specific lines by the technique of two sequential limiting dilution clonings at 0.2 cells per well (17). The antigenic specificity of T cell clones was confirmed by incubating 0.5–1 × 10^5 cells per well with irradiated syngeneic spleen cells (APC) at 1–5 × 10^5 cells per well in the absence or presence of TCR peptides at different concentrations (1 nM to 14 μM). Proliferation of T cell clones was measured by [^{3}H]thymidine incorporation for the last 18 h of a 3-d culture as described above.

Adoptive Transfer Experiments with T Cell Clones Specific for B5. In adoptive transfer experiments, TCR peptide–specific T cells (B5.2 and B4.1) were activated for 3 d with peptide B5 and B4, respectively, in the presence of syngeneic spleen cells. At the end of the culture, cells were passed through Ficoll-Hypaque and the live cells washed thoroughly three times with saline, counted, and injected. 10 d later, lymph node proliferative recall responses were determined as described in the lymph node and splenic proliferation assay section.

Induction of EAE. MBP was isolated from the brains of guinea pigs (Pel-Freez Biologicals, Rogers, AR) as described (18). For induction of EAE, mice were immunized subcutaneously with 100 μg gpMBP in CFA and 0.1 μg pertussis toxin (PTX; List Biological, Campbell, CA) was injected in 200 μl saline intravenously 24 and 72 h later. Mice were observed daily for signs of EAE and until >60 d after MBP immunization. The average disease score for each group was calculated by averaging the maximum severity of all of the affected animals in the group. Also, the maximum disease score of each animal is given in parentheses. Disease severity was scored on a five-point scale (19): 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, whole body paralysis; 5, death. Onset of disease is defined as the first signs of loss of tail tone or hind limb weakness, averaged over the affected group.

Flow Cytometry Analysis. Antibodies were purified from hybridoma supernatants by protein A chromatography. Anti-Vβ14 antibody was biotinylated with NHS-LC-biotin (Pierce, Rockford, IL) according to the manufacturer's recommendations. Antibodies were used in PBS containing 1% FCS. 10^6 cells were stained with 0.5 μg of antibody in a total volume of 50 μl at 4°C for 30 min. Cells were washed twice with PBS and then resuspended in a 50 μl of a 1:50 dilution of either FITC-conjugated streptavidin or goat anti–mouse Ig-FITC (Southern Biotechnology Associates, Birmingham, AL). After 20 min at 4°C, cells were washed, fixed with 1% paraformaldehyde in PBS, and analyzed using a cytofluorograph (Becton Dickinson & Co.).

Results

Peripheral T Cells from Mice Recovering from EAE Proliferate Only to the Immunodominant TCR Peptide B5. To test the possibility that TCR peptide–specific T cells are naturally primed during recovery from EAE, we synthesized 25–30 residue–long peptides with 10–15 amino acid overlaps from both TCR chains (Vβ8.2 and Vα2.3) of a representative T cell clone predominant in the MBP-specific response in B10.PL mice (2) (see β chain peptides in Materials and Methods). The specific proliferative responses to the 10 TCR peptides were followed in splenic T cells 30 d after immunization with MBP/CFA and PTX, well past the start of recovery from EAE.

A proliferative response to a single TCR peptide, B5 (aa 76–101), was revealed in the peripheral T cells (Fig. 1 *B*). Notably, only B5-specific T cells are primed, although TCR peptides B2 (aa 21–50) and B4 (aa 61–90) from the TCR β chain and A4 and A5 from the TCR α chain were also capable of inducing vigorous T cell proliferation when injected into B10.PL mice as peptides (V. Kumar, R. Tabibiazar, H. M. Geysen, and E. Sercarz, manuscript in preparation). Thus, during the course of recovery from MBP-induced EAE, T cells reactive to a single TCR determinant (within B5) from the Vβ8.2 chain are generated in vivo, despite the presence of several other potential T cell determinants on the TCR. Thus, B5 can be considered physiologically dominant while determinants in B2 and B4 appear cryptic. Importantly, B5-reactive T cells were not detected among spleen cells from MBP/CFA/PTX-immunized mice before the onset of EAE (Fig. 1 *A*) or from unimmunized animals (Fig. 1 *C*).

B5-reactive T Cell Clones Express CD4 and Are MHC Class II Restricted. To further study the characteristics of the B5-specific T cells, we established 2 proliferative T cell lines, 3 clones, and 26 hybridomas specific for this peptide. T cell lines were selected from B10.PL mice that were immunized with B5 as well as with MBP/CFA/PTX (see Materials and Methods). Also, for comparison, T cell clones reactive to B4 (B4.1 and B4.2) were generated after immunization with B4 in CFA. These T cells were examined for MHC restriction, cell surface expression of CD4, CD8, and TCR, and their ability to modulate proliferative responses to Ac1-9 in vivo and EAE. T cell clones B5.1 and B5.2 were derived from two independent lines and were used to study downregulation of the MBP-specific response.

Earlier studies in rats have shown that CDR II region TCR peptide–induced bulk T cell lines appear to be restricted by class I MHC molecules, although they were positive for both CD4 and CD8 markers (7). However, more recently, CD4-enriched splenic cells from recovered rats have been shown to recognize the same TCR peptide from the CDR II region in a class II context (20). Staining of cloned B5-specific T cells with anti-CD4 and anti-CD8 antibodies revealed that B5.2 as well as other B5-reactive T cell clones, hybridomas, and lines only express CD4 and not CD8 molecules (Fig. 2).

Proliferation of T cell clones (B5.1 or B5.2) was blocked by anti-I-A^u (10-2-16) as well as by anti-CD4 antibodies (GK 1.5), but not by anti-I-E^u (14.4.4S) or anti-CD8 antibodies (Fig. 3). To further study MHC restriction, presentation of
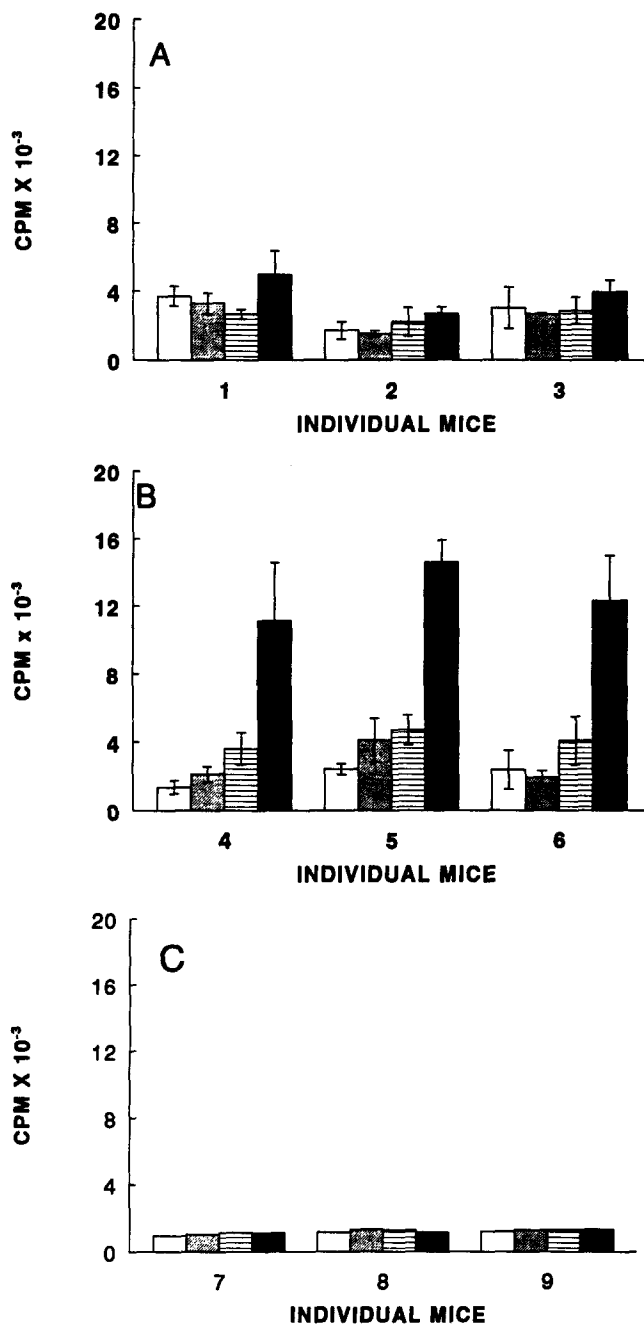
Figure 1. T cells reactive to the immunodominant TCR peptide B5 become primed in mice recovering from EAE. B10.PL mice were immunized with MBP/CFA and PTX (48 and 72 h later). 10 d (*A*) and 30 d (*B*) later, the proliferative recall responses to the immunogenic TCR peptides from the Vβ8.2 chain were tested in the spleen. Proliferation to the TCR peptides was also tested in spleen cells from unimmunized mice (*C*). Mice in the 30-d group had recovered from whole body and hind limb paralysis by the time of the assay. Responses to TCR peptides: none (□), B2 (▨), B4 (▤), and B5 (■), at an optimum concentration (3 μM) in individual mice, are shown. The data are expressed as arithmetic means ± SD of [^{3}H]thymidine incorporation (cpm $\times 10^{-3}$) in triplicate cultures. This experiment is one representative of four separate experiments.
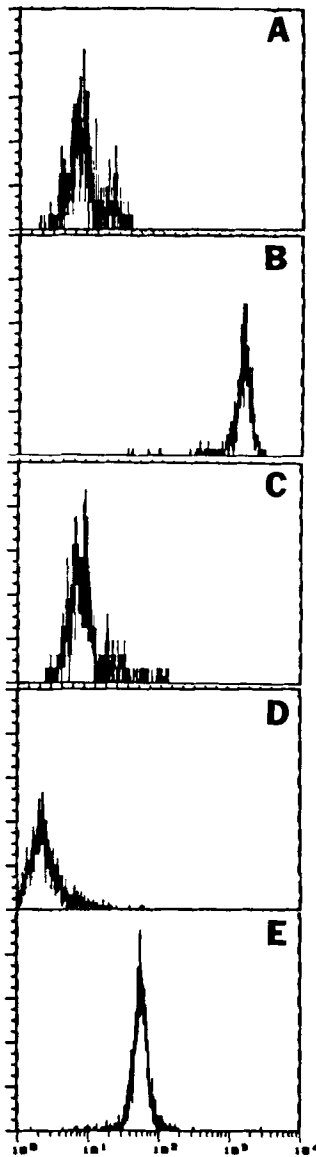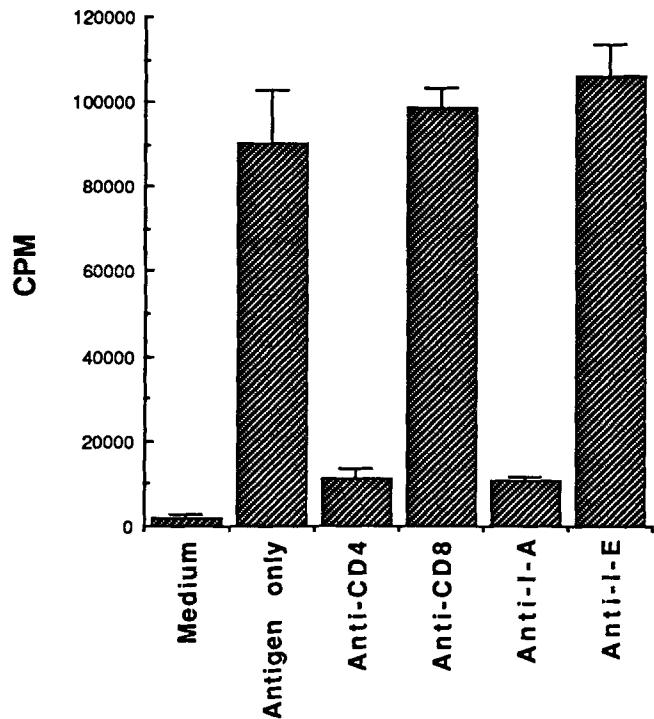
Figure 3. TCR peptide–reactive regulatory T cells are restricted by the class II MHC molecule; B5-specific proliferation of clone B5.2 is inhibited in the presence of anti-I-A^u antibodies. [^{3}H]Thymidine incorporation in response to an optimum concentration of TCR peptide B5 (0.7 μM) and irradiated syngeneic spleen cells was measured in the presence of an optimum concentration of various antibodies: anti-I-A, anti-I-E, anti-CD4, and anti-CD8.

Figure 2. B5-reactive T cells are CD4$^+$, CD8$^-$, and predominantly use the TCR Vβ14 gene segment. T cell clone 5.2 was stained with various mAbs and the fluorescence was measured by flow cytometry analysis; representative histograms are shown. (A) Goat anti–mouse Ig-FITC only; (B) anti-CD4-PE (GK1.5); (C) anti-CD8 (53-6) and goat anti–mouse Ig-FITC; (D) streptavidin-FITC only; (E) biotinylated anti-Vβ14 (14-2) and streptavidin-FITC.

TCR peptide B5 to specific T cell hybridomas was examined using L cell transfectants expressing MHC class II molecules. B5-reactive T cell hybridomas were stimulated by B5 only in the presence of L cell transfectants expressing I-A^u, and not by L cells expressing I-A^d (data not shown).

B5-specific T Cells Predominantly Use the TCR Vβ14 Gene Segment. How heterogeneous is the population of regulatory CD4$^+$ T cells? We have tested Vβ gene segment usage in these cells by staining with various available anti-TCR Vβ chain antibodies. Interestingly, B5-specific T cells predominantly use the Vβ14 gene segment, in that 26 (3 clones and 23 hybridomas) of 29 cloned T cells generated from two different animals stained positively with the anti-Vβ14 antibody (biotinylated-14-2 [12]) and no other biotinylated antibody (Fig. 2). Each of the three T cell hybridomas negative for Vβ14 used the Vβ3 gene segment (R-PE-conjugated KJ-25 (13).

TCR Peptide–specific CD4$^+$ T Cell Clones Specifically Downregulate Proliferative Responses to Ac1-9. Immunization with TCR peptide B5, but not B2 or B4, was able to specifically downregulate Ac1-9 responses in B10.PL mice (V. Kumar, R. Tabibiazar, H. M. Geysen, and E. Sercarz, manuscript in preparation). It was important to test whether the regulatory activity could be adoptively transferred with CD4$^+$ T cells that are able to proliferate to B5. T cell clone B5.2 was transferred intraperitoneally, and on the same day or a day later, mice were injected with MBP/CFA subcutaneously. After 10 d, lymph node or splenic responses were recalled in vitro with Ac1-9 (Fig. 4 *A*), gpMBP (Fig. 4 *B*), or gpMBP 43-57 (Fig. 4 *C*). Clearly, administration of B5-reactive T cell clones, but not B4-specific T cells, was able to downregulate proliferative responses to Ac1-9. Similar data were obtained with another B5-specific clone, B5.1, as well as with T cell lines. However, there was no effect on proliferative recall either to the unique xenogeneic determinants on gpMBP (Fig. 4 *B*) or to a single xenogeneic determinant, gpMBP 43-57 (Fig. 4 *C*), to which the B10.PL mouse has an equivalent response to that of Ac1-9. Furthermore, recall responses either to gpMBP or to 43-57 of gpMBP were not inhibited even when titrated to lower concentrations (0.1 μM). Responses against xenogeneic determinants served as internal controls for any
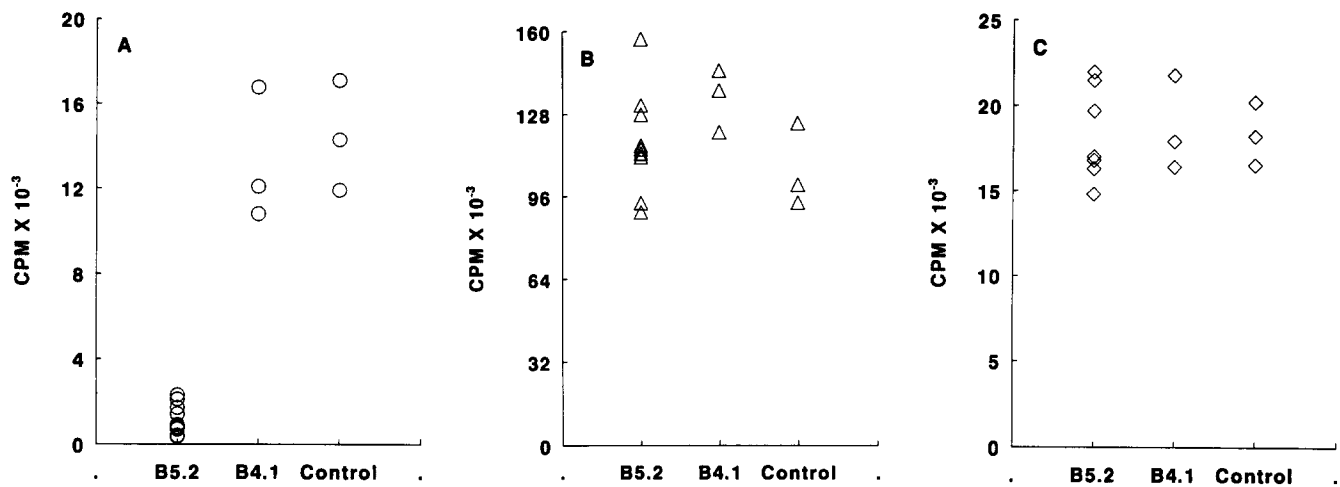
Figure 4. TCR peptide–reactive T cell clone B5.2 specifically downregulates proliferative responses to Ac1-9 in B10.PL mice. Groups of mice were immunized intraperitoneally with the activated B5.2 T cell clone (four animals, 5 × 10⁵ cells; five animals, 1 × 10⁵ cells), B4.1 T cells (5 × 10⁵ cells), in 0.5 ml saline and saline only (*Control*). The next day all the animals were immunized subcutaneously with 100 μg guinea pig MBP in CFA. 9–10 d later, lymph node proliferative responses to Ac1-9 (*A*) or to the xenogeneic determinants on MBP (*B*) or to a single xenogeneic determinant of gpMBP, p43-57 (*C*), were measured at an optimum concentration of antigen (7 μM). Background incorporation with medium alone ranged from 754 to 2,178 cpm. The data are expressed as arithmetic means of [³H]thymidine incorporation (cpm ×10⁻³) in triplicate cultures. These data are representative of three separate experiments.

generalized, nonspecific effect on proliferation and clearly argue against ergotypic regulation (21) in this model. It is noteworthy that the downregulation was efficient after transfer of as few as 10⁵ B5-specific T cells.

Regulatory T Cells Do Not Anergize All Vβ8.2⁺ T Cells. We have asked whether the B5-reactive T cells induce inactivation of all T cells expressing the Vβ8.2 receptor. Since B10.PL T cell proliferative responses that predominantly use the Vβ8.2 gene segment other than to Ac1-9 are not known, using mAbs to Vβ8.2 as well as to CD3, we have studied proliferation induced by receptor crosslinking. Clearly, lymph node T cells from mice transferred with B5.2 clones proliferated to a similar extent in comparison to lymphocytes from mice receiving saline only (Fig. 5). In four separate experiments, although the incorporation varied, there was no change in the profile. This suggests that B5-reactive T cells did not anergize or inactivate a majority of Vβ8.2⁺ T cell population. This is in contrast to the result with a CDR II region Vβ8 peptide that induced clonal unresponsiveness in all Vβ8.2⁺ T cells in DBA 2 or (PL/J × SJL)F₁ mice (23).

B5-specific CD4⁺ T Cell Clones Protect Mice from MBP-induced EAE. To determine the physiological significance of regulatory T cell clones in modulation of EAE, after transfer of 10⁵ B5.2 cells, mice were tested to see if they were protected from MBP-induced EAE. In two separate experiments, a total of 16 mice that had received activated, B5-specific cloned T cells were protected significantly from EAE (Table 1). In contrast, parallel experiments with adoptively transferred B4-reactive T cells (B4.1) showed no effect on the course of the disease (Table 1). Apparently, the response to the xenogeneic determinants on guinea pig MBP is of little or no consequence for the disease process.

Discussion

EAE serves as a prototype for T cell–mediated autoimmune diseases. Although a great deal is known about the induction of EAE in experimental animals, little is understood about
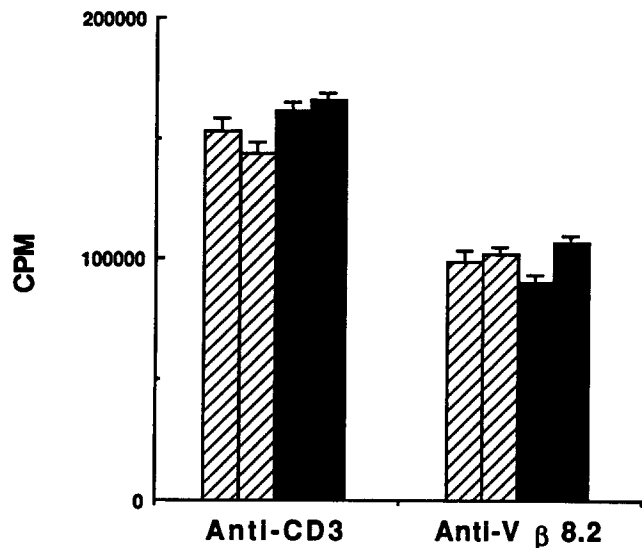

Figure 5. B5 peptide–specific T cells do not induce generalized clonal unresponsiveness in Vβ8.2⁺ T cells. TCR antibody–induced proliferation of lymph node T cells from B10.PL mice were transferred with clone B5.2 in saline (▨) or saline only (■), as in the legend for Fig. 4. TCR crosslinking experiment using plate-bound anti-Vβ8.2 or anti-CD3 (see Materials and Methods) was performed on the same day as proliferation to Ac1-9. The data from groups of two mice each are expressed as arithmetic means of [³H]thymidine incorporation (cpm ×10⁻³) in triplicate cultures. These data are representative of four separate experiments.

Table 1. *Regulatory T Cell Clones Protect Mice from MBP-induced EAE*

Exp.	Treatment*	Mice with EAE/total mice	Average score (individual scores)	Time of onset
				d
1	B5.2 T cells	0/6	N/A	N/A
	B4.1 T cells	4/6	2.7 (4,3,3,1)	20
2	None	4/5	3.0 (5,3,3,1)	20
	B5.2 T cells	1/10	1.0	21
	B4.1 T cells	9/10	3.2 (5,5,5,4,3,2,2,2,1)	19

* Age-matched female B10.PL mice were treated intraperitoneally with activated and Ficoll-Hypaque–purified TCR peptide–specific T cells (B5.2, 1–5 × 10^5 cells; and B4.1, 5 × 10^5 cells) in 500 μl saline. 24 h later, EAE was induced with MBP as described in Materials and Methods.

the physiological mechanisms involved in regulation of MBP-reactive T cells in vivo. This study takes an initial step towards analyzing anti-TCR responses in murine EAE and their possible role in the regulation of the MBP-specific response. We have studied the dynamics of the spontaneous induction of an anti-TCR peptide response during MBP-induced EAE. We have demonstrated the antigen-dependent, in vivo priming of anti-TCR regulatory T cells in B10.PL mice recovering from EAE. We have shown that downregulation by these CD4+ T cells is determinant specific. The physiological relevance of these findings was confirmed by adoptive transfer experiments, where cloned T cells prevented MBP-induced autoimmunity.

The regulatory T cells reactive to the TCR-peptide B5 (aa 76–101) are primed naturally during the course of MBP-induced disease, despite the presence of many other potential T cell determinants on both the TCR α and β chains. Importantly, only T cells reactive to the immunodominant peptide (B5) are capable of modulating anti-MBP responses while T cells specific for other TCR peptides, for example, B4 (61–90) or B2 (21–50), are not. It may be that B2 and B4 are cryptic determinants in that they can induce T cells upon immunization as peptides but fail to do so naturally in the context of the whole TCR molecule and therefore are not part of the regulatory network. Recently, it has been reported that CDR II peptide 39–59 from the TCR Vβ8.2 chain may be a cryptic determinant because it failed to elicit regulatory T cells in Lewis rats (10). In B10.PL or (B10.PL × SJL)F$_1$ mice, the TCR Vβ8.2 peptide (B3, aa 41–70) almost encompassing the entire CDR II region (aa 39–59) did not elicit a proliferative response (V. Kumar, R. Tabibiazar, H. M. Geysen, and E. Sercarz, manuscript in preparation), although such a response has recently been shown in (PL/J × SJL)F$_1$ mice (22). Background genes between B10.PL and PL/J mice may be responsible for such differences. Therefore, CDR II region peptides from the TCR Vβ chains may not induce regulation in all strains of mice and rats.

Fine specificity mapping of the minimal determinant in B5 (aa 76–101) indicated that amino acid residues PSQTSV-

YFCA (aa 83–92) constitute a core essential for recognition by the regulatory T cells (V. Kumar, R. Tabibiazar, H. M. Geysen, and E. Sercarz, manuscript in preparation). These residues are near the COOH terminus of the Vβ8.2 chain in the putative Vβ framework region III (23) not including the D-J (CDR III) region. Interestingly, it has been suggested that residues in framework region III are relatively superficial and accessible; for example, they are involved in binding to superantigens (24). Also, autoantibodies from individuals with certain autoimmune diseases have been shown to bind to this antigen. (25).

B5-reactive T cells are of limited heterogeneity in that they predominantly use a single TCR Vβ gene segment, Vβ14. This limited diversity of TCR peptide–reactive T cells may be simply owing to their restricted specificity. It may also reflect that these T cells themselves are a highly focused target for regulation (26, 27).

This study demonstrates that the regulation of the MBP-specific response by TCR peptide B5–reactive CD4+ T cells only affects T cells using the TCR Vβ8.2 gene segment, but does not affect all such T cells. Clearly, the proliferative response to Ac1-9 was inhibited while responses to unique xenogeneic determinants on guinea pig MBP, presumably using other TCR Vβ gene segments, were not affected. Likewise, Vβ14+ regulatory T cells do not appear to downregulate all Vβ8.2+ T cells but rather those specific for Ac1-9. In contrast to a recent report that TCR peptide (CDR II region) immunization in DBA/2 or (PL × SJL)F$_1$ mice inhibited the Vβ8.2-restricted response to peptide 110–121 of sperm whale myoglobin and led to clonal unresponsiveness in all Vβ8.2+ T cells (22), adoptive transfer of B5-reactive T cells did not result in generalized anergy. These findings argue against generalized suppression or ergotypic regulation, which has been proposed in other systems (21).

The physiological induction of B5-reactive T cells during the recovery from MBP-induced EAE, and the fact that cloned CD4+, Vβ14+ T cells are capable of protecting mice from the disease even when transferred at a low number, suggest their involvement in regulating the response to the im-

munodominant Ac1-9. Thus, natural priming and very effective protection from EAE suggest that B5-specific T cells are involved in a TCR-based regulatory network responsible for the transient nature of the disease. Other investigators have found that a polyclonal, nylon wool-adherent, CD4$^+$-enriched splenic suppressor population (10^8 cells), from post-recovery rats, did not protect animals from EAE unless B cells from immunized rats were also transferred (20).

Mouse T cells do not generally express class II MHC molecules and therefore may not be able to present self-TCR peptides (28, 29). How then do the TCR peptide-reactive CD4$^+$ T cells become primed in vivo? We favor the view that professional APC, for example, TCR-specific B cells or macrophages, could pick up the TCR or TCR-Ab complex at the inflammatory sites and present TCR peptides to the regulatory CD4$^+$ T cells in the context of MHC class II. We have found that if a higher number (2–5 × 10^6/200 μl) of splenic APCs is used, the B5.2 T cell clone can be stimulated without the addition of exogenous TCR peptide B5 (unpublished observation). We are currently attempting to define the molecular form of the B5 peptide that is taken up by the splenic APCs. It should however be emphasized here that whatever the mechanism of processing and presentation of TCR peptides, B5-reactive T cells become primed in vivo in the absence of any external challenge with the peptide.

How do regulatory CD4$^+$ T cells actually downregulate the Vβ8.2$^+$ effector T cells? There are at least two possibilities: first, regulatory T cells could secrete modulating cytokines, e.g., TGF-β (30) or IL-10 (31), acting locally and directly on targets at the site of inflammation. Second, B5-reactive T cells could recruit CD8$^+$ T cells that specifically recognize peptides from either the native α or β chain of the TCR in the context of MHC class I at the surface of MBP-reactive effector T cells (32). The display of TCR peptide-class I complexes on activated Ac1-9-specific Vβ8.2$^+$

effector T cells may provide the basis for specific recognition and downregulation by CD8$^+$ T cells. Recently, CD8 knock-out or antibody-mediated depletion have demonstrated discrete effects but have not indicated an obligatory role for CD8$^+$ T cells in the recovery from EAE (33, 34). In preliminary experiments, we have found that the adoptive transfer of B5.2 T cells did not effectively downregulate the proliferative response to Ac1-9 in the recipients when CD8$^+$ T cells were deleted in vivo after anti-CD8 mAb treatment. Demonstration of this requirement for CD8$^+$ T cells in our system is consistent with a recent report of Gaur et al. (22), who showed requirement of CD8$^+$ T cells in TCR peptide-induced clonal unresponsiveness.

We have found that B5-specific regulatory cells are not revealed before the onset of disease in mice that received MBP and PTX injections. Also, we were unable to detect a proliferative response in vitro to the TCR peptide B5 in splenic cells from naive B10.PL mice. There are at least two possibilities to explain these findings. First, regulatory T cells may only become reactivated or primed after many effector T cells have been expanded during EAE. Second, these cells may be normally activated and constitute an integral part of a regulatory homeostatic mechanism maintaining tolerance to MBP, and initially may be inhibited in some way (maybe in the presence of strong adjuvants).

What is evident from our results is that during the course of activation of the disease-inducing arm of the immune response to autoantigen, networks for its downregulation are engaged. Such antigen-initiated regulation that works through recognition of dominant TCR determinants suggests the presence of preexisting idiotypic mechanisms (26). It has been suggested that certain dominant idiotypic motifs are favored in the regulatory network presumably because they can provide a necessary focus for regulation (26, 27). Thus, a TCR-based network may be responsible for the transient and self-limiting nature of autoimmune encounters.

Address correspondence to Vipin Kumar or Eli Sercarz, Department of Microbiology and Molecular Genetics, College of Letters and Science, 5304 Life Sciences Building or Molecular Sciences Building, Los Angeles, CA 90024.

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