A Pathogenic Role for Myelin-specific CD8+ T Cells in a Model for Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) characterized by plaques of infiltrating CD4+ and CD8+ T cells. Studies of MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS, focus on the contribution of CD4+ myelin-specific T cells. The role of CD8+ myelin-specific T cells in mediating EAE or MS has not been described previously. Here, we demonstrate that myelin-specific CD8+ T cells induce severe CNS autoimmunity in mice. The pathology and clinical symptoms in CD8+ T cell–mediated CNS autoimmunity demonstrate similarities to MS not seen in myelin-specific CD4+ T cell–mediated EAE. These data suggest that myelin-specific CD8+ T cells could function as effector cells in the pathogenesis of MS.

Key words: autoimmunity • central nervous system • experimental autoimmune encephalomyelitis • myelin basic protein • cytotoxic T cell

Introduction

Multiple sclerosis (MS)* is a demyelinating disease of the central nervous system (CNS) of unknown etiology that is characterized by a wide spectrum of neurological symptoms (1). Clinical problems in MS patients frequently result from upper motor neuron disease and include hyperreflexia, ataxia, spasticity, and visual defects (2). MS is believed to be an autoimmune disease because inflammatory infiltrates of the CNS contain T and B lymphocytes (3–5) and because genetic susceptibility has been linked to MHC class II genes (6). In addition, experimental autoimmune encephalomyelitis (EAE), an animal model of MS, is induced by generating T cell–mediated immunity to myelin antigens.

Oligoclonal CD4+ and CD8+ T cell populations have been detected within MS plaques, however, the function and antigenic specificity of these T cells is not known (7, 8). Attention has focused on CD4+ T cells because susceptibility to MS is associated with MHC class II genes (9–11) and CD4+ T cells are critical to induction of EAE. In addi-

tion, a subset of TCR V gene sequences detected within MS plaques have been reported to express TCR sequence motifs similar to peripheral CD4+ T cells specific for myelin basic protein (MBP; reference 7). CD4+ T cells may not be the only cells that mediate MS pathology, however, as depletion of CD4+ T cells in MS patients made only minor improvements in relapse rates or levels of MRI activity (12, 13).

We chose to investigate the role of CD8+ T cells in CNS autoimmunity for several reasons. First, CD8+ T cell clones specific for myelin antigens have been isolated from the periphery of MS patients and normal donors (14, 15). Second, MHC class I genes are in linkage disequilibrium with the MHC class II genes associated with susceptibility to MS (16). Third, the ability of CD8+ myelin-specific T cells to function as effector cells in CNS autoimmunity has not been investigated. Earlier studies of EAE in CD8-deficient mice suggested that CD8+ T cells with undefined antigen specificity may function as suppressor/regulatory T cells in CNS autoimmune disease (17–19). CD8+ T cells derived from rodents fed MBP also suppress in vitro immune responses to MBP and protect animals from induction of EAE via a TGF-β–mediated pathway (19). Here we show that MBP-specific CD8+ T cells isolated from wild-type mice do not function as regulatory T cells but instead mediate severe CNS autoimmunity. The disease induced by CD8+ MBP-specific T cells exhibits some similarities to MS not seen in myelin-specific CD4+ T cell–mediated EAE.

Materials and Methods

Mice. C3HeB/FeJ, C3HeB/FeJ-MBP[shi/+], C3HeB/FeJ-*lpr*, and C3HSmn.C-*scid*/J were purchased from The Jackson Laboratory. All mice were housed in a specific pathogen free animal facility at the University of Washington. All procedures have been approved by the animal care and use committee of the University of Washington.

Peptides. HPLC-purified MBP79-87 peptide was purchased from Research Genetics.

Immunization and Isolation of MBP-specific T Cell Clones. 4–6-wk-old C3H and C3H.*shi* mice were immunized in the flank with 50 μg MBP79-87 peptide emulsified in Complete Freund's adjuvant. Draining lymph nodes from four immunized C3H and C3H.*shi* mice were harvested 10 d later and 3×10^7 draining lymph node cells were restimulated in vitro with 10^7 MBP 79–87-pulsed splenocytes and 10 U/ml IL-2 (Hoffman-La Roche) in 10 mls RPMI supplemented with 10% FCS. After 1 wk in culture, T cell lines were cloned by limiting dilution by culturing 10^4, 10^3, and 10^2 T cells with 10^6 syngenic spleen cells, 10^4 L cells infected with Vaccinia virus expressing MBP, and 50 U/ml IL-2 per well in 200 μl final volume. T cell clones from positive wells were then restimulated, expanded in 24-well plates, and tested in a ^{51}Cr release assay (20).

Flow Cytometry. T cell clones were stained with FITC-labeled anti-TCR-αβ, biotin-conjugated anti-CD8, and PE-labeled anti-CD4 mAbs (BD PharMingen). Biotin-conjugated anti-CD8 was visualized with streptavidin-conjugated Tricolor (Caltag). Data were collected on a FACScan™ (Becton Dickinson) and analyzed using CELLQuest™ software (Becton Dickinson).

T Cell Adoptive Transfers. For disease induction experiments using intravenous transfer of MBP–CTL, recipient mice were sublethally irradiated (400 rads) on day −1. 2×10^7 T cells that had been stimulated for 2 d in vitro were transferred intravenously on day 0. 10^4 U IL-2 were administered intraperitoneally at the time of T cell transfer. For disease induction experiments using intrathecal transfer, 10^6 T cells were transferred (4 d after in vitro stimulation) with or without neutralizing reagents intrathecally into T4/5 lumbar section of the spinal cord of recipient mice in 20 μl PBS. An additional dose of neutralizing reagents was intrathecally injected on day 1. Cytokine blocking experiments used 40 ng of anti–IFN-γ (Clone H22, provided by Dr. R. Schreiber) or hamster IgG anti-TNP (BD PharMingen); 100 ng TNFR:Fc (Immunex) or human IgG1 (Sigma-Aldrich) per injection.

Clinical Scoring Scale for CTL-induced CNS Autoimmunity. Clinical scoring scale for CTL-induced CNS autoimmunity was as follows. Grade 1, ataxia, loss of coordinated movement, and spastic reflexes seen in only one rear leg, with spinning and head-tilt in some mice; Grade 2, a loss of coordinated movement and spastic reflexes of both rear legs; Grade 3, paralysis of one leg (frequently sensation is retained); Grade 4, paralysis in two legs (frequently sensation is retained); and Grade 5, moribund. All Grade 5 mice were killed.

Histologic Analysis of Brain and Spinal Cords. Brain and spinal cord of 15 recipient mice of MBP–CTL (affected) and eight recipient mice of VAC-CTL (control) were dissected, fixed in 10% Millonig's modified phosphate-buffered formalin for 48 h, sectioned, and embedded in paraffin. To ensure reproducibility, a rodent brain matrix (Electron Microscopy Sciences) was used for all brain sectioning. 4–6 serial sections of five brain cross-sections (20–30 total sections), two cross-sections of the spinal cord (T2, L2) and three longitudinal sections (C1-6, T3-13, L3-cauda equina) per mouse were stained with hematoxylin and eosin (H&E), Luxol Fast Blue (LFB), Crystal Violet, and anti-GFAP (Vector Laboratories). Sections from other major organs from these mice were stained with H&E.

Results

MBP-specific CD8+ T Cells Circulate in the Periphery in Wild-Type Mice. Previously we demonstrated that MHC class I–restricted, MBP-specific CD8+ T cells can be isolated from C3H.*shi* (MBP-deficient) mice primed with recombinant virus encoding MBP (20). The CD8+ CTLs are specific for a single epitope, MBP79-87, associated with the K^k MHC molecule. In this study MBP79-87–specific T cells were not readily detected in primary T cell lines derived from C3H wild-type mice infected with virus expressing MBP. This observation indicated that the MBP79-87–specific T cells normally undergo substantial tolerance induction in vivo, similar to many T cells specific for MHC class II–restricted myelin epitopes (21–24).

To determine whether some MBP-specific CTLs escape tolerance, we immunized wild-type C3H and MBP-deficient C3H.*shi* mice with MBP79-87 peptide and cloned T cells from the draining lymph nodes by limiting dilution. Six independent MBP-specific T cell clones were established from C3H wild-type mice and 10 T cell clones were established from C3H.*shi* mice using this protocol. All T cell clones derived from C3H and C3H.*shi* mice were of the CD8α/β+ TCR α/β+ lineage (Fig. 1 A and data not shown). All wild-type clones lysed H-2K^k expressing, MHC class II–deficient target cells infected with recombinant vaccinia virus encoding MBP as well as target cells coated with MBP79-87 peptide in a similar dose-dependent manner as T cell clones derived from C3H.*shi* mice (Fig. 1, B and C). These data indicate that CD8+ MHC class I–restricted MBP-specific cytotoxic T cells circulate in the periphery of wild-type mice and exhibit the same sensitivity to antigen as T cells from MBP-deficient mice.

MBP-specific CD8+ T Cells Induce CNS Autoimmunity. To investigate whether CD8+ T cells could induce CNS autoimmunity, activated MBP-specific CD8+ T cell clones isolated from C3H wild-type mice (MBP-CTLs) were transferred intravenously into recipient mice. Both C3H wild-type and C3H.*scid* recipient mice exhibited pronounced neurological disease. The clinical symptoms were primarily associated with upper motor neuron impairment including ataxia, spastic reflexes, loss of coordinated movement, spinning, and head-tilt, with some mice developing hind limb paralysis. Mice often lost motor function in their hind legs yet retained sensory feelings. Because the clinical disease course is distinct from classic EAE in which the primary symptom is ascending flaccid paralysis, we developed a new scale for scoring the severity of clinical signs (see Materials and Methods). The disease course was rapid and severe in both wild-type and C3H.*scid* mice, with significant weight loss and a high incidence of mortality observed
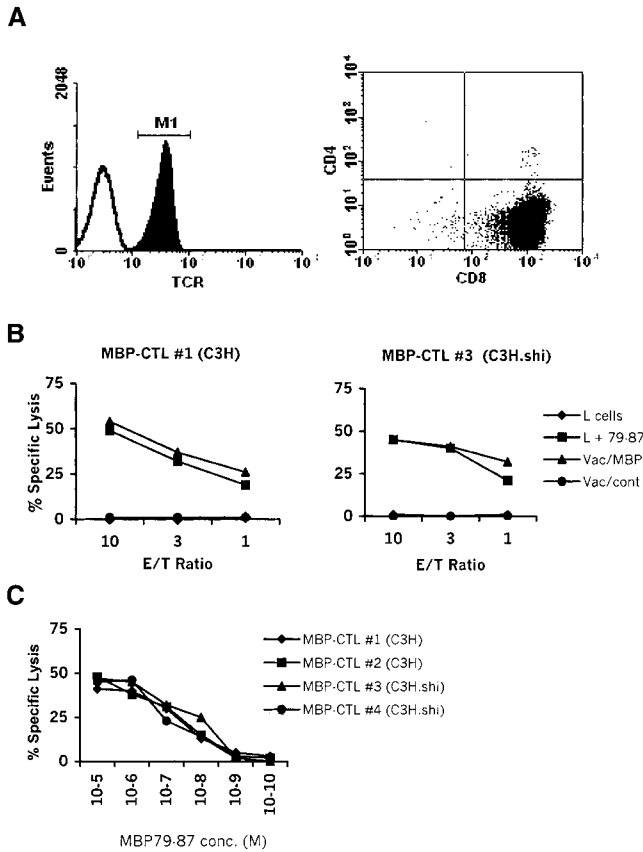
Figure 1. MBP-specific CD8^{+} cytotoxic T cells are present in wild-type mice. (A) T cell clones derived from C3H wild-type mice express an α/β^+ TCR as well as CD8. All MBP79-87 T cell clones isolated from C3H and C3H.*shi* mice were of the CD8^{+} TCR α/β^+ lineage. Data is shown for the T cell clone used for disease transfer experiments. (B) MBP-specific CD8^{+} T cells derived from wild-type mice lyse MBP-expressing target cells and (C) target cells coated with MBP79-87 peptide in a similar dose response as T cell clones derived from C3H.*shi* mice in a standard ^{51}Cr release assay. An effector to target (E/T) ratio of 10:1 was used for the peptide dose response. Data shown is representative of six independent MBP-specific T cell clones isolated from C3H wild-type mice and 10 T cell clones isolated from C3H.*shi* mice.

(Fig. 2). Wild-type mice receiving vaccinia-specific CTLs or C3H.*shi*$^{-/-}$ mice receiving MBP–CTLs were clinically normal and experienced no weight loss.

The clinical signs described previously are very distinct from those seen in CD4^{+} T cell–mediated disease. Histologic analyses shown in (Fig. 3) also revealed striking differences between MBP-specific CTL-mediated autoimmune disease and EAE in both the distribution and type of lesions in the CNS. Lesions of clinically affected mice that received MBP–CTLs were confined to the brain and involved small blood vessels and the surrounding nervous tissue. These lesions were most frequent within the white matter of the cerebellum (Fig. 3 B), but widely scattered, focal involvement of gray (Fig. 3 J) and white matter of the brain stem, midbrain, and cerebral cortex was also observed. Focal inflammation of the meninges with involvement of adjacent nervous tissue occurred in some severely affected animals. Spinal cord lesions were not seen in this series of animals.
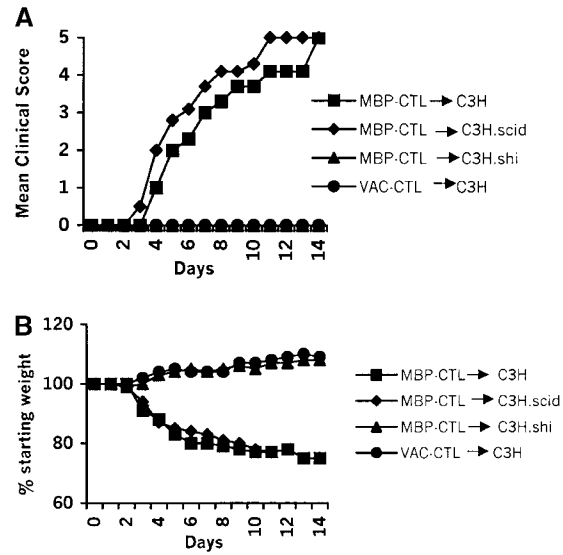

Figure 2. MBP-specific CTLs isolated from C3H wild-type mice transfer CNS autoimmunity. (A) Mean clinical score of recipient mice after transfer of MBP–CTL or Vaccinia-specific CTLs (VAC–CTL) into C3H, C3H.*scid*, or C3H.*shi* recipient mice. (B) MBP–CTLs induced severe weight loss in recipient mice expressing endogenous MBP. The mean clinical score and weight loss is the average of three independent experiments using the same T cell clone. Of two independent T cell clones studied, both induced similar CNS disease. Incidence of disease after transfer of MBP–CTLs into recipient mice was 17/19 in C3H, 12/13 in C3H.*scid*, 0/15 in C3H.*shi*, and 0/14 after transfer of VAC–CTLs into C3H.

The distribution of lesions in mice receiving MBP–CTLs at various times post disease onset is summarized in Table I. The average number of lesions in each anatomical compartment per slide did not vary significantly between mice at different time points after disease onset with the cerebellum having an average of six independent lesions per slide, the brain stem with two, the midbrain with four, and the cerebral cortex with two lesions per slide.

The vascular lesions in MBP-specific CTL-mediated disease involved capillaries and venules and consisted of perivascular cuffing with rare disruption of the vascular wall. The perivascular cuffs were composed of mixtures of lymphocytes, macrophages, and a few neutrophils (Fig. 3 C). Inflammatory cells were focally marginated to the prominent endothelial surface of cuffed vessels. The changes seen in nervous tissue surrounding these affected vessels were consistent with focal cytotoxic or ischemic injury, although blood clots were not observed. These changes included the presence of degenerative, necrotic, and apoptotic cells (Fig. 3, D, G, and H) and loss of staining intensity (Fig. 3, B and F). Few inflammatory cells were present outside of the perivascular cuff. Vacuolation of the surrounding nervous tissue was common and consisted of demyelination and cytoplasmic swelling (Fig. 3, F, G, and J). Astrogliosis was occasionally noted in tissues surrounding affected vessels (Fig. 3 L) although in a few instances perivascular injury manifests as a loss of all nucleated cells. Brain tissues from control mice were histologically normal (Fig. 3, A, E, I, and K). Examination of lung, liver, kidney,
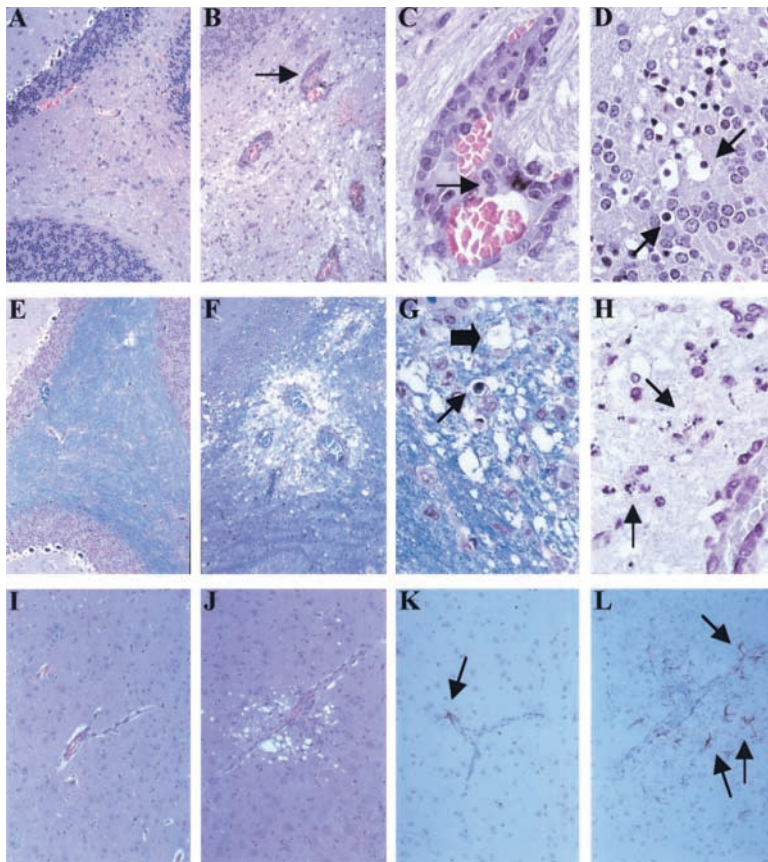
Figure 3. Histologic analysis of brain from control (VAC–CTLs transferred) and clinically affected (MBP–CTLs transferred) mice. All sections were stained with H&E unless otherwise noted. (A) Section of cerebellum from a control mouse. The darkly stained nuclei of the granular layer outline the central white matter. (B) Multiple vessels within the cerebellar white matter of an affected mouse are prominent due to perivascular cuffing. Loss of staining intensity of the surrounding tissue is evident. The arrow designates a vessel shown at a higher magnification in C. (C) Venule with a perivascular cuff composed of lymphocytes and macrophages. The arrow indicates one of several inflammatory cells marginated to the endothelium. (D) Apoptotic granular cell nuclei (arrows) in a zone peripheral to an involved venule similar to those in B. (E) Section of cerebellum from a control mouse stained with LFB. (F) LFB-stained section of an affected mouse demonstrates myelin loss in an area similar to that in B. (G) LFB-stained section of white matter showing loss of myelin around an axon remnant (large arrow) and an apoptotic glial cell nucleus (small arrow). (H) Crystal Violet–stained section demonstrates fragments of nuclear debris (arrows) in a focus of cerebellar white matter adjacent to an affected vessel. (I) Section of normal gray matter from a control mouse. (J) Section of gray matter from an affected mouse showing perivascular cuffing and vacuolation. (K) Immunohistochemical detection of GFAP demonstrates a solitary astrocyte (arrow) adjacent to a vessel in the gray matter of a control mouse. (L) GFAP staining of a serial section to that shown in J demonstrates marked perivascular astrogliosis. Sections are shown at original magnification: 20× (A, B, E, F, I, J, K, and L) or original magnification: 100× (C, D, G, and H). The perivascular lesions shown are highly representative of lesions found in all anatomical compartments in all affected mice.

and myocardium from affected and control mice revealed no significant lesions.

Effector Mechanism of MBP-specific CTL-mediated CNS Autoimmunity. The major differences between the pathology described here and that seen in EAE are the predominance of lesions in the brain versus the spinal cord, the general lack of inflammation (except that directly associated with vascular walls) and the severe demyelination and perivascular cell death suggesting a cytotoxic or ischemic injury. It is likely that some of these differences are due to different effector mechanisms used by CD8$^+$ versus CD4$^+$ T cells. To begin to address effector mechanisms in MBP-specific CTL-mediated disease, we assessed the requirements for IFN-γ, TNF-α, and Fas–FasL interactions. Disease was induced in recipient mice by intrathecally coinjecting MBP–CTLs with and without neutralizing reagents specific for IFN-γ and TNF-α. Intrathecal transfer of MBP–CTLs alone induced a potent CNS autoimmune disease (Fig. 4 A). Clinical signs appear more rapidly after intrathecal versus intravenous transfer, presumably because the T cells do not have to traffic to the CNS from the periphery. Coinjecting a neutralizing Ab to IFN-γ with MBP–CTLs significantly reduced severity of disease compared with control mice (Fig. 4 B), indicating that IFN-γ plays an important role in CD8$^+$ T cells specific for MBP (MBP–CTL)–mediated CNS autoimmune disease. In contrast to the results with IFN-γ, coinjection of TNFR:Fc fusion protein with MBP–CTLs had no effect on disease course compared with mice receiving isotype-matched control Abs (Fig. 4 C). MBP–CTLs also do not depend on Fas–FasL signaling as CNS autoimmunity was induced in C3H.*lpr* mice that was indistinguishable from wild-type C3H mice in time course and severity (Fig. 4 D).

Table I. *Location of Lesions After Intravenous Transfer of MBP-specific CTLs*

Days after disease onset	Cerebral cortex	Midbrain	Brain stem	Cerebellum
Preonset	0/4	0/4	0/4	0/4
1–4	4/4	4/4	3/4	4/4
5–8	2/4	3/4	4/4	4/4
9–14	1/3	3/3	2/3	2/3

Data shown are the number of mice per time point with lesions in each anatomical compartment. Preonset mice were analyzed 4 d after transfer and were asymptomatic. Mice in all other groups had a clinical score of a two or greater. See paper for average number of lesions per brain section. 4–6 serial sections of each cross-section were analyzed.

Discussion

The role of CD8$^+$ T cells in MS is not known. The question is of considerable interest because significant num-
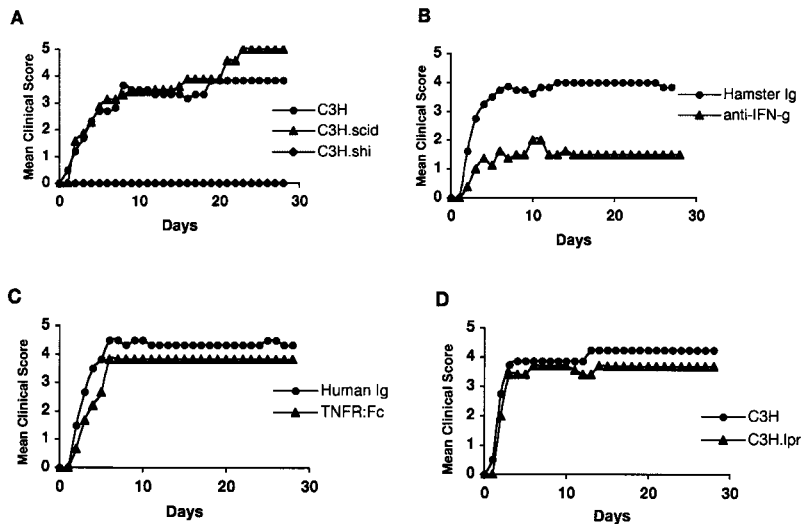
Figure 4. Mechanism of MBP-specific CTL-mediated autoimmunity. Mean clinical scores are shown for recipient mice after intrathecal transfer of MBP–CTLs. Recipient mice are (A) C3H, C3H.*scid*, or C3H.*shi* mice, (B) C3H mice coinjected with anti–IFN-γ or control Ab, (C) C3H recipients coinjected with TNFR:Fc fusion protein or control Ab, and (D) C3H or C3H.*lpr* mice. These experiments have been repeated twice. Incidence of disease was 35/43 for C3H mice; 14/19 for C3H.*scid* mice; 0/24 for C3H.*shi*; 14/18 for C3H plus anti–IFN-γ; 15/17 for C3H plus hamster IgG; 7/9 for C3H plus TNFR:Fc; 7/10 for C3H plus human IgG; and 9/10 for C3H.*lpr* mice.

bers of CD8^{+} T cells of unknown function and antigen specificity are present in MS plaques (3, 4, 8). Our results demonstrate that MBP-specific CD8^{+} T cells can function as effector cells to induce CNS autoimmune disease in mice. Demonstrating a pathogenic role for CD8^{+} T cells in CNS autoimmunity contrasts with observations made in EAE that suggest that CD8^{+} T cells inhibit the induction of CNS autoimmunity or alter the clinical course of disease (17–19). Finding divergent roles for CD8^{+} T cells in CNS autoimmunity suggests that functionally distinct subsets of CD8^{+} T cells contribute to disease in different ways. TGF-β–expressing CD8^{+} T cells appear to function as regulatory cells (19) while our results indicate that IFN-γ producing MBP-specific CD8^{+} T cells induce severe CNS autoimmunity. This hypothesis has a direct corollary with the role of CD4^{+} T cells in autoimmune disease. CD25^{+}CD4^{+} T cells of undefined antigen specificity perform a regulatory function in inhibiting autoimmunity (25, 26) while CD4^{+} T cells specific for CNS antigens function as effector cells in inducing EAE.

The disease mediated by adoptive transfer of CD8^{+} MBP-specific T cells described here is characterized by very different clinical signs and CNS pathology than classic EAE mediated by transfer of CD4^{+} T cells. C3H mice have been considered fairly resistant to EAE induced by active immunization, however, adoptive transfer of CD4^{+} Th1 MBP-specific T cell lines into C3H mice results in classic EAE (27). These differences between CD4 T cell– and CTL-mediated EAE may be due to recognition of different cell types within the CNS by CD4^{+} and CD8^{+} T cells and/or the different effector mechanisms employed by CD4^{+} versus CD8^{+} T cells. The MBP–CTL-mediated disease described here is independent of CD4^{+} T cells as well as nonspecific CD8^{+} T cells, as shown by the induction of disease in C3H.*scid* recipients. This result indicates that MBP-specific CTLs can directly induce CNS autoimmunity by interacting with MHC class I molecules presenting the MBP epitope within the CNS and not by activating endogenous myelin-specific CD4^{+} T cells. Interestingly,

there are some reports of "atypical" EAE in some EAE-resistant mouse strains immunized with certain PLP peptides that exhibits some similarities to the CTL-mediated disease described here (28, 29). T cells mediating this atypical EAE were not characterized and it would be interesting to determine if they were CD4^{+} or CD8^{+} T cells. In human autoimmune disease, both CD4^{+} and CD8^{+} T cells may contribute to disease, such that the pathology reflects the activities of both types of effector cells.

CD8^{+} T cell–mediated CNS autoimmunity is largely inhibited by neutralizing IFN-γ activity. IFN-γ may function to increase MHC class I expression which is extremely limited in the CNS but is upregulated on resident cells by exposure to IFN-γ (30, 31). IFN-γ may also promote demyelination through activation of macrophage and microglia or by a deleterious effect on oligodendrocytes as described for transgenic mice that express IFN-γ under the control of the MBP promoter (32). In contrast to the dependence on IFN-γ of CTL-mediated disease, IFN-γ is a protective cytokine in CD4^{+} T cell–mediated EAE (33). Interestingly, clinical trials of IFN-γ in MS resulted in significant exacerbation of symptoms in patients receiving IFN-γ as compared with controls (34) suggesting a stronger parallel for IFN-γ activity to CD8^{+} T cell-mediated CNS autoimmunity than CD4^{+} T cell–mediated EAE.

In addition to IFN-γ, TNF-α and Fas appear to play disparate roles in CD4^{+} versus CD8^{+} T cell–mediated CNS autoimmunity. Neutralizing TNF-α activity had no effect on CD8^{+} T cell–mediated CNS autoimmune disease course. This result differs from the amelioration of disease that occurs when TNF-α activity is neutralized in EAE (35), and may be more similar to MS in which an essential role for TNF-α has not been established (36, 37). CD8^{+} T cell–mediated CNS autoimmunity is also independent of Fas–FasL interactions, while these interactions have been shown to influence EAE in some mouse models (38, 39).

The observation that CD4^{+} and CD8^{+} T cells employ distinct effector mechanisms within the CNS may have im-

portant implications for understanding the pathogenesis of MS. MS exhibits heterogeneity in clinical course and CNS lesions and it has been difficult to formulate a unifying hypothesis that accounts for this heterogeneity. Recent histological studies have categorized the different types of MS lesions into separate patterns or groups. These efforts led to the suggestion that the heterogeneity in pathology and clinical outcome in MS patients reflects fundamentally different disease mechanisms (40). The MBP–CTL-mediated model that we describe here provides experimental evidence for this idea. The pathology observed in the MBP–CTL model is most similar to that of Pattern IV described by Lucchinetti et al. (41) for three patients with primary progressive MS. In these patients, inflammatory lesions centered on veins and venules and oligodendrocyte death is suggested that is reminiscent of virus or toxin-induced demyelination. Patients exhibiting pattern IV pathology may share a common pathogenic mechanism that includes CD8^{+} lymphocytes. Not all primary progressive MS patients exhibit this pattern, however, and most patients with primary progressive MS have numerous lesions in the spinal cord rather than the predominance of lesions in the brain seen in MBP-specific CTL-mediated disease. Thus, the CTL-mediated model, like CD4^{+} T cell–mediated EAE, is valuable in exploring the pathogenic potential and effector mechanisms of different T cell subsets but does not recapitulate all of the features of human disease.

One reason that accurate animal models of MS are difficult to generate is that the low precursor frequency of both CD4^{+} and CD8^{+} myelin-specific T cells in healthy animals requires artificial stimuli to induce disease and these stimuli tend to focus on activation of one particular T cell subset. Multiple factors are likely to be involved in triggering MS and variation in these factors may preferentially activate different types of myelin-specific T cells in different patients. Predisposing conditions for MS could include perturbations that impair the efficiency of tolerance induction for either MHC class I– or MHC class II–restricted T cells specific for myelin antigens. The type of perturbation may vary among patients such that the precursor frequency of one class of autoreactive T cells may increase more than another class. Therefore, identifying all of the cell types that can contribute to disease as well as the conditions under which these cells may be activated is essential to understand the pathogenesis of MS.

Our results now implicate CD8^{+} MBP-specific CTLs as well as CD4^{+} T cells as potential mediators of CNS autoimmune disease. Importantly, our data indicate that the disease mediated by MBP-specific CTLs is distinct from CD4^{+} T cell–mediated disease, supporting the idea that preferential activation of one T cell subset may emphasize different aspects of MS. The MBP–CTL-mediated model may be particularly useful in delineating events occurring during the initial stages of MS. Widespread inflammation in the CNS is a common feature of chronic MS, however, it has been suggested that demyelination may be triggered by an initial perivascular lymphocyte infiltration (40, 42). Similar to the perivascular infiltrates in the myelin-specific

CD8^{+} T cell model, CD8^{+} T cells present in MS lesions exhibit perivascular localization (3, 4). Endothelial cells as well as resident phagocytic perivascular cells (43) may be able to present MBP epitopes to CTLs via a cross-presentation pathway (44) as both cell types constitutively express MHC class I molecules. Even though endothelial cells and phagocytic antigen presenting cells do not synthesize MBP, cell-associated antigens phagocytosed by antigen presenting cells are processed through the MHC class I pathway (44). Thus, perivascular injury mediated by myelin-specific CD8^{+} T cells may recapitulate events that occur during particular stages of MS that have not been accessible for study in other animal models.

References

1. Steinman, L. 1996. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell.* 85:299–302.
2. Whitaker, J.N., and G.W. Mitchell. 1997. Clinical features of multiple sclerosis. *In* Multiple Sclerosis: Clinical and Pathogenetic Basis. C.S. Raine, H. McFarland, and W.W. Tourtellotte, editors. Chapman and Hall, London. pp. 3–19.
3. Traugott, U., E.L. Reinherz, and C.S. Raine. 1983. Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science.* 219:308–310.
4. Hauser, S.L., A.K. Bhan, F. Gilles, M. Kemp, C. Kerr, and H.L. Weiner. 1986. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol.* 19: 578–587.
5. Esiri, M.M., and D. Gay. 1997. The immunocytochemistry of multiple sclerosis plaques. *In* Multiple Sclerosis: Clinical and Pathogenetic Basis. C.S. Raine, H.F. McFarland, and W.W. Tourtellotte, editors. Chapman and Hall Medical, London. pp. 173–186.
6. McFarland, H.F., R. Martin, and D.E. McFarlin. 1997. Genetic influences in multiple sclerosis. *In* Multiple Sclerosis: Clinical and Pathogenetic Basis. C.S. Raine, H.F. McFarland, and W.W. Tourtellotte, editors. Chapman and Hall Medical, London. pp. 205–219.
7. Oksenberg, J.R., S. Stuart, A.B. Begovich, R.B. Bell, H.A. Erlich, L. Steinman, and C.C. Bernard. 1990. Limited heterogeneity of rearranged T-cell receptor Vα transcripts in brains of multiple sclerosis patients. *Nature.* 345:344–346.
8. Babbe, H., A. Roers, A. Waisman, H. Lassmann, N. Goebels, R. Hohlfeld, M. Friese, R. Schroder, M. Deckert, S.

Schmidt, et al. 2000. Clonal expansions of CD8$^+$ T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med.* 192:393–404.

9. Sawcer, S., H.B. Jones, R. Feakes, J. Gray, N. Smaldon, J. Chataway, N. Robertson, D. Clayton, P.N. Goodfellow, and A. Compston. 1996. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat. Genet.* 13:464–468.

10. Ebers, G.C., K. Kukay, D.E. Bulman, A.D. Sadovnick, G. Rice, C. Anderson, H. Armstrong, K. Cousin, R.B. Bell, W. Hader, et al. 1996. A full genome search in multiple sclerosis. *Nat. Genet.* 13:472–476.

11. Haines, J.L., M. Ter Minassian, A. Bazyk, J.F. Gusella, D.J. Kim, H. Terwedow, M.A. Pericak-Vance, J.B. Rimmler, C.S. Haynes, A.D. Roses, et al. 1996. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. The multiple sclerosis genetics group. *Nat. Genet.* 13:469–471.

12. Lindsey, J.W., S. Hodgkinson, R. Mehta, D. Mitchell, D. Enzmann, and L. Steinman. 1994. Repeated treatment with chimeric anti-CD4 antibody in multiple sclerosis. *Ann. Neurol.* 36:183–189.

13. van Oosten, B.W., M. Lai, S. Hodgkinson, F. Barkhof, D.H. Miller, I.F. Moseley, A.J. Thompson, P. Rudge, A. McDougall, J.G. McLeod, et al. 1997. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology.* 49:351–357.

14. Tsuchida, T., K.C. Parker, R.V. Turner, H.F. McFarland, J.E. Coligan, and W.E. Biddison. 1994. Autoreactive CD8$^+$ T-cell responses to human myelin protein-derived peptides. *Proc. Natl. Acad. Sci. USA.* 91:10859–10863.

15. Dressel, A., J.L. Chin, A. Sette, R. Gausling, P. Hollsberg, and D.A. Hafler. 1997. Autoantigen recognition by human CD8 T cell clones: enhanced agonist response induced by altered peptide ligands. *J. Immunol.* 159:4943–4951.

16. Bugawan, T.L., W. Klitz, A. Blair, and H.A. Erlich. 2000. High-resolution HLA class I typing in the CEPH families: analysis of linkage disequilibrium among HLA loci. *Tissue Antigens.* 56:392–404.

17. Jiang, H., S. Zhang, and B. Pernis. 1992. Role of CD8$^+$ T cells in murine experimental allergic encephalomyelitis. *Science.* 256:1213–1215.

18. Koh, D.R., W.P. Fung Leung, A. Ho, D. Gray, H. Acha Orbea, and T.W. Mak. 1992. Less mortality but more relapses in experimental allergic encephalomyelitis in CD8$^{-/-}$ mice. *Science.* 256:1210–1213.

19. Miller, A., O. Lider, A.B. Roberts, M.B. Sporn, and H.L. Weiner. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor β after antigen- specific triggering. *Proc. Natl. Acad. Sci. USA.* 89:421–425.

20. Huseby, E.S., C. Ohlen, and J. Goverman. 1999. Cutting edge: myelin basic protein-specific cytotoxic T cell tolerance is maintained in vivo by a single dominant epitope in H-2k mice. *J. Immunol.* 163:1115–1118.

21. Harrington, C.J., A. Paez, T. Hunkapiller, V. Mannikko, T. Brabb, M. Ahearn, C. Beeson, and J. Goverman. 1998. Differential tolerance is induced in T cells recognizing distinct epitopes of myelin basic protein. *Immunity.* 8:571–580.

22. Targoni, O.S., and P.V. Lehmann. 1998. Endogenous myelin basic protein inactivates the high avidity T cell repertoire. *J. Exp. Med.* 187:2055–2063.

23. Klein, L., M. Klugmann, K.A. Nave, V.K. Tuohy, and B. Kyewski. 2000. Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nat. Med.* 6:56–61.

24. Anderson, A.C., L.B. Nicholson, K.L. Legge, V. Turchin, H. Zaghouani, and V.K. Kuchroo. 2000. High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire. *J. Exp. Med.* 191:761–770.

25. Takahashi, T., T. Tagami, S. Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, T.W. Mak, and S. Sakaguchi. 2000. Immunologic self-tolerance maintained by CD25$^+$CD4$^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* 192:303–310.

26. Shevach, E.M. 2000. Regulatory T cells in autoimmunity. *Annu. Rev. Immunol.* 18:423–449.

27. Clark, R.B., M.L. Grunnet, and E.G. Lingenheld. 1997. The generation of encephalitogenic T cell lines from experimental allergic encephalomyelitis-resistant strains of mice. *Int. Immunol.* 9:1415–1422.

28. Greer, J.M., R.A. Sobel, A. Sette, S. Southwood, M.B. Lees, and V.K. Kuchroo. 1996. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. *J. Immunol.* 156:371–379.

29. Sobel, R.A. 2000. Genetic and epigenetic influence on EAE phenotypes induced with different encephalitogenic peptides. *J. Neuroimmunol.* 108:45–52.

30. Wong, G.H., I. Clark Lewis, A.W. Harris, and J.W. Schrader. 1984. Effect of cloned interferon-gamma on expression of H-2 and Ia antigens on cell lines of hemopoietic, lymphoid, epithelial, fibroblastic and neuronal origin. *Eur. J. Immunol.* 14:52–56.

31. Massa, P.T., K. Ozato, and D.E. McFarlin. 1993. Cell type-specific regulation of major histocompatibility complex (MHC) class I gene expression in astrocytes, oligodendrocytes, and neurons. *Glia.* 8:201–207.

32. Horwitz, M.S., C.F. Evans, D.B. McGavern, M. Rodriguez, and M.B. Oldstone. 1997. Primary demyelination in transgenic mice expressing interferon-γ. *Nat. Med.* 3:1037–1041.

33. Krakowski, M., and T. Owens. 1996. Interferon-γ confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol.* 26:1641–1646.

34. Panitch, H.S., R.L. Hirsch, J. Schindler, and K.P. Johnson. 1987. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology.* 37:1097–1102.

35. Sean, R.D., H. Korner, D.H. Strickland, F.A. Lemckert, J.D. Pollard, and J.D. Sedgwick. 1998. Challenging cytokine redundancy: inflammatory cell movement and clinical course of experimental autoimmune encephalomyelitis are normal in lymphotoxin-deficient, but not tumor necrosis factor-deficient, mice. *J. Exp. Med.* 187:1517–1528.

36. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. 1999. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. *Neurology.* 53:457–465.

37. van Oosten, B.W., F. Barkhof, L. Truyen, J.B. Boringa, F.W. Bertelsmann, B.M. von Blomberg, J.N. Woody, H.P. Hartung, and C.H. Polman. 1996. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor anti-

body cA2. *Neurology*. 47:1531–1534.

38. Sabelko, K.A., K.A. Kelly, M.H. Nahm, A.H. Cross, and J.H. Russell. 1997. Fas and Fas ligand enhance the pathogenesis of experimental allergic encephalomyelitis, but are not essential for immune privilege in the central nervous system. *J. Immunol*. 159:3096–3099.

39. Waldner, H., R.A. Sobel, E. Howard, and V.K. Kuchroo. 1997. Fas- and FasL–deficient mice are resistant to induction of autoimmune encephalomyelitis. *J. Immunol*. 159:3100–3103.

40. Lucchinetti, C.F., W. Bruck, M. Rodriguez, and H. Lassmann. 1996. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol*. 6:259–274.

41. Lucchinetti, C., W. Bruck, J. Parisi, B. Scheithauer, M. Rodriguez, and H. Lassmann. 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol*. 47:707–717.

42. Prineas, J.W. 1979. Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science*. 203:1123–1125.

43. Weller, R.O., B. Engelhardt, and M.J. Phillips. 1996. Lymphocyte targeting of the central nervous system: a review of afferent and efferent CNS-immune pathways. *Brain Pathol*. 6:275–288.

44. Carbone, F.R., and M.J. Bevan. 1990. Class I–restricted processing and presentation of exogenous cell- associated antigen in vivo. *J. Exp. Med*. 171:377–387.