Brain Pathology 10: 402-418(2000)

RESEARCH ARTICLE

Antibody Association with a Novel Model for Primary Progressive Multiple Sclerosis: Induction of Relapsing-Remitting and Progressive Forms of EAE in *H2*^{s} Mouse Strains

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Multiple sclerosis (MS) can be divided into 4 clinical forms: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR). Since PP-MS is notably different from the other forms of MS, both clinically and pathologically, the question arises whether PP-MS is immunologically similar to the other forms. The pathogenesis of the PP-MS remains unclear, partly due to a lack of highly relevant animal models. Using an encephalitogenic peptide from myelin oligodendrocyte glycoprotein (MOG)$_{92\text{-}106}$, we have established animal models that mimic different forms of MS in 2 strains of *H-2*^{s} mice, SJL/J and A.SW. We induced experimental allergic encephalomyelitis (EAE) using MOG$_{92\text{-}106}$ in the presence or absence of supplemental *Bordetella pertussis* (*BP*). Although, SJL/J mice developed RR-EAE whether *BP* was given or not, A.SW mice developed PP-EAE without *BP* and SP-EAE with *BP*. Histologically, SJL/J mice developed mild demyelinating disease with T cell infiltration, while A.SW mice developed large areas of plaque-like demyelination with immunoglobulin deposition and neutrophil infiltration, but with minimal T cell infiltration. In A.SW mice without *BP*, high titer serum anti-MOG antibody was detected and the anti-MOG IgG2a/IgG1 ratio correlated with survival times of mice. We hypothesized that, in A.SW mice, a Th2 response favors production of myelinotoxic antibodies, leading to progressive forms with early death. Our new models indicate that a single encephalitogen could induce either RR-, PP-, or SP- forms of demyelinating disease in hosts with immunologically different humoral immune responses.

Introduction

The major cause of demyelinating disease of the central nervous system (CNS) is multiple sclerosis (MS) (67). The spectrum of clinical disease of MS is diverse. The clinical course of MS can be classified into 4 forms: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR) (34). RR-MS is defined by disease relapses with full recovery or with sequelae. In contrast, PP-MS progresses continuously from the onset. Initial RR disease is often followed by progression (SP-MS). In contrast to RR-MS, comparatively little information is available on the clinical and pathological features and the pathogenesis of the other forms of MS, particularly those of PP-MS (10). This is partly because the definition of this disease subtype has only recently achieved some consensus (34). The mechanism of the transition and differences between the various forms are not well understood (59). Since the clinical, epidemiological, and pathological findings in PP-MS are notably different from those described for other forms of MS (41), the question arises whether the PP-MS is immunologically the same; i.e., are PP-MS and RR-MS two distinct disease entities (27, 40, 46).

Experimental allergic encephalomyelitis (EAE) can be induced in a variety of species and strains of animals using various CNS antigens (60). EAE is a useful model for MS and serves as an immunologic study of CNS inflammation and demyelination. Like MS, the clinical course of EAE can be quite variable (60). In most EAE models, however, the course can be as short as an acute monophasic event (acute EAE) to a long protracted RR process preceded by an early acute attack. Acute EAE is considered more as an animal model for acute disseminated encephalomyelitis than for MS. The pathological lesions generally seen in EAE, particularly during the

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Mice	*BP*[a]	Clinical Course[b]	Acute Disease		Chronic Disease			Death (days)[f]
			Number[c]	Score[d]	Number	Score	Onset[e]	
SJL/J	-	RR	1/22	0.2 ± 0.2	15/17	3.8 ± 0.3	45.1 ± 4.7	1/17 (32)
SJL/J	+	RR	5/8	3.8 ± 0.5	7/7	3.8 ± 0.3	45.1 ± 6.5	1/7 (81)
A.SW	-	PP	0/16	0	14/14	4.7 ± 0.2	26.4 ± 2.1	13/14 (42.8 ± 4.5)
A.SW	+	SP	3/8	0.4 ± 0.2	8/8	4.3 ± 0.7	46.3 ± 8.4	6/8 (84.8 ± 13.5)

[a] *Bordetella pertussis* intravenous injection
[b] RR – relapsing-remitting, PP – primary progressive, SP – secondary progressive
[c] Number of mice with clinical signs/total number of mice examined
[d] Mean maximum clinical score ± SEM
[e] Onset day of chronic disease
[f] Average day of death or moribund

Table 1. Clinical disease in MOG-induced EAE.

acute disease, show only inflammation without demyelination or less extensive demyelination than MS plaques. Furthermore, few EAE models have been available for chronic demyelinating disease without the preceding acute disease (53). Thus, these models do not provide an ideal system for studying the progressive clinical forms of MS: PP-, SP-, and PR-MS.

Myelin oligodendrocyte glycoprotein (MOG) belongs to the immunoglobulin (Ig) superfamily (15) and is encoded within the major histocompatibility complex (MHC) (47). MOG is detected on the surface of CNS myelin and oligodendrocytes (33). In MS, T and B cells reactive for MOG have been demonstrated in cerebrospinal fluid (CSF) and in demyelinating lesions (17, 56, 71). EAE can be induced in animals using either whole MOG or encephalitogenic peptides derived from MOG (3, 24). In MOG-induced EAE, large confluent plaque-like demyelinating lesions are frequently observed. As with the other forms of EAE, $CD4^+$ helper T (Th)1 cells appear to be essential for initiation of CNS inflammation. However, for the development of the plaque-like demyelinating lesions, anti-MOG antibodies play an important role. It has been shown that passive transfer of anti-MOG antibody can augment demyelination *in vivo* (25, 28, 31) and anti-MOG antibody can lyse oligodendrocytes *in vitro* (32).

$CD4^+$ Th cells can be divided into 2 groups depending on the types of cytokines these cells produce (1, 43). Th1 cells produce interleukin (IL)-2, interferon (IFN)-γ, and lymphotoxin, and mediate delayed type hypersensitivity (DTH) responses; Th2 cells produce IL-4, 5, and 10, and provide help for humoral immune responses. IL-4 producing $NK1.1^+$ T cells contribute to Th2 cell differentiation (65, 73). In most forms of EAE, Th1 cells or Th1-type cytokines have been shown to promote disease, while Th2 cells or Th2-type cytokines down-regulate EAE (57). In MOG-induced EAE, however, both cellular and humoral immune responses are essential for the complete expression of the disease. Therefore, Th2 cells can also play an important role in disease progression in this model.

In this paper, using two *H-2*^{s} strains of mice that have different Th phenotypes, we have established MOG-induced EAE models for the different clinical courses of MS: RR-, PP-, and SP-MS. The first mouse strain, SJL/J, is known to lack $NK1.1^+$ T cells and has a low Th2 phenotype (73). The second strain, A.SW, has more of a Th2-responding phenotype (22). For immune modulation, during sensitization for EAE, we injected mice with *Bordetella pertussis* (*BP*), an inducer of Th1 response (50). SJL/J mice showed RR-EAE, whether *BP* was given or not. In contrast, A.SW mice developed PP-EAE without *BP* supplementation, and developed SP-EAE with *BP* supplementation. In A.SW mice, we found Ig deposition in CNS tissue with high circulating anti-MOG antibody titers. The anti-MOG IgG2a/IgG1 ratio correlated with survival times of mice. We conclude that a single encephalitogen can induce RR-, PP-, or SP-forms of demyelinating disease in hosts with immunologically different backgrounds, which is dependent on the humoral immune responses.

Materials and Methods

Animal Experiments. Seven- to eight-week-old female SJL/J and A.SW mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were immunized subcutaneously in the base of the tail with

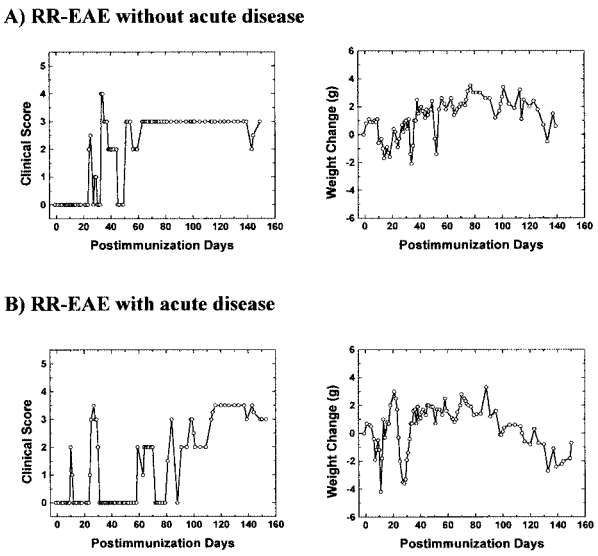


Figure 1. Clinical course of MOG-induced EAE in SJL/J mice. Mice were sensitized with MOG_{92-106} peptide in CFA with (**b**) or without (**a**) *Bordetella pertussis* (*BP*) supplementation. Mice were observed for clinical signs (left) and weight changes (right) for 5 months. Both groups developed relapsing-remitting (RR) disease. Without *BP* supplementation, most mice did not show acute disease (**a**), while half of the *BP* supplemented mice showed moderate acute disease (**b**). Shown is the clinical course of a representative animal from each group in 3 independent experiments.

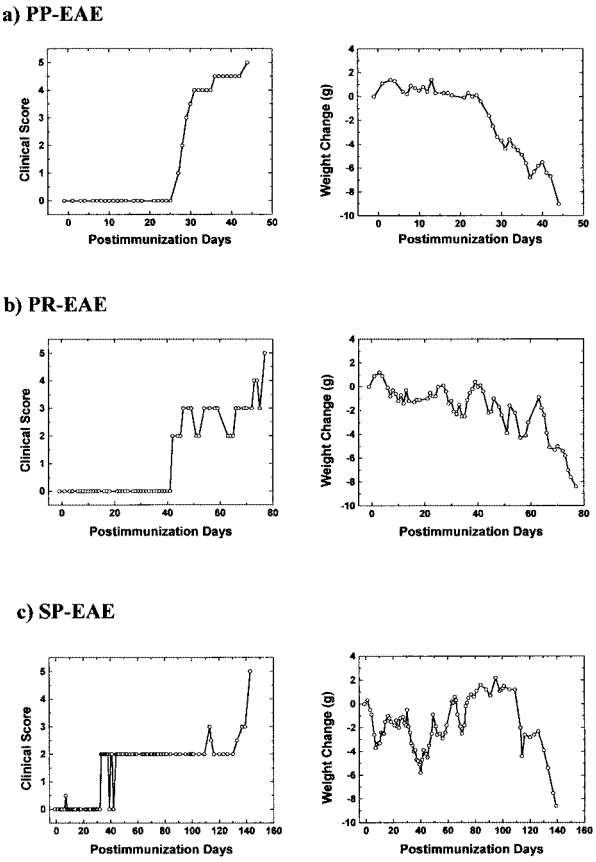
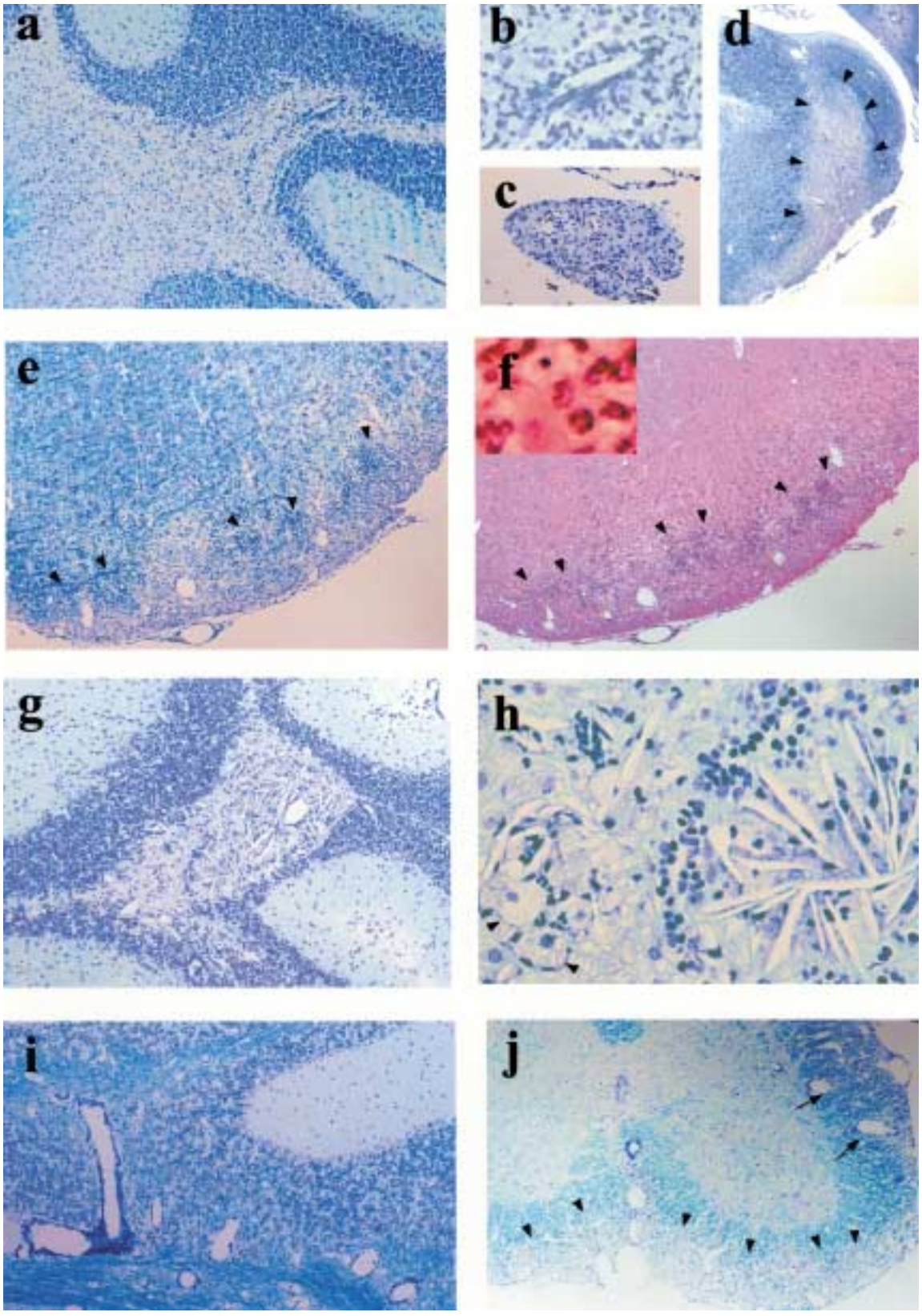


Figure 2. Clinical course of MOG-induced EAE in A.SW mice. Mice were immunized with MOG_{92-106} peptide in CFA with (**c**) or without (**a**, **b**) *Bordetella pertussis* (*BP*) supplementation. Mice were observed for clinical signs (left) and weight changes (right) for 5 months. Without *BP* supplementation, most mice developed primary progressive (PP)-EAE without acute disease (**a**), while some mice developed progressive disease with relapses, progressive relapsing (PR)-EAE (**b**). With *BP* supplementation, mice initially showed RR disease followed by progression, secondary progressive (SP)-EAE (**c**). Half of the *BP* supplemented mice showed mild acute disease. Shown is the clinical course of a representative animal of each group in 3 independent experiments.

100 nmol of MOG_{92-106} peptide (DEGGYTCF-FRDHSYQ) (Core Facility of the University of Utah Huntsman Cancer Institute, Salt Lake City, UT) (3) in complete Freund's adjuvant (CFA) with or without intravenous injection of 5×10^9 *Bordetella pertussis* cells (Michigan Department of Public Health, Lansing, MI) on days 0 and 2. Mice were weighed and observed for clinical signs for 5 months. Classical EAE signs were assessed according to the following criteria (61): 0=no clinical disease; 1=loss of tail tonicity; 2=mild hind leg paresis; 3=moderate hind leg paralysis; 4=complete paraplegia; and 5=quadriplegia, moribund state or death. A second clinical phenotype (ataxic form) of EAE, originally described by Brown and McFarlin (8) and Endoh *et al* (11), was quantified as specified by

Figure 3. (Opposing page) Neuropathology of MOG-induced EAE mice. Without *BP* supplementation (**a-f**), A.SW mice developed large demyelinating lesions in cerebellum (**a**, ×60). Most perivascular cuffs contained less than 2 layers of inflammatory cells (**b**, ×200). Optic nerve (**c**, ×70) and vestibulochlear nerve root and nucleus (**d**, arrowhead, ×20) were often totally demyelinated. Dense rim of polymorphonucelar cells (arrowhead) were noted at the leading edge of the severely demyelinating lesions in the pontine base (**e**, **f**, ×40, inset ×1000). With *BP* supplementation (**g**, ×60), we could find large chronic demyelinating lesions, containing foam cells (**h**, arrowhead, ×300) and cholesterol crystals (h, right) particularly in the cerebellar white matter. In contrast, SJL/J mice developed small, if any, demyelination with perivascular infiltration of mononuclear cells in the brain (**i**, ×60), while large subpial (**j**, arrowhead, ×50) and perivascular demyelination (arrow) were seen in the spinal cord. Luxol fast blue stain (**a-e**, **g-j**). Hematoxylin and eosin stain (**f**).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Greer *et al* (19) with minor modifications. This is described in the text (Results). The clinical course of EAE was determined according to the definition of clinical course of MS (34).

Histology. Mice were euthanized with halothane when moribund, or after the 5-month observation period. We perfused mice with phosphate-buffered saline (PBS), followed with a phosphate-buffered 4% paraformaldehyde solution. We divided brains into 5 coronal slabs and spinal cords into 10 to 12 horizontal slabs, and tissues were embedded in paraffin. Four-μm thick tissue sections were stained with hematoxylin and eosin, or luxol fast blue for myelin visualization. Histologic scoring was performed as described previously (61, 64). Brain sections were scored for meningitis (0=no meningitis; 1=mild cell infiltrates; 2=moderate cell infiltrates; 3=severe cell infiltrates), perivascular cuffing (0=no perivascular cuffing; 1=1-10 lesions; 2=11-20 lesions; 3=21-30 lesions; 4=31-40 lesions; 5=over 50 lesions), and demyelination (0=no demyelination; 1=mild demyelination; 2=moderate demyelination; 3=severe demyelination). For scoring spinal cord sections, each spinal cord section was divided into quadrants: the ventral column, the dorsal column, and each lateral column. Any quadrant containing meningitis, demyelination or perivascular cuffing was given a score of 1 in that pathologic class. The total number of positive quadrants for each pathologic class was determined, then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for each pathologic class. An overall pathologic score was also determined by giving a positive score if any lesions were present in the quadrant.

Immunohistochemistry. T cell, B cell and Ig deposition were visualized by the avidin-biotin peroxidase complex (ABC) technique, using anti-CD3ϵ antibody (following trypsinization, 1:10 dilution, Dako corporation, Carpinteria, CA) (35), biotin-conjugated anti-mouse CD45R/B220 antibody (1:3000 dilution, PharMingen, San Diego, CA) (14), and biotin-conjugated anti-mouse IgG (H+L) antibody (1:200 dilution, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), respectively. DNA fragmentation was detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) as described previously (62, 63). Sections were analyzed by *Image-Pro® Plus* version 3.0 (Silver Spring, MD).

Serum Anti-MOG$_{92\text{-}106}$ Antibody Assay. MOG-immunized mice were bled when sacrificed. We used an enzyme-linked immunosorbent assay (ELISA) to measure the level of serum anti-MOG$_{92\text{-}106}$ antibody as described previously (64). Ninety-six well plates were coated with MOG$_{92\text{-}106}$ peptide overnight. After blocking, serial dilutions of sera were added to the plates and incubated for 90 minutes. After washing, a peroxidase-conjugated anti-mouse IgG1 or IgG2a antibody (Caltag Laboratories, South San Francisco, CA) was added for 90 minutes. The plates were colorized with *o*-phenylenediamine dihydrochloride (Sigma Chemical Co., St. Louis, MO) and were read at 492 nm on a Titertek Multiskan Plus MK II spectrophotometer (Flow Laboratories, McLean, VA). Correlations between IgG2a/IgG1 ratio and mouse survival period were calculated by linear regression analysis.

Modulation of EAE with Bacterial DNA. Plasmid pCMV11 was derived by excision of the β-galactosidase gene from pCMVβ (Clontech, Palo Alto, CA) (61, 64, 72). Plasmid pCMV11 contains the immediate early gene promotor/enhancer from human cytomegalovirus, an intron (splice donor/splice acceptor) and the polyadenylation signal from simian virus 40, an ampicillin resistance gene, and 20 CpG motifs. Instead of β-galactosidase, pCMV11 encodes myelin basic protein 1-11, which is a non-encephalitogenic epitope in *H-2*^{s} mice. We injected A.SW mice with either PBS or 100 μg of plasmid intramuscularly into the gastrocnemius muscles. Two weeks after intramuscular injection, we induced EAE with MOG$_{92\text{-}106}$ without *BP* supplementation.

Results

Clinical Course of EAE. We assessed the clinical course of EAE by weight change and clinical score. The clinical course of MOG-induced EAE was different among the groups (Table 1). SJL/J mice developed a RR disease, whether *BP* was or was not given (Figure 1). Generally, SJL/J mice immunized with MOG did not develope acute disease in the absence of *BP* supplementation (Figure 1a). About half of the *BP* supplemented SJL/J mice, however, showed obvious clinical signs beginning around 2 weeks following sensitization (acute EAE) (Figure 1b).

In contrast, MOG-immunized A.SW mice without *BP* supplementation developed progressive disease. The mice showed no clinical signs within the first 3 weeks post-immunization; most mice showed clinical signs

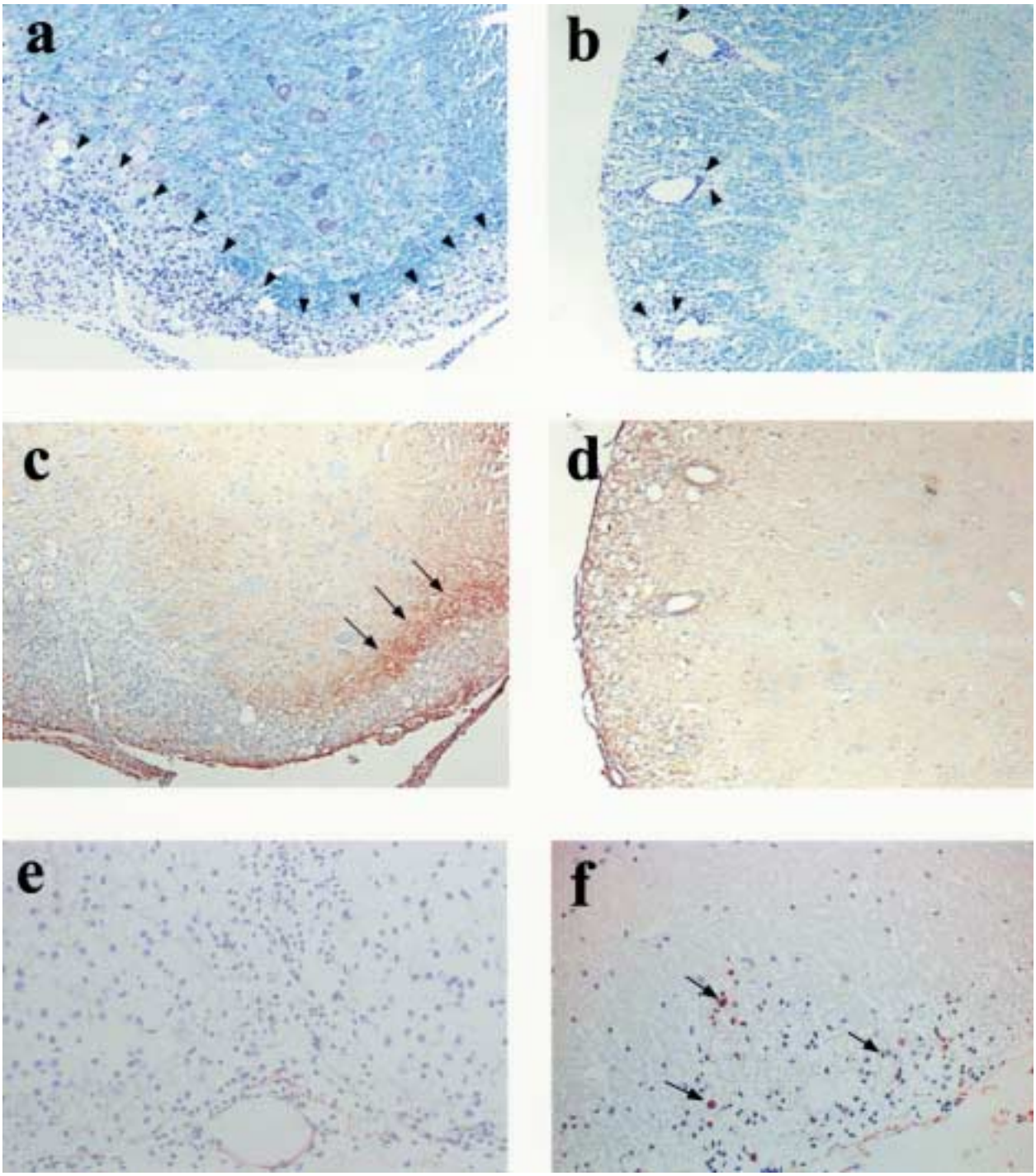

Figure 4. Demyelination (**a**, **b**), immunoglobulin (Ig) deposition (**c**, **d**), and apoptosis in the spinal cord of MOG-induced EAE mice. In A.SW mice, we could detect Ig deposition (**c**, arrow) at the plaque margin (**a**, arrowhead without *BP* supplementation). Ig deposition was found in the meninges and endothelial cells of perivascular demyelinating lesions (arrowhead) in SJL/J mice (**b**, **d**, with *BP* supplementation). Small number of TUNEL positive cells were detected in the CNS of MOG immunized A.SW mice with *BP* supplementation (**f**). In contrast, no apoptosis was found in the A.SW mice without *BP* supplementation, even in active fresh lesions (**e**). Luxol fast blue stain for myelin visualization (**a**, **b**). Ig deposition and apoptosis were detected by anti-mouse IgG immunohistochemistry (**c**, **d**) and the TUNEL method (**e**, **f**), respectively (**a-d**, ×50, **e**, **f**,×150).

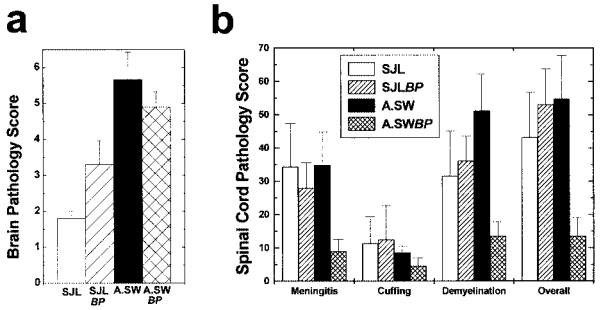

Figure 5. Neuropathology score of MOG-induced EAE mice. SJL/J and A.SW mice were subcutaneously immunized with $MOG_{92\text{-}106}$/CFA with (A.SW*BP*; cross hatched, SJL*BP*; hatched) or without (A.SW; closed, SJL; blank) *Bordetella pertussis* (*BP*) supplementation. (**a**) MOG-immunized A.SW mice showed higher brain pathology score than SJL/J mice, whether *BP* was or was not given. (**b**) Although both MOG-immunized SJL/J mouse groups as well as A.SW mice without *BP* supplementation showed high meningitis and demyelinating scores, *BP*-supplemented A.SW mice showed small involvement in the spinal cord. Perivascular cuffing was inconspicuous in all the groups.

and weight loss 1 month after immunization. By and large mice developed progressive disease from the onset and died within 20 days after initial clinical signs. Thus, the disease was defined as PP-EAE (Figure 2a). Some mice showed progressive disease with relapses, occasional plateaus and temporary minor improvements (Figure 2b). Therefore, this disease pattern (PR-EAE) is similar to that of PR-MS.

With *BP* supplementation, MOG immunized A.SW mice initially showed RR disease with half of the mice having mild acute disease (Figure 2c). The RR disease was followed by progression with or without occasional relapses, minor remissions, and plateaus; the disease was therefore defined as SP-EAE.

Clinical Signs of EAE. In addition to the distinct clinical patterns, we also noted unique clinical signs in mice with MOG-induced EAE. EAE has been induced in several animal species either with adoptive transfer of encephalitogenic cells or by active sensitization with CNS antigens. Their clinical signs in classical EAE, however, are quite similar whether they are of the acute monophasic type or RR-EAE. The symptomatology of classical EAE is characterized by atony of the tail, flaccid paralysis of the hind limb(s), complete paraplegia, and incontinence (61). Recently, however, Greer *et al* (19) described a second disease type or form of EAE, where mice develop gait abnormalities with rolling.

Without *BP* injection, A.SW mice showed a different symptomatology from the classical EAE signs, similar to those described by Brown and McFarlin (8), Endoh *et al* (11) and Greer *et al* (19). The clinical disease (ataxic phenotype) generally commenced with mice turning their heads or bodies to one side (scored as 1 or 2 depending on the degree to which the head was turned) with or without a waddling gait. Disease progressed to the point where mice continuously rolled by twisting their bodies or rotated laterally in a circle (score 3) and advanced such that the mice could not stand but would lay on their sides with or without rolling (score 4). The disease progressively developed and all mice became moribund and were euthanized or died (score 5). During the clinical course, mice showed moderate spastic paralysis of the hind and/or front limbs. Mice rarely developed the classical clinical signs of EAE: atony of the tail, flaccid paralysis of the hind limb(s), complete paraplegia, and incontinence. This scoring system for the ataxic type of EAE correlated well with changes in weight (Figure 2a, 2b).

With *BP* supplementation, A.SW mice also showed the ataxic phenotype of EAE during the latter stage. Although rolling and twisting was not typical in this group, mice developed disequilibrium with a wide-based or waddling gait, or with body rotation. Interestingly, half of the mice showed atony of the tail, a classical EAE sign, during the early stage.

MOG-sensitized SJL/J mice without *BP* supplementation had both classical and ataxic signs of EAE. The mice developed flaccid paralysis of the tail and the hind limb(s) as well as ataxia. On the other hand, with *BP* supplementation, SJL/J mice developed the clinical signs of classical EAE. Signs of the ataxic phenotype were atypical, or mild, if any.

Neuropathology. To determine whether different lesions were responsible for the distinct clinical disease among the groups, we assessed neuropathology in EAE mice. Cardinal features of MOG-immunized A.SW mice without *BP* supplementation were large plaque-like areas of demyelination in the cerebellar white matter, the cerebellar peduncle, and the vestibulocochlear nerve root and nucleus (Figure 3a, d). In some mice, the cerebellar white matter appeared totally demyelinated. Demyelinating lesions were also detected in regions of the CNS not usually affected in classical EAE induced with encephalitogenic peptides (61): those were the lateral olfactory tract, the optic nerve and chiasm (Figure 3c) (44, 54), the basal cerebral peduncle, and the spinal trigeminal tract. In the spinal cord, demyelination was seen in the ventral root entry zone and the anterior and posterior faniculi (Figure 4a). Subpial demyelination was common in the spinal cord. Meningitis was gener-

Treatment	Clinical Score			Brain Pathology	Spinal Cord Pathology			
	Disease /mice	Maximum score[b]	Death (Day)		Meningitis	Cuffing	Demyelination	Overall
PBS	5/5	4.8 ± 0.2	4/5 (42.5 ± 10.7)	3.5 ± 0.4	20.5 ± 4.7	15.5 ± 4.7	31.8 ± 10.1	37.5 ± 10.3
Plasmid	2/4	2.3 ± 1.3	1/4 (67)	1.8 ± 1.2	13.3 ± 7.3	3.5 ± 2.1	15.0 ± 8.7	23.3 ± 13.2

[a]A.SW mice were immunized with MOG_{92-106} peptide in CFA following either PBS or plasmid DNA intramuscular injection.
[b]Values are expressed as mean ± SEM.

Table 2. Modulation of primary progressive MOG-induced EAE with immunohistostimulatory DNA[a].

Strain	*BP*[a]	Clinical Course[b]	Disease Type	Acute EAE	Demyelination	T cell Infiltration	CNS IgG Deposition	Serum Anti-MOG IgG	Th type
SLJ/J	-	RR	Paralytic + Ataxic	-	Perivascular	++	+	+	Th1?
SJL/J	+	RR	Paralytic	±	Perivascular	+++	+	+	Th1?
A.SW	-	PP	Ataxic	-	Plaque-like	±	+++	+++	Th2?
A.SW	+	SP	Ataxic[c]	±	Plaque-like	±	+++	+	Th1→Th2?

[a] *Bordetella pertussis* intravenous injection
[b] RR – relapsing remitting, PP – primary progressive, SP – secondary progressive
[c] Mice showed classical (paralytic) signs during the acute EAE stage

Table 3. Clinical, histological and immunological comparison between SJL/J and A.SW mice with MOG-induced EAE.

ally mild and was seen around the hippocampus, and the basal cerebral peduncle, and the pontine base. Lymphocytic infiltrates, a cardinal feature of classical EAE, were sparse in both meningeal and perivascular spaces. Large demyelinating areas were not accompanied by perivascular cuffing (Figure 3a, 4a). Perivascular cuffs contained less than 2 layers of inflammatory cells, if any (Figure 3b). Demyelinating lesions included enormous numbers of polymorphonuclear neutrophils (PMN), many macrophages and reactive glial cells, but few lymphocytes. Demyelinating lesions sometimes contained a dense rim of neutrophils at the border between the plaque and the periplaque white matter (Figure 3e, 3f). MOG-sensitized A.SW mice without *BP* supplementation were also sacrificed on days 12 and 21, prior to mice having clinical signs. Neither inflammation nor demyelination was evident during the subclinical stage.

With *BP* supplementation, A.SW mice had severe lesions in brain similar to those of the mice without *BP* supplementation. This was most likely due to mice being sacrificed when moribund following secondary disease progression (Figure 5a). In addition to active lesions, this group exhibited chronic lesions, having foam cell accumulation with cholesterol crystals and gliosis (Figure 3g, 3h). Spinal cord lesions were less marked (Figure 5b) but were similar in nature to the brain lesions.

In contrast to A.SW mice, spinal cord lesions in SJL/J mice were more conspicuous than those of brain (Figures 3j, 4b, 5b). Although we found perivascular infiltration of mononuclear cells (MNC), a typical neuropathological feature in classical EAE, in the midbrain, the cerebellum, and the pons, only small demyelinating lesions, if any, were seen around the cuffs in the brain (Figure 3i). In contrast, in the spinal cord, mice developed many large subpial and perivenular demyelinating lesions with meningitis but perivascular cuffing was mild. While neutrophils were seen in the lesions, the infiltrates were predominantly composed of MNCs (Figure 6a). Neuropathology was essentially the same in MOG-immunized SJL/J mice whether *BP* was or was not given, except that the extent of perivascular cuffing in the brain was higher in the group having *BP* supplementation.

Lymphocyte Infiltration in the CNS. To compare lymphocyte infiltrates among the groups, we stained and enumerated T and B cells using immunohistochemistry. Since T cells predominate in CNS infiltrates in the classical type of EAE, T cells are believed to act not only as

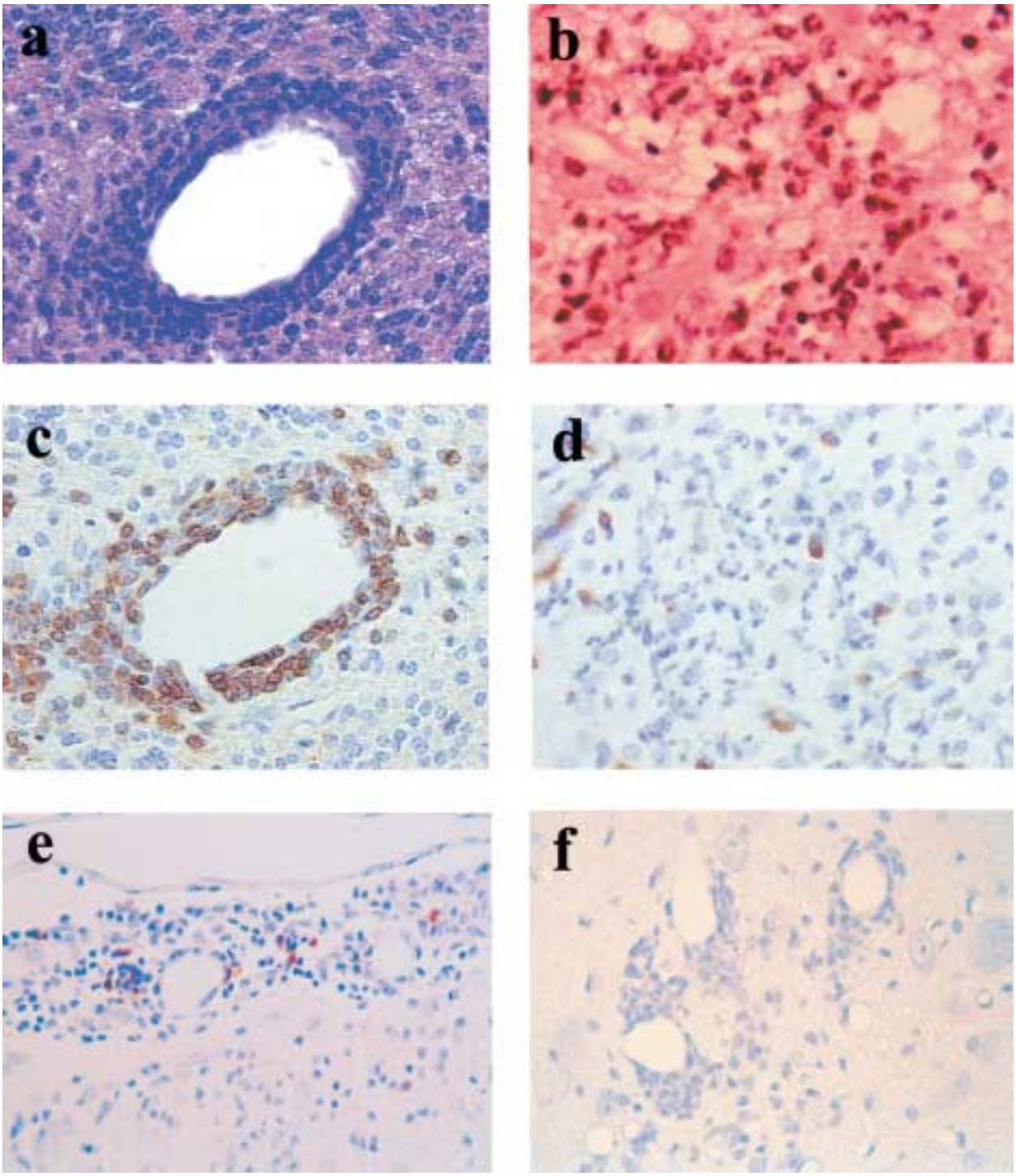

Figure 6. Immunohistochemical staining of CD3+ T cells (**c**, **d**) and B220+ B cells (**e**, **f**) in MOG immunized SJL/J (**a**, **c**, **e**, **f**) and A.SW mice (**b**, **d**). We found prominent perivascular and parenchymal T cell infiltrates in SJL/J mice (**a**, **c** without *BP* supplementation). In constrast, T cells were sparse in the lesions of A.SW mice (**b**, **d** without *BP* supplementation) (**a**, **b**, Hematoxylin and eosin). B cell infiltration was found only in meningeal space (**e**, SJL/J with *BP*), but not in the perivascular space (**f**, SJL/J with *BP*) or in demyelinating areas both in SJL/J and A.SW mice (**a-d**, ×500, **e**, **f**, ×250).

an initiator of CNS inflammation but also as effector cells causing demyelination. In accordance with this hypothesis, in MOG-immunized SJL/J mice with or without *BP* supplementation, $CD3^+$ T cells predominated both in meningeal and perivascular infiltrates (Figures 6a, 6c, 7a). Such T cells were further seen infiltrating into the brain parenchymal demyelinating lesions. In contrast, in A.SW mice immunized with $MOG_{92\text{-}106}$, whether *BP* was given or not, $CD3^+$ T cells comprised a small portion of the infiltrates in both meningeal and perivascular spaces (Figure 6b, 6d) and were virtually absent in parenchymal demyelinating lesions (Figure 7a). Thus, in A.SW mice, T cells are unlikely the effector cells of demyelination.

The contribution of B cells was analyzed using anti-CD45R/B220 antibody. In contrast to T cells, there were no significant differences among the groups (Figure 7b). B cells comprised about 10% of meningeal infiltrates (Figure 6e), while they were virtually absent in the CNS parenchyma, including perivascular cuffing (Figure 6f). Although the expression of adhesion molecules on T cells versus B cells could explain the different composition and extent of infiltration in perivascular space, the precise mechanism was not addressed in this study.

Ig Deposition and TUNEL Positive Cells in the CNS. Humoral immune-mediated pathology, more likely necrosis, is believed to be important in MOG-induced EAE (32). On the other hand, apoptosis of encephalitogenic T cells is important to down-regulate inflammation in the CNS and apoptosis of oligodendrocytes could be responsible for demyelination in some instances of classical EAE (4, 18, 60). To examine apoptotic cells and Ig deposition in the CNS, we used TUNEL staining combined with immunohistochemistry using anti-mouse IgG (H+L).

In MOG-immunized A.SW mice without *BP* supplementation, we detected an intense Ig deposition in certain regions of the myelin and endothelial cells but not within totally demyelinating lesions. In other areas, Ig was deposited only at plaque margin but not in the demyelinating zones (Figure 4a, 4c). This finding is highly reminiscent of the neuropathology observed in MS brains (51, 54). In contrast, we also found the presence of Ig deposited in demyelinating lesions accompanied with foam cells and cholesterol crystals in A.SW mice supplemented with *BP*. In SJL/J mice showing RR-EAE, we generally detected Ig deposition only in the meninges and endothelial cells (Figure 4b, 4d). In SJL/J mice that developed SP-EAE, we found moderate to intense Ig staining in the white matter. In all groups,

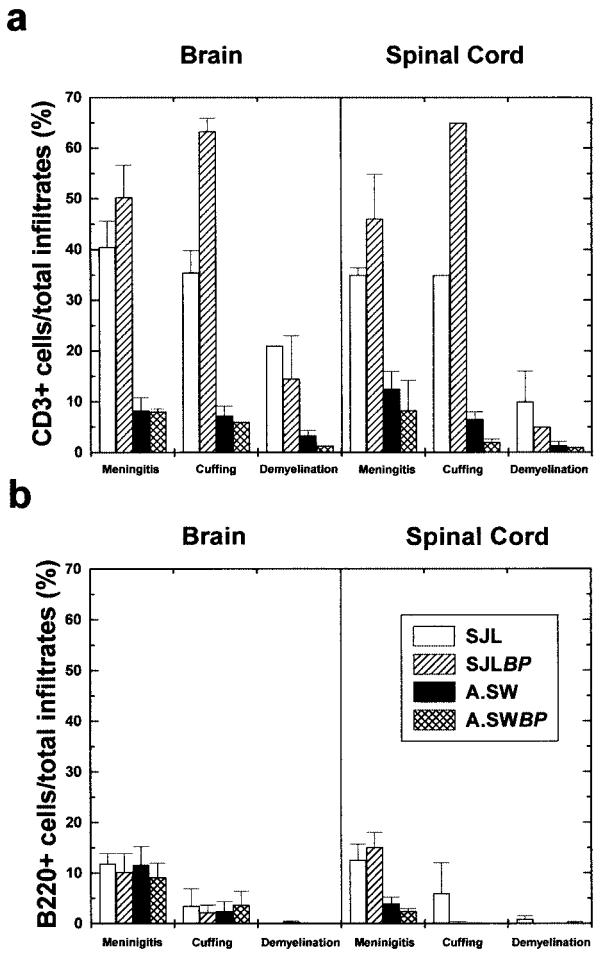

Figure 7. T and B cell infiltration in MOG-induced EAE lesions. We detected T cells and B cells in brain (left) and spinal cord (right) sections with anti-CD3 and anti-CD45R/B220 antibody, respectively. (**a**) T cell infiltration was evident in all pathological lesions in SJL/J mice, with (SJL*BP*, hatched) or without *BP* supplementation (SJL, blank). T cells were virtually absent in demyelinating lesions of A.SW mice either with (A.SW*BP*, cross hatched) or without (A.SW, closed) *BP* supplementation. (**b**) B cells were detected only in meningeal space in all groups.

some meningeal infiltrates were intensely Ig positive, suggesting the presence of plasma cells and secretion of antibody.

Only a small number of TUNEL positive cells were seen in the CNS of *BP* supplemented A.SW mice (Figure 4f). None were detected in the other groups sensitized with MOG. Even though, A.SW mice without *BP* supplementation have fresh-active lesions, both infiltrating cells and oligodendrocytes were TUNEL negative (Figure 4e).

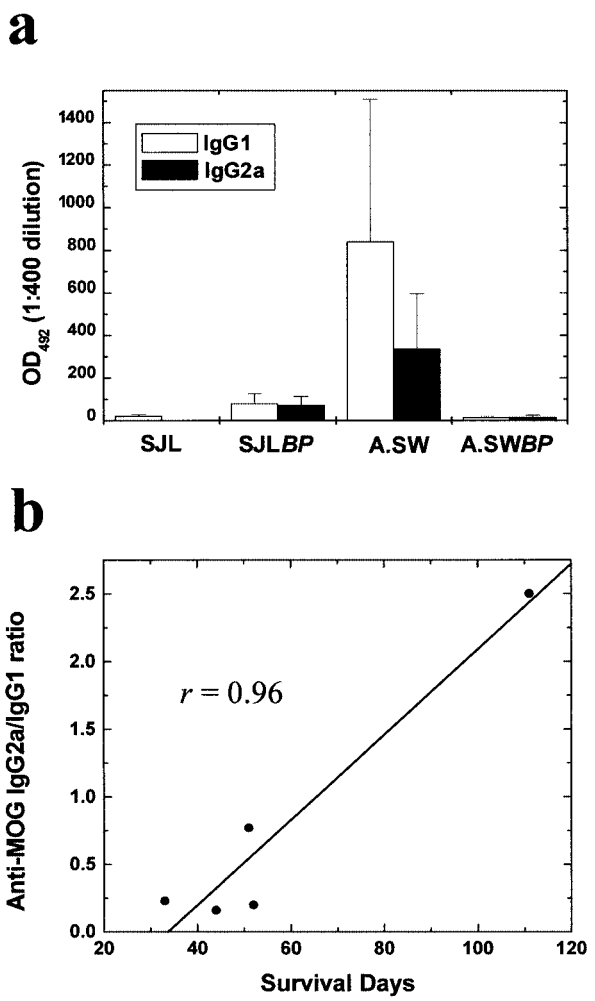

Figure 8. Serum anti-MOG_{92-106} antibody responses in MOG-induced EAE mice. (**a**) Serum anti-MOG IgG1 (open column) and IgG2a (closed column) were measured by ELISA. High serum anti-MOG antibody responses were detected only in MOG-immunized A.SW mice without *BP* supplementation. Values are mean optical density $(OD)_{492}$ ± SEM. (**b**) A significant correlation between the serum anti-MOG_{92-106} IgG2a/IgG1 ratio and survival date of MOG-immunized A.SW mice without *BP* supplementation (r=0.96). The longer mice survived the higher their serum anti-MOG IgG2a/IgG1 ratio was.

Anti-MOG Antibody Responses. Since we found more Ig deposition in mice with progressive disease, we tested whether serum anti-MOG antibody titers correlated with clinical and pathological findings. As seen in Fig 8a, we could detect high serum anti-MOG antibody titers only in MOG-immunized A.SW mice without *BP* supplementation. However, there was a large range among individual mice even within the same group.

Interestingly, there was a significant correlation between the anti-MOG IgG2a/IgG1 ratio and survival length (r=0.96, Figure 8b) in A.SW mice without *BP* supplementation. The longer the mice survived the higher their serum IgG2a/IgG1 ratio was. Since Th1 and Th2 cells help with the Ig isotype switch to IgG2a and IgG1, respectively (9), our results are consistent with a Th2-type response favoring production of myelinotoxic antibodies, leading to disease progression and early death. Alternatively, a Th1 response could contribute to suppression of anti-MOG antibody, leading to longer survival.

Modulation of PP-EAE by plasmid DNA. Since a Th1-type immune response appeared to prevent mice from developing progressive disease, PP-EAE, we tested whether a Th1-promoting approach could modulate its clinical course. Bacterial DNA, which encodes CpG motifs, have been demonstrated to promote Th1-type immune responses. The bacterial DNA can also exacerbate myelin proteolipid protein (PLP)-induced EAE, a $CD4^+$ Th1 cell mediated disease, with enhancement of the production of Th1-promoting cytokines (64). Therefore, 3 weeks prior to EAE induction, we injected A.SW mice with either PBS or bacterial plasmid DNA. As expected 4 of 5 control A.SW mice immunized with MOG developed fatal PP or PR-EAE. Interestingly, half of the mice injected with bacterial DNA showed no clinical signs during a 108-day observation period and only 1 mouse died with SP-EAE (Table 2). These results further support the hypothesis that a Th1-type immune response plays a protective role against PP-EAE.

Discussion

Here, we establish animal models for PP-, SP-, and RR-MS, using a single encephalitogenic peptide MOG_{92-106} (Table 3). While PP-MS and RR-MS have been argued to be different diseases, our models demonstrate that the same self-antigen can induce both PP- and RR-form of demyelinating disease in hosts with different immune response phenotypes. Using these models we are provided clues as to how relapsing-remitting disease could passage to a secondary progress course. Without *BP* supplementation, MOG-immunized mice developed PP-EAE without clinical signs of acute disease. This is in contrast to what is commonly seen in most other models of EAE. The lack of acute disease renders the A.SW model more typical for what is observed in MS, since the initial acute attack is generally caused by inflammation and edema, and not by demyelination. With *BP* supplementation, mice initially developed RR-EAE, followed by secondary progression, whose clinical course

was defined as SP-EAE. On the other hand, SJL/J mice developed RR-EAE, whether *BP* was or was not given. Since A.SW and SJL/J mice have the same *H-2*^{s} haplotype, the models will be useful to investigate the factors that contribute to different clinical courses. In MOG-induced EAE in rats, although major histocompatibility complex (MHC) genes are strongly involved in disease susceptibility (52), non-MHC genes also influence both clinical disease and T cell function (66).

In addition to its unique disease course, MOG-immunized A.SW mice show a different symptomatology, an ataxic type. In MS, the cerebellum and brain stem are frequently involved; ataxia is one of the most common symptoms (37). The ataxic type in MOG-induced EAE was similar to the ones described by Brown and McFarlin (8), Endoh *et al* (11) and Greer *et al* (19), although the clinical disease seen in our study was much more severe and lead to death. The lesions responsible for the phenotype was likely due to the demyelination observed in either the cerebellar white matter or the vestibulocochlear nerve root and nucleus. It is of note that the former is frequently involved in the classical phenotype of EAE, while the latter is rarely involved (61).

Although the particular distribution of the lesions in MOG-induced EAE contributes to the ataxic phenotype, the nature of the lesions could also promote the disease phenotype, and more importantly to the disease course. We found plaque-like areas of demyelination with Ig deposition in PP-EAE and SP-EAE mice with high anti-MOG antibody titers in the circulation of PP-EAE mice. In addition, in MOG-immunized A.SW mice, most perivascular cuffs contained less than 2 layers of inflammatory cells and CD3^{+} T cell infiltration was absent in demyelinating lesions. This histology is similar to that of PP-MS. Revesz *et al* (49) showed that, in PP-MS, inflammation was less intense and perivascular cuffs containing at least 2 continuous layers of mononuclear inflammatory cells were sparse. Moreover, Booss *et al* (5) reported a single case of PP-MS whose T cell content in the CNS did not correlate with the demyelinating activity.

In contrast, in SJL/J mice with RR-EAE, we found T cell infiltration with little Ig deposition in and around the lesions and low serum anti-MOG antibody responses. Thus, in MOG-immunized SJL/J mice, humoral immune responses against MOG seems an unlikely contributing factor for pathogenesis. Bystander or cytotoxic killing by T cells (58) appears to be more responsible for demyelination. This is similar to what has been described in other forms of classical EAE. In support of this, Hjelmström reported that B cell-deficient μMT mice develop acute EAE with demyelination after MOG immunization (21). In A.SW mice with MOG-induced EAE, however, myelinotoxic antibody most likely plays an important role as one of the effectors as discussed above. In this context, MOG-specific T cells might act as inducers, but not as the final effectors, in the MOG-induced EAE in A.SW mice. Therefore, the contribution of Ig and T cell to the pathogenesis between A.SW and SJL/J mice with MOG-induced EAE is different. Our data could explain the fact that demyelination in some MOG-EAE models is anti-MOG antibody dependent, while in other models MOG-EAE can be induced in Ig depleted animals (21). We also observed some apoptotic cells in the CNS of *BP*-supplemented A.SW mice, but none in the other groups, in spite of the fact that most lesions were recent and active in A.SW mice with PP-EAE. Lack of oligodendrocyte apoptosis supports the hypothesis of Ig-mediated demyelination in MOG-induced EAE, since Ig-mediated tissue injury causes necrosis rather than apoptosis. In addition, the lack of apoptotic cell death of the inducer and effector cells in the CNS is consistent with the progressive nature of the disease we observe (60).

With *BP* supplementation, A.SW mice had low serum anti-MOG antibody levels in spite of a secondary progressive disease course with Ig deposition in the CNS. The discrepancy between CNS Ig deposition and low serum anti-MOG titer could be due to: *1)* deposition of irrelevant anti-myelin antibody in the CNS, produced by antigen spreading; *2)* a dominant intrathecal production of anti-MOG antibody; or *3)* deposition of anti-MOG antibody in the CNS from the plasma leading to a decrease in antibody in the circulation. Thus, although anti-MOG antibody levels tended to be associated with a PP course, both IgG1 and IgG2a titers by themselves did not correlate with disease progression or Ig deposition in the CNS.

In PP-EAE mice, the anti-MOG IgG2a/IgG1 ratio correlated with survival; the shorter the survival time, the lower the IgG2a/IgG1 ratio. Th1 and Th2 responses are known to favor IgG2a and IgG1 isotype switching, respectively. Thus, this further supports the important role of the humoral response in MOG-induced EAE, since Th2 deviation could favor the production of myelinotoxic antibodies, leading to progressive disease. Genain *et al* (16) has suggested that Th2 immune deviation can increase the amount of pathogenic autoantibodies and that this leads to exacerbation of demyelinating disease. Moreover, we noted that *BP* supplementation in A.SW mice suppressed primary progression of the disease and mice had decreased anti-MOG antibody

responses. *BP* has been used to enhance EAE, most likely due to enhancement of blood-brain-barrier permeability and deviation to Th1 immune responses. The former is unlikely to be our experiments, since *BP* supplementation suppressed the primary progressive disease. The deviation to Th1 immune responses in the induction phase could suppress the production of myelinotoxic antibody leading to limit progression. The protective role of Th1 cytokines have been reported in some forms of MOG-induced EAE. Willenborg *et al* (68, 69) demonstrated that IFN-γ but not Th2 cytokines, down regulate disease progression in MOG_{35-55}-induced EAE in mice lacking the ligand binding chain of the IFN-γ receptor (IFN-$\gamma R^{-/-}$).

In addition, favoring a Th2 immune response could contribute to ataxic clinical signs, while a Th1 response would favor the classical disease phenotype in MOG-induced EAE. With *BP* supplementation (Th1 deviation), SJL/J mice (Th1 phenotype mouse strain) developed classical disease signs without ataxia. While A.SW mice (Th2 responding phenotype mouse strain) developed ataxic progressive disease, they showed classical signs during the acute EAE stage only if they were given *BP* supplementation.

Although cytokine profile analyses are essential to clarify the role of Th1 versus Th2 responses in disease progression (55, 68), this could not be undertaken in the current study. In A.SW mice, particularly those without *BP* supplementation, we found severe atrophy of lymphoid organs, including thymus, lymph node, and spleen (Tsunoda I and Fujinami RS, manuscript in preparation). When mice were autopsied, the thymus and lymph nodes were severely atrophic. We found a 90% decrease in weight of the spleen, and $CD3^+$ T cells were severely depleted. Together with the fact that few $CD3^+$ T cells were detected in the CNS, we could not perform cytokine analyses using isolated T cells from A.SW mice. We are currently performing a kinetic time course study of chemokine and cytokine mRNA analyses in MOG-induced EAE using RNase protection assays.

As noted above, the influence of the immunomodulation on the clinical disease course of MOG-induced EAE in A.SW mice seemed to be opposite to that of classical EAE, where Th1 response exacerbates the disease and Th2 response contributes to disease suppression. To confirm this, we modulated MOG-induced EAE using bacterial plasmid DNA, which has been demonstrated to enhance Th1-mediated disease in PLP-induced EAE, and is widely used in DNA immunization studies (64). Bacterial DNA vaccination suppressed the primary disease progression in A.SW mice without *BP* supplementation. Clinically, it is known that in RR-MS, both IFNβ and copolymer I are effective therapeutic treatments. However, the usefulness of IFNβ and copolymer I in PP- and SP-MS is controversial and sometimes can have adverse effects on disease progression (6, 7, 12, 26). Taken together, patients with PP- and SP-MS could respond differently to some forms of immunomodulatory therapies than patients with RR-MS.

In addition, we found a massive neutrophilic infiltration in A.SW mice. This observation is interesting because there are a few previous reports that reference PMN involvement in EAE and MS. Neutrophilic involvement in the classical forms of EAE and MS is absent or sparse. However, in a subgroup of patients with Devic's neuromyelitis optica, granulocytes have been found in the CSF (45) and in a patient with Marburg's type of MS, occasional neutrophilic infiltrates were evident (23). Interestingly anti-neutrophilic antibody and polymorphonuclear neutral protease activity have also been reported in MS (13, 20, 42). Recently, McColl *et al* (39) reported that anti-granulocyte antibodies suppressed several types of EAE. This suggests a role for PMNs during the effector phase, but not the induction phase of EAE. Moreover, PMN infiltration has been reported in some MOG-associated EAE models (48, 54) and in EAE in BALB/c mice, which are usually a resistant strain (38). The latter model has some similarity in histology with our models, since T cell infiltration in the CNS is limited in the BALB/c mouse EAE model. Neutrophilic infiltration without T cells in the CNS has also been reported in the hyperacute form of EAE (29, 30). In this model, T cells were depleted with cyclophosphamide before the induction of EAE; mice developed primary progressive disease. In our PP-EAE model, T cell infiltration was virtually absent in the demyelinating lesions and T cells were also severely depleted in the peripheral lymphoid organs (Tsunoda I and Fujinami RS, manuscript in preparation). Although T cell depletion generally prevents immune-mediated demyelinating diseases, it should be stressed that T cell depletion could potentially exacerbate the disease whether it is caused artificially, such as by cyclophosphamide administration, or without treatment as seen in our model.

The differences observed between A.SW and SJL/J mice with an identical *H-2* haplotype demonstrate contributing factors that involve genes outside of the MHC. We have not identified the gene(s) that is responsible for the disease modulation in MOG-induced EAE. This disease susceptibility is, however, similar to that of mercu-

ry-induced autoimmunity (22). In mercury-induced autoimmune disease, IL-4 producing A.SW mice are susceptible to the disease, while SJL mice, a poor Th2 responder, are resistant. This difference is likely due to SJL/J mice lacking IL-4 producing $NK1.1^+$ T cells (73). Recently, although the role of NK and NKT cells in autoimmune diseases and demyelinating diseases, including MS and EAE, has been investigated, it still remains controversial (36, 70, 74). The identification of Th2-promoting cytokine producing cells, including NKT cells, would help in understanding of the mechanism of disease progression in MOG-induced EAE.

Although the pathomechanism of RR-MS and their animal models has been investigated extensively, those of progressive forms of MS are still unclear, at least partly due to the lack of highly relevant animal models. Here we establish animal models for different clinical forms of MS, including primary and secondary progressive MS. The progressive form of EAE was associated with humoral anti-MOG immune responses. Moreover, abnormalities in humoral immune responses, such as increased autoantibody and intrathecal IgG production, have also been associated with PP-MS (2, 59). Therefore, in some instances Th2 responses would be detrimental for the outcome of MS and could help explain the transition from the relapsing-remitting form of MS to the chronic progressive state. Our system is novel and has direct application in understanding the mechanism of why some individuals with MS have a remitting-relapsing course and why others have a chronic progressive course.

Acknowledgements

The authors would like to thank Jane E. Libbey, Neal D. Tolley, and Tobias Derfuss for many helpful discussions, and Thomas S. Cannon, Loren S. Jack, Kristie M. Parker, Timothy S. Alexander and Jana L. Bryner for their technical assistance. We are grateful to Ms. Kathleen Borick for preparation of the manuscript. This work was supported by the grants from the University of Utah Research Foundation, National Multiple Sclerosis Society PP0364 and the National Institutes of Health AI42525.

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