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A MONOCLONAL ANTIBODY AGAINST A MYELIN OLIGODENDROCYTE GLYCOPROTEIN INDUCES RELAPSES AND DEMYELINATION IN CENTRAL NERVOUS SYSTEM AUTOIMMUNE DISEASE[1]

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The factors contributing to chronic relapsing inflammatory disease processes of the central nervous system (CNS) and demyelination are poorly understood. In addition to cellular immune reactions, humoral factors such as antibodies might quantitatively or qualitatively influence the disease process. We therefore investigated the effects of administration of a monoclonal antibody specific for a CNS autoantigen on both acute and chronic experimental autoimmune encephalomyelitis (EAE) in mice and rats. This monoclonal antibody, 8-18C5, specific for a myelin/oligodendrocyte glycoprotein, was observed to accelerate clinical and pathologic changes of CNS autoimmune disease. In SJL mice with chronic relapsing EAE, injection of antibody into animals recovering from an attack induced fatal relapses; in Lewis rats, acute EAE was enhanced and associated with a hyperacute inflammatory response with demyelination, a feature not commonly seen in acute EAE. The demonstration that relapses and demyelination can be induced by administration of a white matter-reactive monoclonal antibody offers new possibilities to study processes resulting in CNS damage during autoimmune disease. Furthermore, these findings support the immunopathogenic potential of antibody to myelin components in inflammatory CNS disease processes and, specifically, in causing demyelination.

Myelin basic protein and proteolipid protein are known to be major autoantigens in experimental central nervous system (CNS) autoimmune disease (1–7), but other CNS autoantigens might also be targets of the immunopathologic process. In addition, whereas the central role of cellular immune reactions mediating CNS experimental autoimmune disease is clearly established (1–3), humoral factors may contribute to the disease process (8–21). Early experiments with myelinated cerebellar cultures detected antibodies which caused demyelination in vitro. Such antibodies are produced during experimental autoimmune encephalomyelitis (EAE)[3] in guinea pigs and other animals immunized with spinal cord tissue, but a complete dissociation of neurologic signs of EAE and the generation of in vitro demyelinating antibodies was noted (8). In vivo, demyelination was observed with sera from guinea pigs with acute and chronic EAE if injected into the lumbosacral space of rats (9). In this system, a significant correlation between the appearance of antibodies against myelin in serum or cerebrospinal fluid and demyelination has been reported (10). Interestingly, a synergy between antibody-dependent and cell-mediated immune processes has been established by an experimental protocol for the induction of primary demyelination in the rabbit eye (11). A focal demyelination could be induced in rabbit eyes by injection of a supernatant from activated lymphocyte cultures and IgG from rabbits immunized with spinal cord tissue. Either agent was ineffective in induction of demyelination (11). However, the specificity of these demyelinating antibodies remained unknown, and such sera contain abundant antibodies against a variety of CNS components, some of which are only tentatively characterized (14–16), and the role of these antibodies in the pathogenesis of the demyelination remains unclear.

Recently, a novel myelin/oligodendrocyte glycoprotein (MOG) has been defined by a mouse IgG1 monoclonal antibody, 8-18C5. This antigen is a minor glycoprotein of myelin, detectable in white matter tracts of the CNS of most vertebrate species but not in peripheral myelin (22). Antibody 8-18C5 did not cause pathologic effects when injected in normal animals, but its effect in animals with acute and relapsing EAE was pronounced. In acute EAE in Lewis rats, 8-18C5 induced a hyperacute inflammatory response with selective white matter involvement and extensive demyelination, something not seen in animals with conventional acute EAE. In chronic relapsing EAE in the SJL mouse, 8-18C5 antibody induced fatal relapses. The definition of a new autoantigen involved in demyelinating processes and the availability of an antibody that quantitatively and qualitatively affects CNS autoimmune disease presents a new avenue to study the

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; CFA, complete Freund's adjuvant; BBB, blood brain barrier; MOG, myelin/oligodendrocyte glycoprotein.

synergistic interaction of a T cell-mediated encephalitic processes and antibody-mediated demyelination in vivo.

MATERIALS AND METHODS

Animals and immunizations. SJL mice were obtained from The Jackson Laboratory, Bar Harbor, ME and Lewis rats from Charles River Labortories, Wilmington, MA. Mice were immunized with 400 μg guinea pig myelin basic protein (MBP), contained in an emulsion of MBP/complete Freund's adjuvant (CFA) (5 mg/ml *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, MI) and 5 mg/ml guinea pig MBP). Lewis rats were immunized with 50 μg guinea pig MBP/CFA, contained in 100 μl of an emulsion of MBP/CFA containing 5 mg/ml *M. tuberculosis* and 0.5 mg/ml MBP.

Passive transfer of EAE. SJL mice were immunized with MBP as described above. At nine to 10 days later, lymph nodes were removed and cells restimulated in vitro (1, 3) with 50 μg/ml MBP. To minimize variance in experiments on chronic relapsing EAE animals, pairs of animals matched for comparable disease course were selected and animals of each group were randomly assigned to receive an injection of either control antibody or antibody 8-18C5. Activation of cells was monitored by assessing [^{3}H]methyl-thymidine (Amersham Corp., Arlington Heights, IL, specific activity 5 Ci/mmol) incorporation of stimulated cells. Briefly, 5×10^5 cells were distributed into round-bottomed microtiter plates in a total volume of 200 μl RPMI 1640 supplemented by 1% fresh autologous serum. Antigen was added to a final concentration of 50 μg/ml. Cells were incubated for 3 days and then pulsed with 0.2 μCi [^{3}H]methyl-thymidine and incubated for an additional 24 hr. Thereafter, cells were harvested and incorporated radioactivity was determined by liquid scintillation counting. Only those cultures with a stimulation index of more than 8 were used for experiments. SJL mice received a single i.v. injection of 35×10^6 activated lymph node cells (in a total volume of 1 ml phosphate-buffered saline).

Purification and injection of antibody 8-18C5. Hybridoma cell line 8-18C5 was grown in RPMI 1640 supplemented with 10% fetal calf serum. Antibody 8-18C5 was purified from cell culture supernatant by immunoaffinity chromatography on columns of rabbit anti-mouse-Ig (Tago Inc., Burlingame, CA). Before coupling of the rabbit anti-mouse Ig to Tresyl-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) antibodies cross-reacting with fetal calf serum were removed by solid phase adsorption on columns of fetal calf serum/Sepharose 4B. Antibodies were injected i.p. in a total volume of 1 ml RPMI 1640 at a dose of 200 μg/g body weight. Alternatively, 8-18C5 and the control antibody used, polyclonal mouse IgG1, were prepared from normal mouse serum and ascites on anti-IgG1 Agarose columns (Sigma Chemical Co., St. Louis, MO). Antibody was buffered and concentrated on YM5 membranes (Amicon Corp., Danvers, MA).

Scoring of EAE. EAE was scored as follows: 0: no disease; 1: decreased activity, limp tail; 2: mild paraparesis, unsteady gait; 3: moderate paraparesis, limbs splayed apart, but voluntary movements still possible; 4: tetraplegia; 5: moribund.

Histologic analysis. Brain and spinal cord tissue samples were fixed in 4% paraformaldehyde, embedded in paraffin, and 8-μm sections were stained with Luxol fast blue hematoxylin-eosin. Counts of parenchymal inflammatory foci (20 or more inflammatory cells) in standard cerebrum, midbrain, brainstem/cerebellum, and upper and lower spinal cord sections were made as in a previous study (4). Additionally, in the white matter, each of these inflammatory foci was assessed for demyelination, as judged by loss of blue staining in the area immediately around the focus. Histologic evaluation was done by a single observer (R.A.S.) without knowledge of the treatment the animal received.

RESULTS

SJL mice immunized with MBP/CFA do not develop overt neurologic autoimmune disease, even though they harbor autoimmune T lymphocytes reactive with MBP. We investigated whether injection of 8-18C5 antibody resulted in clinical or pathologic manifestations of CNS disease in MBP/CFA-sensitized mice. SJL mice were immunized with 400 μg MBP/CFA and randomly assigned to treatment groups (three to five animals per group, mean body weight 21 ± 1 g). Groups were treated on different days, starting from the day of immunization until day 12. Mice received a single i.p. injection of 4 mg of 8-18C5 antibody. SJL mice did not develop EAE after immunization with MBP/CFA and injection of antibody 8-18C5 did not result in clinical disease in MBP/CFA sensitized mice observed for up to 60 days after immunization (Table I). Furthermore, no CNS pathologic changes were observed in these animals (data not shown).

Lewis rats (mean body weight 192 ± 12 g) were immunized with MBP/CFA and treated at various times with 200 μg/g body weight of 8-18C5 antibody. Untreated animals developed EAE within 11 days after immunization with MBP/CFA (Table I; Fig. 1). The disease course was acute and transient, with approximately two-thirds of animals recovering from neurologic symptoms within 1 to 2 wk. However, injection of 8-18C5 antibody greatly accelerated the disease process. Animals that received a single injection either on day 0, 1, 2, 3, 4, 5, 6, or 7 developed clinical symptoms as early as day 8, 3 days before control animals showed signs of EAE. The disease progressed very rapidly and tetraparesis and death occurred within 24 to 48 hr, often without clear expression of the early symptoms of EAE (caudal to cranial progressive paralysis, tail atonia, and hind limb weakness) (Table I; Fig. 1). At this time, animals treated with control antibody showed no clinical signs of EAE. Antibody 8-18C5 injections given just before disease onset on days 8, 9, or 10 resulted in rapid disease development, again with death of most animals. Injection of antibody on day 11 or 12 in animals with clinical symptoms induced rapid worsening of the disease. The disease course of representative groups of animals is given in Figure 1. Autopsy revealed no gross abnormalities in organ systems other than the CNS, apart from obstructive uropathy (a common complication of EAE) mostly in antibody 8-18C5-treated animals.

Histologic examination was carried out on days 10 and 12 in animals injected with antibody 8-18C5 on day 8 after immunization and compared with animals immunized with MBP/CFA alone. As shown in Table II, the total number of inflammatory foci in animals treated with antibody 8-18C5 was higher than that seen in EAE animals. Furthermore, although equal numbers of parenchymal inflammatory foci without demyelination was present in both 8-18C5- and control antibody-treated rats at day 10, there was a marked increase in inflammatory foci with demyelination in 8-18C5-treated rats. Representative histologic examination of 8-18C5-treated rats is seen in Figure 2. Edema was more pronounced in the CNS of 8-18C5-treated rats, and sheets of polymorphonuclear leukocytes were found in parenchymal perivascular cuffs and subpial bands of white matter (Fig. 2). Fibrinoid degeneration of the vessel wall and marked neutrophil infiltrations were found in the spinal cord of 8-18C5-treated animals, whereas in EAE rats treated with control immunoglobulin only typical mononuclear cell infiltrates were present.

Only three MBP/CFA sensitized, 8-18C5-treated rats survived beyond the acute phase of EAE. These were observed for a period of 13 wk, but chronic disease progression or relapses did not occur. Antibody 8-18C5 itself, when injected into normal rats that had not been immunized with MBP/CFA, caused no clinical or pathologic effects.

To study the effect of antibody on chronic relapsing

TABLE I

Effect of antibody injection on MBP-immunized animals[a]

Treatment of Animals		No. of Animals		Onset of Disease (Days)	Dead
		Total	Diseased		
SJL mice					
	8-18C5 antibody	8	0		0
MBP/CFA		15	0		0
MBP/CFA	8-18C5 antibody	8	0		0
Lewis rats					
	8-18C5 antibody	6	0		0
MBP/CFA		18	18	11	6
MBP/CFA	Control antibody	14	14	11	6
MBP/CFA	8-18C5 antibody	34	34	8–9	31

[a] Animals were immunized with myelin basic protein as described in *Materials and Methods*. Groups of animals received a single i.p. injection (200 μg/g body weight) of antibody on the day of immunization or on days 1 to 8 after immunization. Control antibody was injected into animals from control groups on days 0 and 8. Data presented are pooled from all animals injected from day 0 to 8. The disease process of individual groups of rats injected on days 0, 4, 8, and 10 is given in Figure 1. SJL mice received antibody injections on day 0 or day 6 after immunization.

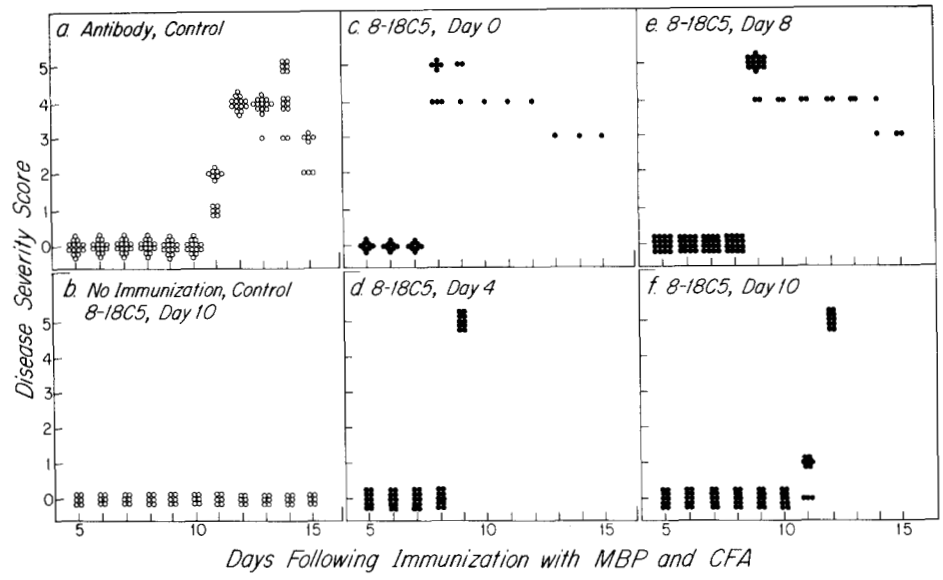

Figure 1. Effect of antibody 8-18C5 on rat EAE. Rats were treated as described in Table I. Animals are represented individually by *symbols*.

EAE, relapsing disease was induced by injecting in vitro activated, MBP-specific lymph node cells into normal SJL mice. Animals were treated with antibody at various times after they had recovered from a relapse. Relapses generally occurred every 4 to 6 wk after transfer of MBP-specific T cells. Matched pairs of animals with a comparable disease course and disease severity score were selected and a treatment schedule was randomly assigned to partners of each pair. In totally recovered animals (disease severity score = 0) injection of 8-18C5 antibody did not induce relapses within an observation period of 3 wk and no differences between 8-18C5 and control antibody-treated animals were evident in the number or degree of subsequent relapses (Table III, Fig. 3*a*). However, as described below, if 8-18C5 antibody was injected into animals that did not fully recover from their last attack, lethal relapses occurred.

As shown in Figure 3 *a* and *b*, animals recovering from an attack were treated with 8-18C5 or control antibody when their clinical condition stabilized at a disease severity score of 1 (Fig. 3*b*) or 2 (Fig. 3c). On the *horizontal axis* of Figure 3 *b* and *c*, *days* represents the observation period after the pairing of animals into treatment and nontreatment groups. As shown in Table III and Figure 3*b*, animals treated with control antibody continued to improve and recover from the previous EAE attack (*open circles*), whereas 8-18C5-treated animals suddenly worsened 2 to 5 days after 8-18C5 injection and died within 3 days. Even more severe relapses were induced in animals with a higher disease severity score (atonia and hind limb weakness, disease severity score = 2). In these animals, injection of antibody 8-18C5 also induced a relapse within 2 to 5 days, but the disease course progressed more rapidly, resulting in death within 24 hr. The disease course of a representative group of animals is given in detail in Figure 3. Although the effects of antibody treatment on the relapses were dramatic clinically, the pathologic effects were less easy to isolate, since all mice had large areas of CNS demyelination as well as chronic inflammation, features which are commonly seen in the relapsing EAE model.

DISCUSSION

The present study provides evidence that an antibody to a MOG (22) can amplify demyelination and modify the

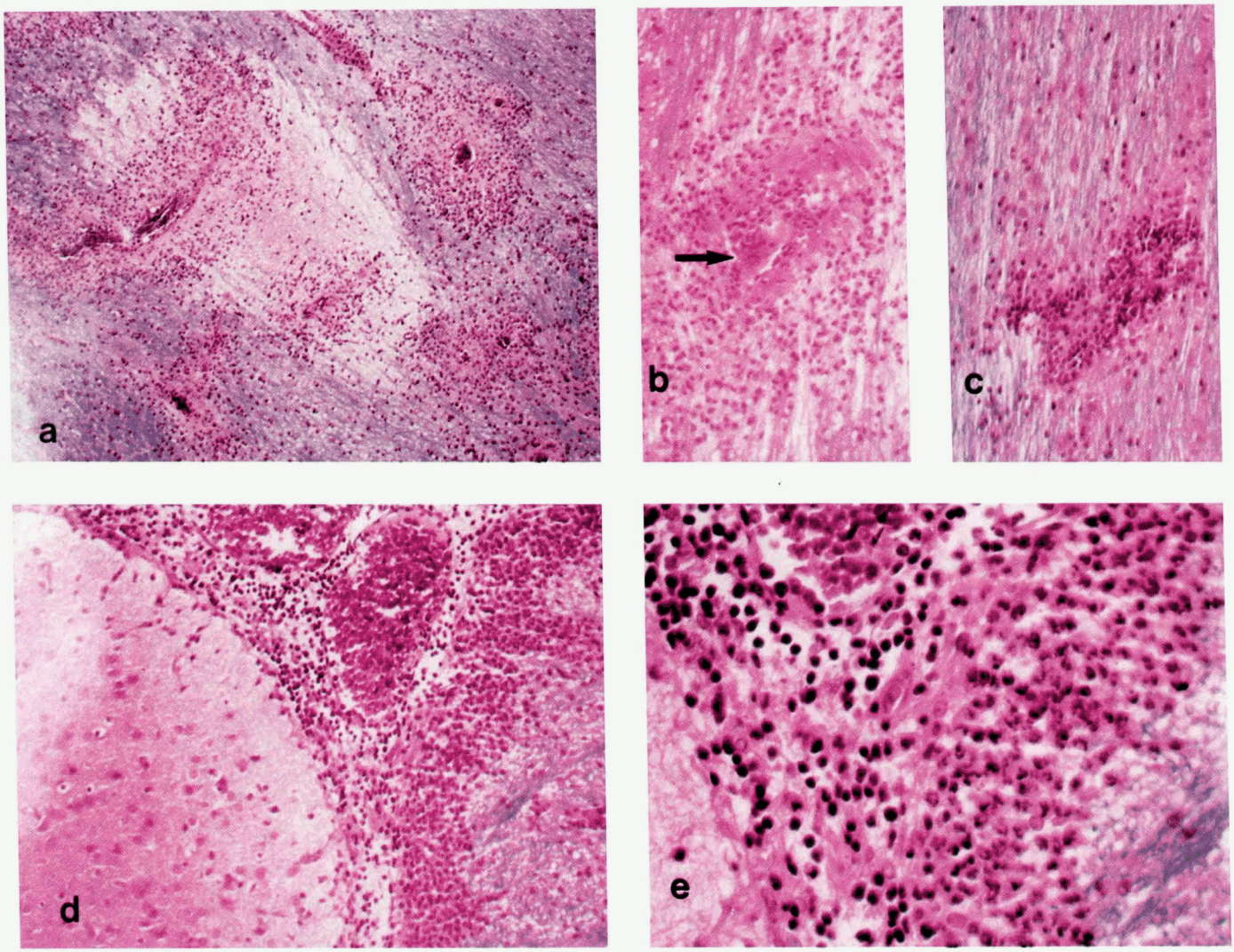

Figure 2. *a*, Brain of rat with acute EAE treated with anti-MOG monoclonal antibody 8-18C5. There is extensive perivascular edema and demyelination indicated by vacuolation and loss of blue staining around vessels. Luxol fast blue H & E, ×80. *b*, Spinal cord of rat with acute EAE treated with 8-18C5. There is fibrinoid degeneration of the vessel wall (*arrow*) and a marked neutrophil infiltrate. Luxol H & E, ×320. *c*, Spinal cord of control antibody-treated rat with acute EAE. A typical mononuclear cell infiltrate is present. Luxol H & E, ×320. *d*, Brain of rat with acute EAE treated with 8-18C5. There is a mononuclear cell infiltrate in the leptomeninges; the large gray matter (*pink*) area of the brain is spared whereas the white matter (*blue*) has a band of subpial and perivascular neutrophil infiltrate. Luxol H & E, ×320. *e*, Higher power of *d* showing mononuclear cell infiltrate in leptomeninges on the *left* and intense neutrophil infiltrate in white matter on the *right*. Luxol H & E, ×800.

TABLE II

Quantification of CNS inflammation in EAE rats[a]

Days after Immunization	Antibody Injection	No. of Animals	Total	No. of Parenchymal Inflammatory Foci	
				With demyelination	Without demyelination
10	Control	4	20.5 ± 8.2	1 ± 1.2	19.5 ± 8.1
10	8-18C5	7	102.4 ± 20.9	81.7 ± 21.7	20.7 ± 7.2
12	Control	3	62 ± 11.1	9.7 ± 8.4	52.3 ± 6.5

[a] Animals were immunized as described in *Materials and Methods*. Antibodies were injected i.p. at day 8, and animals were killed, as indicated above, for histologic analysis.

clinical expression of acute EAE in Lewis rats and the severity of clinical relapses of chronic EAE in the SJL mouse.

The degree of demyelination associated with EAE is variable among different experimental models. In mice, passive transfer of activated MBP-specific lymph node cells or T cell clones results in a chronic relapsing disease process associated with demyelination (1, 2). In rats, immunization with MBP or transfer of MBP-specific T cells induces an acute, monophasic disease with little apparent demyelination. The mechanisms inducing demyelination in vivo are still unresolved, although in vitro and in vivo experiments suggest that humoral factors as well as T cells contribute to demyelinative processes (8–11, 15, 17–21).

In acute EAE, encephalitis induced by MBP-reactive T cells is accompanied by massive influx of leukocytes across the blood brain barrier (BBB). In some species, this

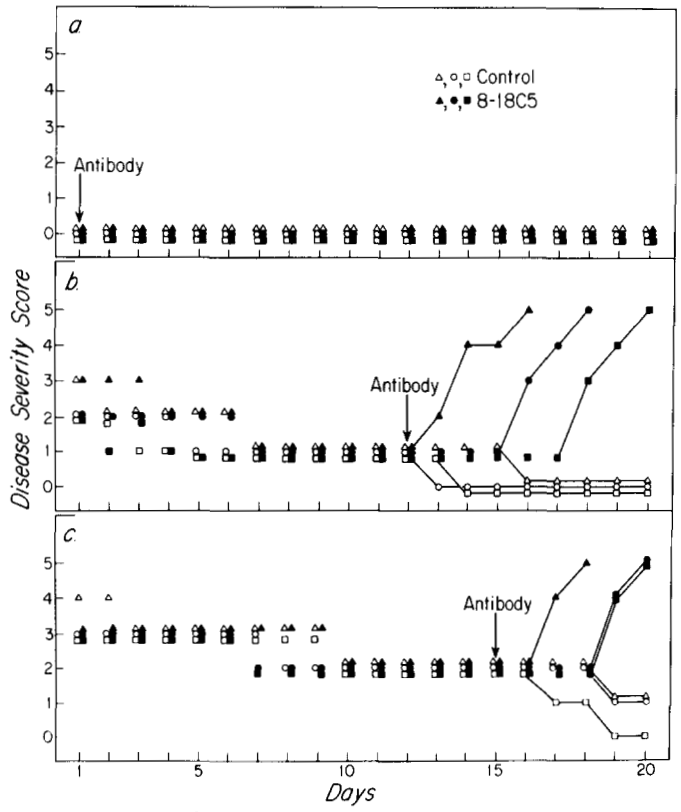

Figure 3. Effect of antibody 8-18C5 on chronic relapsing EAE in SJL mice. EAE was induced in SJL mice by passive transfer of 35×10^6 in vitro MBP-activated lymph node cells taken from animals immunized with MBP. Matched pairs of chronic-relapsing animals were injected as described in Table II (*open symbols*: control antibody injections; *closed symbols*: antibody 8-18C5). Data of antibody injection is indicated by *arrows*. On the *horizontal axis* of *b* and *c*, *days* represents the observation period after the pairing of animals into treatment and nontreatment groups.

TABLE III

Effect of antibody 8-18C5 on chronic relapsing EAE in mice[a]

Antibody Treatment	Disease Severity Score on Day of Injection	No. of Animals		No. of Attacks Before treatment
		Total	Lethal relapse	
8-18C5	0	10	0	1
Control	0	10	0	1
8-18C5	0	4	0	3
Control	0	4	0	3
8-18C5	1	9	9	3
Control	1	9	0	3
8-18C5	2	6	6	3
Control	2	6	0	3

[a] Animals were treated as described in Table I and Figure 3. Treatment schedule of animals and EAE score is given in Table I and Figure 3. Matched pairs of mice with comparable disease course were randomly assigned to 8-18C5 or control antibody injection.

might result in an in situ autoimmunization against CNS antigens, associated with a variety of secondary immunologic reactions, possibly including antibody formation. It has been demonstrated, indeed, that plasma cells present intrathecally produce specific antibody against CNS antigens (9). Due to the very close spatial relationship between site of antibody production, target antigen as well as effector cells such as macrophages, the necessary components exist for a specific immune response to occur within the CNS.

The model of demyelinating EAE in rats that we have described, creates an experimental system in which both cellular and humoral components of the pathologic reaction can be induced and studied separately. Encephalitis is induced by immunization with MBP or by transfer of encephalitogenic T cells and extensive demyelination by injection of antibody.[4] The juxtaposition of a meningeal chronic inflammatory infiltrate with a neutrophil infiltrate that selectively involves white matter is consistent with the presence of two distinct immunopathologic processes.

In vivo activity of 8-18C5 antibody after systemic injection apparently requires opening of the BBB. In rats injected with antibody 8-18C5, clinical signs of EAE first became apparent 3 days earlier than in the control groups and appeared at the same time whether antibody was injected on day 1 or day 8 after immunization. The rapid development of lethal disease, often without the early stages of caudal to cranial progressive paralysis, suggests that the antibody directly meets its target structure throughout the CNS. These findings suggest that the BBB becomes penetrable for antibodies around day 8 before the appearance of neurlogic symptoms. Autoantibodies against CNS antigens can be present systemically and cause no effect, but become pathogenic as soon as the BBB is opened. Actually, increased vascular permeability associated with exudation of γ-globulin has been observed in rats as early as 7 days after immunization (23).

In mice, relapses could only be induced in animals not fully recovered from EAE, which again suggests that antibody is only active as long as the BBB is penetrable. In addition, an ongoing encephalitis might be necessary to facilitate demyelination, which might be due to direct activity of the antibody or mediated indirectly by antibody-dependent cellular cytotoxicity. Such a mechanism has been proposed previously for focal demyelination in the rabbit eye, induced by a synergistic action of IgG from EAE animals and cell culture supernatant from activated leukocytes (11). Of note is that antibody 8-18C5 induced not only a marked increase in the number of inflammatory demyelinating foci but an increase in the total number of inflammatory foci. This increase might be due to antibody-initiated breakdown of myelin, which in turn activates the complement system (24). Furthermore, chemotactic complement components might then attract more leukocytes into the parenchyma with further increase in inflammatory foci and demyelination, associated with pronounced perivascular tissue damage, marked edema, and the high number of polymorphonuclear leukocytes seen within the infiltrates. However, not all white matter-specific antibodies appear to induce demyelination as monoclonal antibodies against MBP did not modify disease course or histopathology of EAE in Lewis rats (H. J. Schluesener, unpublished observation). In addition, it has been reported that antibodies against proteolipid protein, the major CNS protein, or against myelin-associated glycoprotein did not induce demyelination or inhibit myelination, although such antibodies have been shown to bind to CNS tissue (25–28). Demyelination has been induced in vitro by antibodies against galactocerebroside, but in this case demyelination is affecting both CNS and peripheral nerve tissue (21).

It remains to be established whether antibodies against

[4] **Linington, C., M. Bradl, H. Lassmann, C. Brunner, and K. Vass.** 1987. Augmentation of demyelination in acute experimental allergic encephalomyelitis by a monoclonal antibody specific for a surface myelin antigen. *Submitted for publication.*

MOG or other CNS constituents play a role in demyelinating CNS disease in other species. In guinea pig EAE, a correlation between degree of demyelination and antibody titers to MOG has been reported (29). MOG appears to be a general constituent of myelin, at least in vertebrates (22), and might therefore be regarded as a potential autoantigen in a variety of species, including man.

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