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# *Augmentation of Demyelination in Rat Acute Allergic Encephalomyelitis by Circulating Mouse Monoclonal Antibodies Directed Against a Myelin/Oligodendrocyte Glycoprotein*

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**In this study the authors have developed a model with which can be studied directly the influence of circulating anti-myelin antibody on the clinical and pathologic course of inflammatory T-cell–mediated experimental allergic encephalomyelitis (EAE) in the rat. EAE was induced by passive transfer of either myelin basic protein (MBP)-activated spleen cells derived from sensitized donors or long-term-cultured MBP-specific T-cell lines. At the onset of the disease, monoclonal antibodies against a myelin/oligodendrocyte glycoprotein (MOG) were injected intravenously. This antigen is exposed on the surface of central nervous system myelin and oligodendrocytes. Intravenous injection of the antibody in the course of T-cell–mediated transfer EAE augmented the severity and duration of clinical signs and resulted in the formation of large, confluent demyelinated plaques. (Am J Pathol 1988, 130:443–454)**

EXPERIMENTAL allergic encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS), induced in susceptible animals by sensitization with either brain tissue[1] or purified myelin components such as myelin basic protein (MBP)[2] or proteolipid protein (PLP).[3] In its different acute and chronic variants, EAE is the animal model that most closely resembles inflammatory demyelinating diseases in man, including multiple sclerosis.[4,5]

Recent studies using antigen-specific T-lymphocyte cell lines and clones have demonstrated that autoreactive $CD4^+$ T lymphocytes ("helper/inducer" T-cells) induce EAE and may initiate both acute and chronic (relapsing) inflammatory lesions in the CNS.[6,7] The lesions seen in these T-cell-line–mediated models of EAE are accompanied by variable degrees of primary demyelination,[8,9] which in the case of the Lewis rat is restricted to occasional perivenous myelin sheaths.

However, indirect evidence suggests that immune reactions against CNS antigens other than MBP and PLP may play an additional role in the pathogenesis of actively-induced EAE, especially by augmenting demyelination. In general, demyelination is more pronounced when, instead of MBP, total CNS tissue or myelin is used for sensitization. Studies in chronic EAE models showed that addition of other CNS antigens to the MBP inoculum or parallel sensitization with MBP and nonencephalitogenic brain antigens results in pronounced demyelination.[10,11] Furthermore, some demyelination was noted in guinea pig acute EAE models when certain myelin lipids were incorporated in the MBP inoculum used for sensitization.[12,13] Circulating myelin-specific antibodies,[14,15,16] which are able to induce demyelination both *in vitro*[17,18] and *in vivo*,[19,20] have been described

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in animals with EAE. The titer of these autoantibodies is especially high during the chronic stage of chronic relapsing EAE, when demyelination is most pronounced[15,20,21,22]; and in one model of EAE a correlation between the serum myelination inhibitory activity *in vitro* and demyelination *in vivo* in the CNS has been described.[23]

The aim of the present study was to develop a model in which we can study directly the influence of circulating anti-myelin antibody on the clinical and pathologic course of inflammatory T-cell–mediated EAE in the rat. For this purpose we induced EAE by passive transfer of MBP-reactive lymphocytes. At the onset of the disease we injected a mouse monoclonal antibody, (8-18C5) which defines a minor myelin/oligodendrocyte glycoprotein (MOG)[24] that is biochemically distinct from other well-characterized myelin probes (MBP, PLP, or myelin-associated glycoprotein [MAG][24], is shown in the present study to recognize an epitope on the surface of CNS myelin sheaths and oligodendrocytes and can induce demyelination *in vivo* following intrathecal injection.[25]

## Materials and Methods

### Animals and Reagents

Inbred Lewis rats were obtained from the animal breeding facilities of the Max Planck Institut für Immunobiologie (Freiburg, FRG). Guinea pig MBP was prepared as described by Eylar and Jackson[26] and stored lyophilized at −80 C. Polyclonal mouse IgG and 8-18C5 IgG were isolated from normal mouse serum and ascitic fluid, respectively, by ammonium sulfate precipitation and further purified by ion-exchange chromatography on a 1.2 × 25-cm column of S-Sepharose (Pharmacia, Piscataway, NJ) using an NaCl gradient (10 mM to 1 M) in 10 mM Tris/HCl, pH 7.3, at a flow rate of 5 ml/min. The IgG preparations were dialyzed against phosphate-buffered saline (PBS) for 24 hours, concentrated by ultracentrifugation, adjusted to a final concentration of 5 mg/ml with PBS and stored at −20 C. Protein was determined by the method of Lowry et al.[27] Tuberculin-purified protein derivative (PPD) was obtained from Difco Laboratories (Detroit, Mich) and concanavalin A (Con A) from Yeda (Rehovot, Israel).

The monoclonal and polyclonal antisera used in this study were W3/13 (monoclonal, Sera Lab, UK, 1:400), which stains all rat T cells and granulocytes; 8-18C5 (monoclonal, 1:100)[24] for myelin/oligodendrocyte glycoprotein (MOG); NF (monoclonal, 1:400, Labsystems, Finland), specific for a phosphorylated 200-kd neurofilament epitope; PLP (polyclonal, 1:1000)[28]; MBP (polyclonal, 1:300)[29]; MAG (polyclonal, 1:300)[30]; glial fibrillary acidic protein, polyclonal, 1:100).[31] The appropriate species-specific biotinylated anti-mouse, anti-rabbit, and anti-sheep/goat immunoglobulins (Amersham, UK, 1:200) were used as secondary antisera, and binding was visualized by means of an avidin-conjugated horseradish peroxidase (HRP) reagent (Sigma Chemical Co., St. Louis, Mo, 1:70).

### Establishment of Rat MBP-Specific T-Cell Line

In essence, the method of Ben Nun et al[6] was followed. A Lewis rat was immunized with 50 μg guinea pig MBP emulsified in Freund's complete adjuvant (CFA, 4 mg/ml *Mycobacterium tuberculosis*, H-37RA, Difco). Draining lymph nodes were removed 9 days after sensitization, and single-cell suspensions were prepared. The lymphocytes were incubated for 3 days in complete medium (Dulbecco's modified Eagle's medium; GIBCO, Grand Island, NY), enriched with L-glutamine (2 mM), sodium pyruvate (1 mM), 1% vol/vol nonessential amino acids, 5 × $10^5$ M 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin (all additives from GIBCO), 1% normal fresh rat serum, and, as selecting autoantigen, rat MBP (20 μg/ml). Activated lymphoblasts were isolated by centrifugation on a discontinous bovine serum albumin gradient, washed, and cultured in complete medium supplemented with 15% T-cell growth factor.[32] A stable monospecific T-cell line was generated by periodically alternating antigen-dependent activation episodes with T-cell growth factor-driven propagation phases.[6]

### Induction, Modulation, and Quantitation of EAE

Experimental allergic encephalomyelitis was induced in female Lewis rats (body weight 150–200 g; 8–10 weeks old) by the passive transfer of freshly activated MBP-specific T-line blasts ($10^5$–4 × $10^6$) following antigen-specific activation for 72 hours as described above. Alternatively, naive Lewis rats were given intravenous injections at 5 × $10^7$ spleen cells derived from donors injected 12 days previously with 50 μg MBP in CFA. Prior to transfer, the spleen cells were cultured *in vitro* for 3 days in the presence of MBP (10 μg/ml).[33] Control animals received spleen cells from donors given CFA 12 days before, which had been cultured *in vitro* in the presence of PPD (10 μg/ml) or Con A (2.5 μg/ml)[34] for 72 hours prior to transfer.

Animals were examined daily for clinical signs of EAE, which was scored on the following scale: 0.5, partial loss of tail tone; 1, complete loss of tail tone; 2,

hind limb weakness; 3, hind limb paralysis; 4, moribund; 5, dead. At the onset of clinical disease animals were given intravenous injections of either 8-18C5 or polyclonal IgG (approximately 30 μg/g body weight) or PBS.

At appropriate times following cell transfer, animals were perfused with 4% paraformaldehyde in (PBS); and the brain, spinal cord and nerve roots, spleen, and kidneys were dissected, postfixed in the same fixative for 3 hours and finally embedded in paraffin. Neuropathologic evaluation was performed on paraffin sections, using hematoxylin and eosin (H&E), Giemsa, Klüver–periodic acid–Schiff myelin stain and Bodian or Bielschowsky silver impregnantions for axons. For ultrastructural analysis, small blocks of thoracic and lumbar spinal cord were fixed in 3% phosphate-buffered glutaraldehyde, osmicated, and embedded in Spurr resin. Immunocytochemical analysis of the lesions was performed with the antibodies described above. Inflammation and demyelination were quantified in a standardized region of the medulla oblongata adjacent to the fourth ventricle. In this region the numbers of perivascular infiltrates and the area of demyelination were evaluated over a total area of 15 sq mm. The results are expressed in numbers of perivascular infiltrates per square millimeter for inflammation or in square millimeters for the area of demyelination.

### Immunocytochemical Characterization of the 8-18C5 Target Antigen, MOG

The localization of the epitope of MOG recognized by the 8-18C5 antibody was studied immunocytochemically in tissue samples (brain, spinal cord and nerve roots, trigeminal ganglion, peripheral nerves, lymphatic tissue, liver, kidney, lung, and intestine fixed by perfusion with 4% paraformaldehyde in PBS) from the following animal strains and species: rats (Lewis, SD), mice (BALB/c, SJL/J), and guinea pigs (Hartley, Strain 13). Human cortex (adjacent to tumor biopsy) and human brain white matter and spinal cord (autopsy tissue) were also investigated. The expression of MOG during the development of the CNS was studied with paraformaldehyde-fixed brain and spinal cord tissue from 1-, 7-, and 14-day-old and adult SD rats.

### Immunocytochemical Techniques

Immunocytochemistry was performed on paraffin sections using a biotin–avidin peroxidase method, as described previously.[35,36] In brief, deparaffinized sections were incubated consecutively in 10% fetal calf serum in PBS and then in the following reagents, diluted in the same solution: 1) the primary monoclonal or polyclonal antibody, 2) a biotinylated species-specific anti-mouse or anti-rabbit immunoglobulin, and 3) an avidin HRP complex. Incubations were performed in a humid chamber for 1 hour at room temperature, and between each step the sections were rinsed extensively with PBS. Endogenous peroxidase was blocked with 0.2% hydrogenperoxide/methanol for 30 minutes immediately before incubation with the avidin peroxidase complex. Peroxidase was then developed with a diaminobenzidine reagent,[37] and sections were optionally counterstained with hematoxylin.

A similar technique was applied to thin razor blade-cut sections of fixed material for the immunoelectronmicroscopic localization of the 8-18C5 epitope. However, to achieve satisfactory results only recently fixed material could be used and each incubation step was prolonged to 8 hours, while washing was done overnight at 4 C and the hydrogenperoxide/methanol was omitted. After developing with diaminobenzidine, the material was osmicated and embedded in Epon.

In each of the immunocytochemical techniques described above the specificity was controlled by either omitting the primary antiserum or monoclonal antibody or by replacing the primary antiserum with either normal rabbit serum or a hyperimmune serum directed against another antigen (cytoskeletal proteins, neuropeptides). In the case of monoclonal antibodies, irrelevant antibodies of the same IgG class were used for controls.

Blood-brain barrier damage and the distribution of mouse immunoglobulins in rat brain lesions were visualized by means of a modified immunostaining procedure. Biotinylated species-specific anti-mouse and anti-rat immunoglobulin sera (Amersham, UK) were used as the primary antibody layer; but in order to increase sensitivity, a second layer of biotinylated anti-sheep/goat immunoglobulin (preabsorbed with normal mouse or rat serum) was added before incubation with the avidin peroxidase complex. Since considerable cross-reactivity exists between rat and mouse immunoglobulins, the following precautions were followed: The primary biotinylated anti-mouse or anti-rat antibodies are affinity-purified and extensively cross-absorbed against rat or mouse immunoglobulin, respectively. According to the manufacturer (Amersham UK) these antibodies do not cross-react between mouse and rat as tested by ELISA. In immunocytochemistry on paraffin sections the anti-mouse antibody did not recognize rat immunoglobulins in rat lymphatic tissue even when used in tenfold

concentration to that necessary for the detection of mouse immunoglobulins. To further control the problem of possible cross-reactivity, immunostaining was performed by diluting all antisera and reagents with 10% normal rat serum (for detection of mouse immunoglobulins) and 10% normal mouse serum (for rat immunoglobulins).

## Results

### Localization of MOG in the Mammalian CNS

A study at the light-microscopic level demonstrated that the 8-18C5 epitope of MOG is stable to fixation with paraformaldehyde and that the distribution of the antigen in fixed CNS tissue is identical to that seen in native frozen sections.[24]

In the CNS of rats the 8-18C5 antibody not only identified myelin sheaths in the white and gray matter (Figure 1a), but also stained oligodendrocytes (Figure 1b) and satellite cells. Peripheral nervous system myelin and Schwann cells did not react with the antibody (Figure 1a), and no reactivity was detected in tissues other than the CNS (lymphatic organs, liver, kidney, intestine). An identical distribution of the antigen was found in mice, guinea pigs, and man.

During CNS development the reactivity of oligodendrocytes and myelin with the 8-18C5 antibody appears to parallel myelination: prior to the formation of myelin sheaths glial cells did not express detectable levels of MOG.

Immunoelectron microscopy on tissue blocks of the developing rat CNS revealed that the 8-18C5 epitope was located on the extracellular surface of oligodendroglia plasma membranes (on perikarya and processes) as well as the outer surface of the developing myelin sheaths (Figure 1c). In adult animals the distribution of the antigen was altered slightly, staining was restricted almost entirely to the surfaces of the myelin sheaths and adjacent oligodendrocyte processes, and the surface of oligodendrocyte perikarya showed either weak reactivity or remained unstained. In adult human brain the localization of the antigen was at the electron-microscopic level identical to that seen in adult rat CNS (Figure 1d).

No surface staining of myelin or oligodendrocytes was noted in any of the experiments when monoclonal antibodies of irrelevant specificities and the same IgG class were used as controls (eg, Ox6 and Ox8, Sera Lab). Furthermore, polyclonal antisera specific for two other myelin antigens (MBP, PLP) did not reveal reactivity on either the oligodendrocyte plasma membrane or on the surface of the myelin sheath.

### Modulation of EAE by 8-18C5: Effect on Disease Severity and Duration

The effect of intravenous administration of 8-18C5 was studied in two models of passively transferred EAE. In the first, intravenous injection of MBP-activated spleen cells into naive recipients resulted in mild EAE, starting 6 days after injection of the cells. The peak of the disease was reached 5 days after onset of clinical signs, after which the animals rapidly recovered (Figure 2a). A similar course was observed in the second model, in which disease was induced by the transfer of freshly activated MBP-specific T-line cells. In this model the onset of disease was slightly earlier, only 4 days after cell transfer (Figure 2d).

The effect of intravenous injection of 8-18C5 at the onset of clinical signs of EAE was virtually identical in both models. Within 48 hours those animals receiving 8-18C5 exhibited more severe signs of disease than the controls treated either with the same dose of polyclonal mouse IgG or a sham injection of PBS (Figure 2). Those animals with EAE induced by the transfer of sensitized spleen cells and also given 8-18C5 antibody were still clinically ill at the time of sacrifice (6 days after onset of the disease), whereas all the control animals had recovered. The prolonged duration of EAE following treatment with the antibody was more clearly demonstrated when animals with T-cell-line–mediated EAE were allowed to survive for up to 22 days after the onset of EAE: treatment with 8-18C5 not only enhanced the clinical severity of the disease but also doubled its duration (Figure 2d). Intravenous injection of the 8-18C5 antibody into either normal Lewis controls or animals treated with mitogen- or PPD-activated spleen cells did not result in clinical disease.

### Modulation of EAE: Pathology

Pathologically, in the absence of 8-18C5 monoclonal antibody, both models of passive transfer EAE resulted in the formation of predominantly inflammatory lesions in the CNS (Tables 1–3). The development of these lesions was reflected in the course of clinical disease. Lesions were characterized by the formation of perivenous inflammatory cuffs (Figure 3b and c) with some dispersion of T lymphocytes and other hematogenous cells into the surrounding parenchyma (Figure 3b) and reactive gliosis in perivascular areas. Demyelination was either absent (Figure 3a and c), or restricted to a small number of perivascular nerve fibers (Figure 4a, Table 2).

In EAE animals treated with 8-18C5 antibody, the distribution of inflammatory infiltrates was similar, compared with the EAE controls (Figure 3g and h),

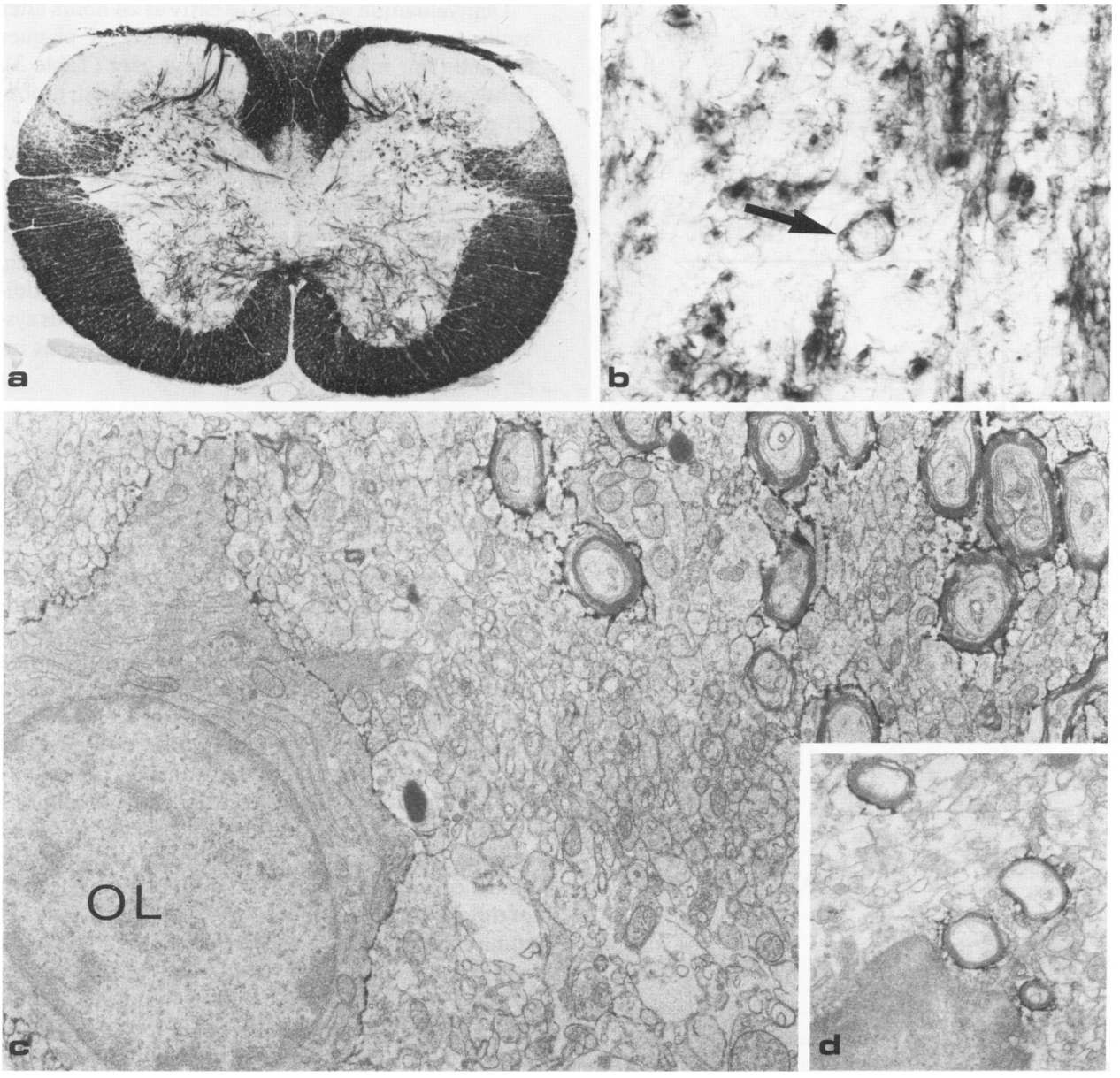


**Figure 1**—Immunocytochemical distribution of MOG in the normal CNS. **a**—Sprague–Dawley rat, 21 days old, thoracic spinal cord; 8-18C5 immunoreactivity on myelin sheaths of the white and gray matter; no staining of peripheral myelin sheaths in spinal roots. (Paraffin section, ×30) **b**—Sprague–Dawley rat, 14 days old, brain stem; myelinated fibers and oligodendrocyte (*arrow*) with 8-18C5 reactivity. (Paraffin section, ×950) **c**—Sprague–Dawley rat, 14 days old, periventricular white matter; 8-18C5 reactivity on the surface of myelin sheaths and on oligodendrocyte (*OL*) perikarya and processes. (Block staining technique, ×17,000) **d**—Cortical biopsy adjacent to human brain tumor (female, 46 years old); 8-18C5 immunoreactivity on the surface of myelin sheaths and oligodendrocyte processes. (Block staining technique, ×9000)

although the number of perivascular cuffs was increased (Tables 1 and 2). The most pronounced difference in the pathology of passive transfer EAE with or without 8-18C5 antibody was found in the degree of demyelination associated with the lesions. Intravenous injection of 8-18C5 antibody at the onset of the disease resulted in massive increase in the extent of demyelination (Tables 1–3). Perivenous demyelination was observed in all brain and spinal cord sections; in the latter, pronounced loss of myelin was also present in subpial areas (Figure 4b). In some locations, especially in the periventricular areas of the cerebellum and medulla oblongata, confluent plaques of demyelination were found (Figure 3d). The demyelinated lesions contained numerous phagocytes with myelin degradation products (Figures 3e and 4b and c), together with many reactive astrocytes (Figures 3i and 4c and d). Axons were in general well

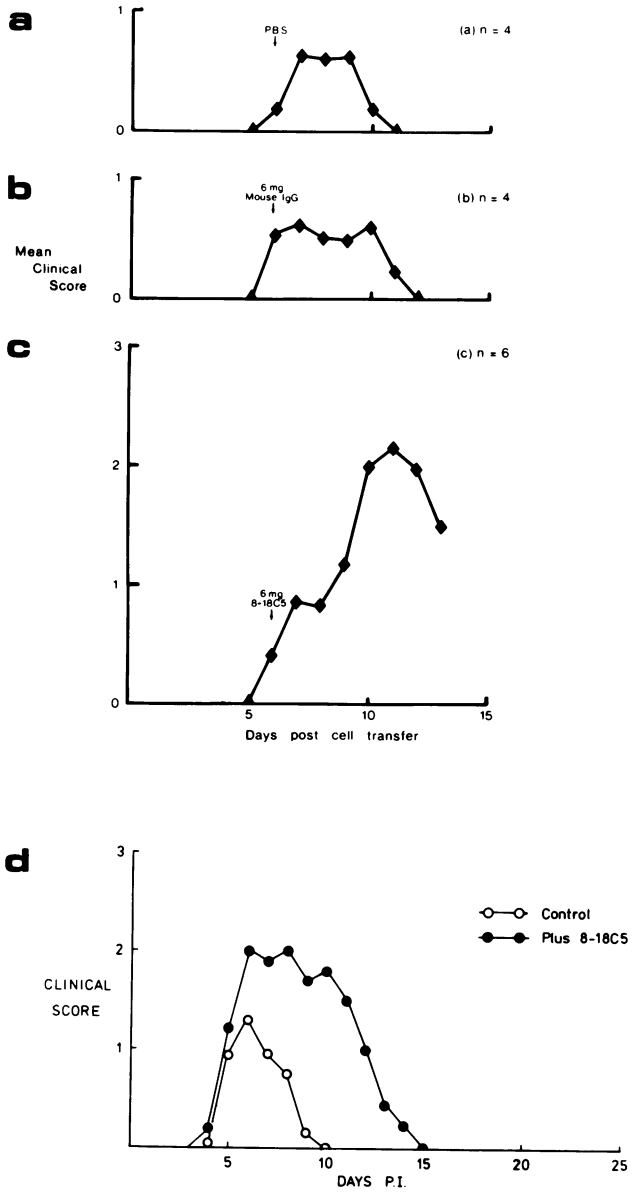


**Figure 2**—Clinical disease of transfer EAE in the presence or absence of circulating 8-18C5 antibody. The values represent the average clinical scores of 4 animals in **a** and **b**, 6 animals in **c**, and 12 animals in each group of **d**. **a–c**—Intravenous injection of $5 \times 10^7$ MBP-activated spleen cells on Day 0. The animals were then treated on Day 6 with either PBS (**a**), 6 mg polyclonal normal mouse IgG (**b**), or 6 mg purified 8-18C5 antibody (**c**). Note the potentiation of clinical signs by circulating 8-18C5 antibody. **d**—Intravenous injection of $10^8$ freshly activated MBP-specific T-line cells followed by intravenous injection of 30 μg/g body weight of either purified 8-18C5 antibody (*dark dots*) or polyclonal normal mouse IgG (*open circles*).

preserved (Figures 3f and 4b–d), although some axonal spheroids were noted. Immunohistologic visualization of various different myelin antigens (MBP, PLP, MAG, and MOG) reflected a loss of myelin sheaths in the lesions like that seen in conventional myelin stains. There was no preferential loss of any one of these myelin antigens in the lesions at any time studied.

Demyelination was noted as early as 24 hours after antibody injection, and the demyelinated plaques reached their maximum size 6 days later (Table 3). Subsequently, the size of the lesions decreased, probably due to remyelination (Figure 4c and d).

These demyelinating lesions were restricted to the CNS, no demyelination was noted in those areas of the peripheral nervous system examined (spinal roots, trigeminal ganglia, or sciatic nerve), and no pathologic alterations were found in the liver, lung, kidney, and lymphatic organs of the animals. Additionally, no pathologic alterations in the nervous system were found in normal rats after intravenous injection of 8-18C5 antibody (Table 1).

### Distribution of Rat and Mouse Immunoglobulins in the Lesions

Immunocytochemistry for rat immunoglobulins reflected the massive blood–brain barrier damage that has previously been reported in animals with EAE.[38-40] The distribution of rat IgG was similar in all experimental groups: immunoreactivity was accentuated in the gray matter and in perivascular areas of the white matter (Figure 5a). A similar, but much fainter staining for mouse immunoglobulins was seen in those EAE animals injected with polyclonal mouse IgG 24 to 48 hours earlier. In contrast, in animals injected 24 to 48 hours earlier with 8-18C5 antibody, mouse IgG was mainly observed in the white matter in broad perivenous sleeves and in the subpial region of the spinal cord (Figure 5b). At later times after injection of the monoclonal antibody (4–6 days) only traces of mouse immunoglobulins were present in the lesions, and this was seen mainly within perivascular macrophages. Areas containing mouse immunoglobulins 24–48 hours after antibody injection were also those in which demyelination was seen at later stages of the disease.

The clearance of the antibody from the circulation was similar in normal and EAE animals. The half-life, as determined in an anti-MOG ELISA (Linington and Lassmann, submitted) was less than 1 day.

## Discussion

Our observations on the ultrastructural distribution of MOG extend previous results with the 8-18C5 antibody[24] in showing the localization of this epitope on the surface of myelin and oligodendrocytes. This is especially important because the requirement for a myelin antigen involved in antibody-mediated demyelination is the accessibility for the immune system, which means the localization on the surface. The

Table 1—Modulation of Acute EAE by Intravenous Injection of 8-18C5: Passive Transfer With EAE Spleen Cells (SC)

| | SC + 8-18C5 (n = 6) | SC + pIgG (n = 4) | SC (n = 4) | 8-18C5 (n = 3) |
|---|---|---|---|---|
| Perivascular cuffs | 0.81 ± 0.18* | 0.28 ± 0.13 | 0.37 ± 0.12 | 0 |
| Demyelination | 0.71 ± 0.27* | 0.01 ± 0.01 | 0.02 ± 0.01 | 0 |
| Demyelination type | Plaques | Perivenous | Perivenous | 0 |

*$P < 0.01$, compared with control groups (SC + pIgG; SC), evaluated by U-test (Mann–Whitney).

Neuropathologic evaluation was performed 7 days after antibody injection (13 days after spleen cell transfer). Perivascular cuffs, number of perivenous inflammatory infiltrates per square millimeter of medulla oblongata. Demyelination, size of demyelinating lesions ($mm^2$) in the periventricular area of the medulla oblongata.

Table 2—Modulation of Acute EAE by Intravenous Injection of 8-18C5 Passive Transfer With 4 × $10^6$ MBP-Reactive T-Cell Line Cells (LBP)

| | LBP + 8-18C5 (n = 9) | LBP + pIgG (n = 6) |
|---|---|---|
| Perivascular cuffs | 4.4 ± 0.6* | 3.4 ± 0.6 |
| Demyelination | 0.53 ± 0.17† | 0.05 ± 0.04 |

*$P < 0.01$, compared with control group.

†$P < 0.0005$, compared with control group (LBP + pIgG), evaluated by Student $t$ test.

Neuropathologic evaluation was performed 2–6 days after antibody injection (5–9 days after transfer of LBP cells). Perivascular cuffs, number of perivenous inflammatory infiltrates per square millimeter of medulla oblongata. Demyelination, size of demyelinating lesions ($mm^2$) in the periventricular area of the medulla oblongata.

present data, however, do not exclude that the antigen is also present in compacted myelin, because penetration of antibodies and immunocytochemical reagents into the compacted myelin lamellae is limited in block staining techniques, used in these experiments.

The concept that antibodies against surface epitopes on the myelin sheath may play an important role in the pathogenesis of demyelination in inflammatory demyelinating disease has been suggested in a number of studies. Sera from animals sensitized with CNS antigens have been shown to induce demyelination *in vitro*[17,18,41–43] and *in vivo* after injection into the cerebrospinal fluid,[20,44] the vitreous of the rabbit eye,[19,45] the optic nerve,[46] and the spinal cord.[47] This demyelinating activity is induced by immunoglobulins and can be mediated via complement[41,48] or via a cooperation with cellular immune reactions.[19,45] Furthermore, in a recent study on a new model of demyelinating EAE, a correlation between the severity of demyelination *in vivo* and the activity of the respective sera to inhibit myelination *in vitro* has been described.[23] These observations led to the concept that demyelination, at least in some models of EAE, may be induced by a cooperation of cell-mediated and humoral immune responses.[4,19,45,49,50] In this study we show for the first time that demyelination in EAE induced by intravenous injection of monospecific MBP-reactive T-cell lines can be significantly enhanced by intravenous injection of a monoclonal antibody directed against an antigen located on the myelin surface.

Antigens involved in immune-mediated demyelination on which most attention has focused are the various glycolipids (galactosyl ceramide, sulfatides, and gangliosides), which can act as the target for antibody-mediated demyelination both *in vitro*[51–53] and *in vivo*.[46,54,55] However, a number of experiments have demonstrated serum demyelinating activity in

Table 3—Time Course of Inflammation and Demyelination in Passive Transfer EAE Modulated by 8-18C5 Antibody: Passive Transfer With $10^6$ MBP Reactive T-Cell Line Cells (LBP)

| | Days after antibody injection | | | |
|---|---|---|---|---|
| | 1 | 6 | 14 | 22 |
| Perivascular cuffs | | | | |
| LBP + 8-18C5 | 3.8 ± 1.6 | 4.0 ± 0.7 | 0.6 ± 0.3 | 0.2 ± 0.08 |
| LBP + pIgG | 2.3 ± 1.4 | 1.9 ± 0.8 | 0.5 ± 0.1 | 0.3 ± 0.2 |
| Demyelination | | | | |
| LBP + 8-18C5 | 0.23 ± 0.09 | 0.25 ± 0.07 | 0.11 ± 0.04 | 0.07 ± 0.01 |
| LBP + pIgG | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 | 0.01 |

Each number represents the mean value obtained from 3 animals. Perivascular cuffs, number of perivenous inflammatory infiltrates per square millimeter of medulla oblongata. Demyelination, size of demyelinating lesions ($mm^2$) in the periventricular area of the medulla oblongata.

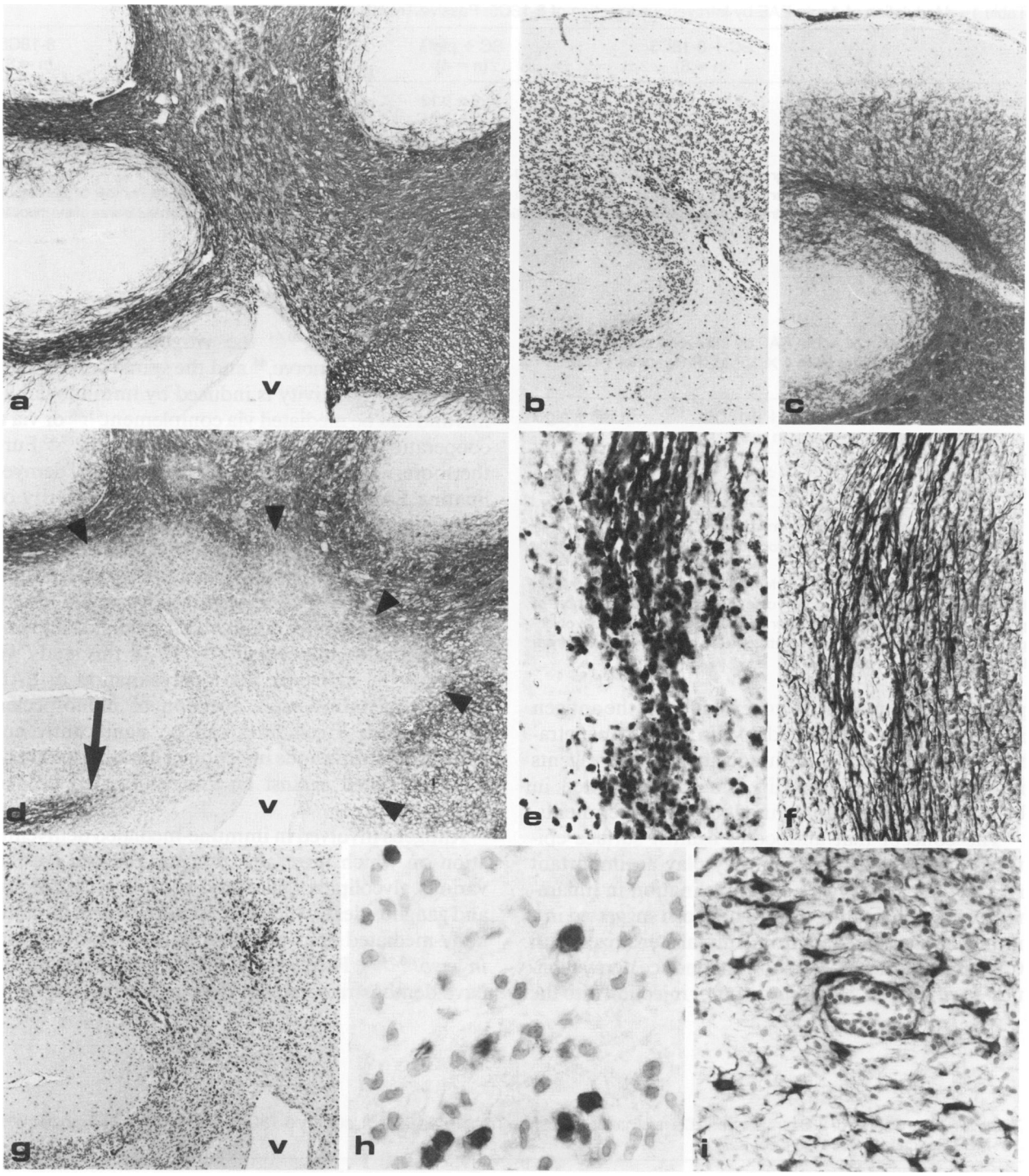


**Figure 3a–c**—Lewis rat injected with $10^5$ freshly activated MBP-specific T-line cells plus polyclonal mouse IgG. **a**—Periventricular area in the cerebellum with normal density of myelin in the white matter and in the area of the dentate nucleus. *v*, fourth ventricle. (Paraffin section, immunostaining with PLP serum, ×40) **b**—Cerebellum of the same animal shown in **a**; perivenous inflammation and some dispersion of inflammatory cells in the CNS tissue. (Immunostaining with W3/13, nuclei counterstained with hematoxylin, ×50) **c**—Serial section adjacent to **b**, immunostained with anti-PLP serum; perivascular inflammatory infiltrates with very limited loss of myelin in perivascular areas. (×50) **d–i**—Lewis rat treated with $10^5$ freshly activated MBP-reactive T-line cells plus 8-18C5. **d**—Same periventricular area of the cerebellum as shown in **a**; large confluent periventricular demyelinated plaque in the cerebellar white matter and dentate nucleus; the plaque borders are indicated by triangles; some perivenous extensions in the periphery of the plaque. the *arrow* indicates the area shown in **e** and **f**. (Paraffin section, immunostained with PLP serum, ×40) **e**—Edge of the demyelinated plaque, shown in **d**; numerous cells with myelin degradation products. (Paraffin section immunostained with MBP serum, ×250) **f**—Serial section adjacent to **e** (Bielschowsky silver impregnation); the axons are well preserved in the lesion in spite of total demyelination. (Interference contrast, ×250) **g**—Detail from the cerebellar lesion shown in **d**; perivenous inflammatory infiltrates and dispersion of inflammatory cells in the tissue. (Immunostaining with W3/13 (T-lymphocytes), nuclei counterstained with hematoxylin, ×50) **h**—Detail from **g**; T lymphocytes with immunoreactivity on their surface, ×550) **i**—Center of the plaque shown in **d**; immunostaining with GFAP antiserum shows pronounced reactive gliosis in the lesions. (×250)

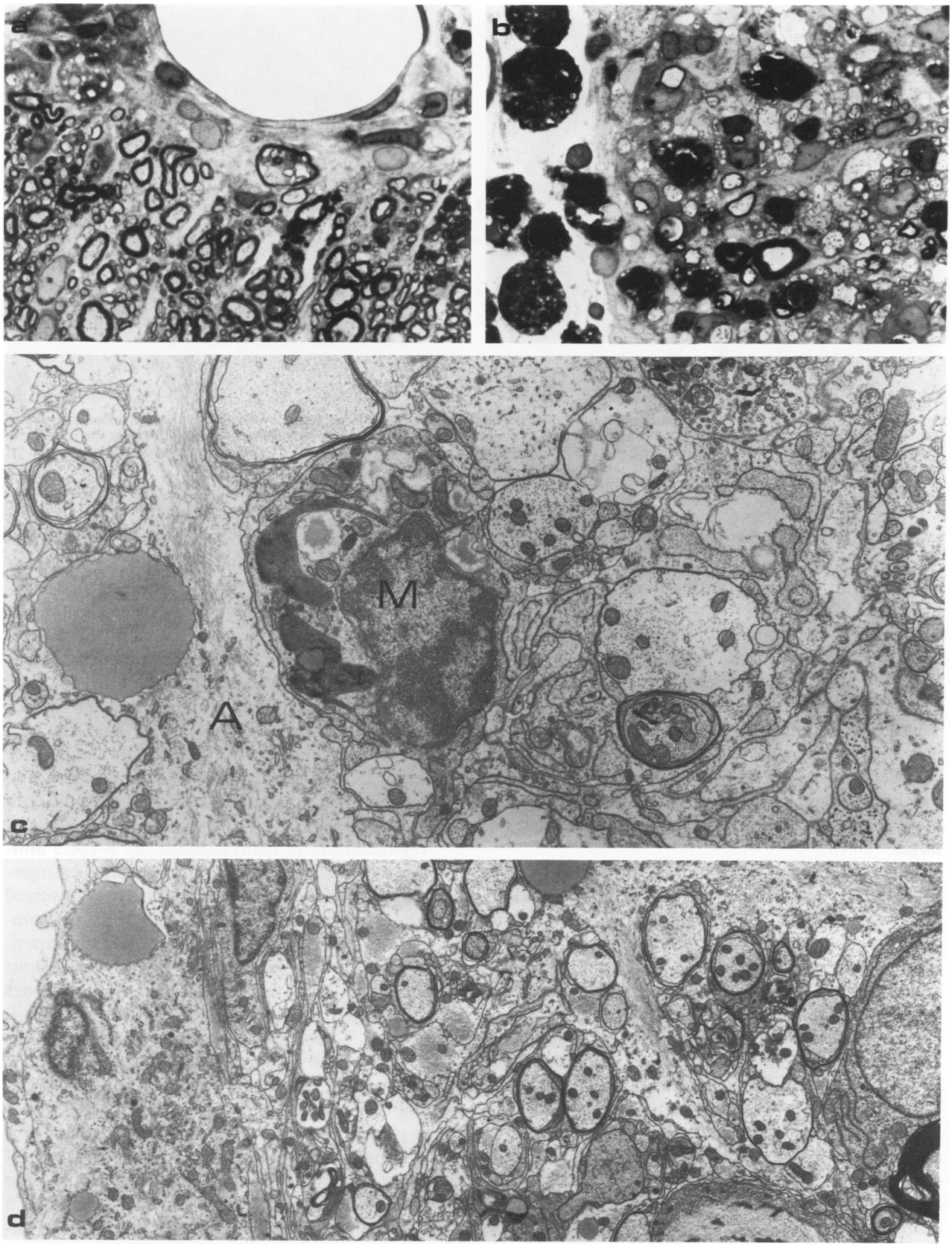


**Figure 4**—Spinal cord lesions of animals given $10^5$ freshly activated MBP-reactive T-line cells in the presence (**b**, **c**, and **d**) or absence of intravenously injected 8-18C5 antibodies. **a**—Subpial surface of the spinal cord with meningeal inflammation and single demyelinated nerve fibers in an animal given T-cells plus irrelevant IgG. (Toluidine blue, ×1000) **b**—Subpial surface of the spinal cord of an animal given encephalitogenic T-cells plus 8-18C5 antibodies; primary demyelination with preservation of axons and numerous macrophages with degradation products in the spinal cord tissue and in meninges. (Toluidine blue, ×1000) **c**—T-cell-mediated EAE plus 8-18C5 antibody, 6 days after antibody injection; subpial spinal cord tissue with demyelinated axons, early remyelination, reactive astrocyte (**A**) and a macrophage with lipid debris (**M**). (×11,000) **d**—T-cell-mediated EAE plus 8-18C5 antibody, 14 days after antibody injection, subpial spinal cord with extensive gliosis and numerous remyelinating axons. (×4200)

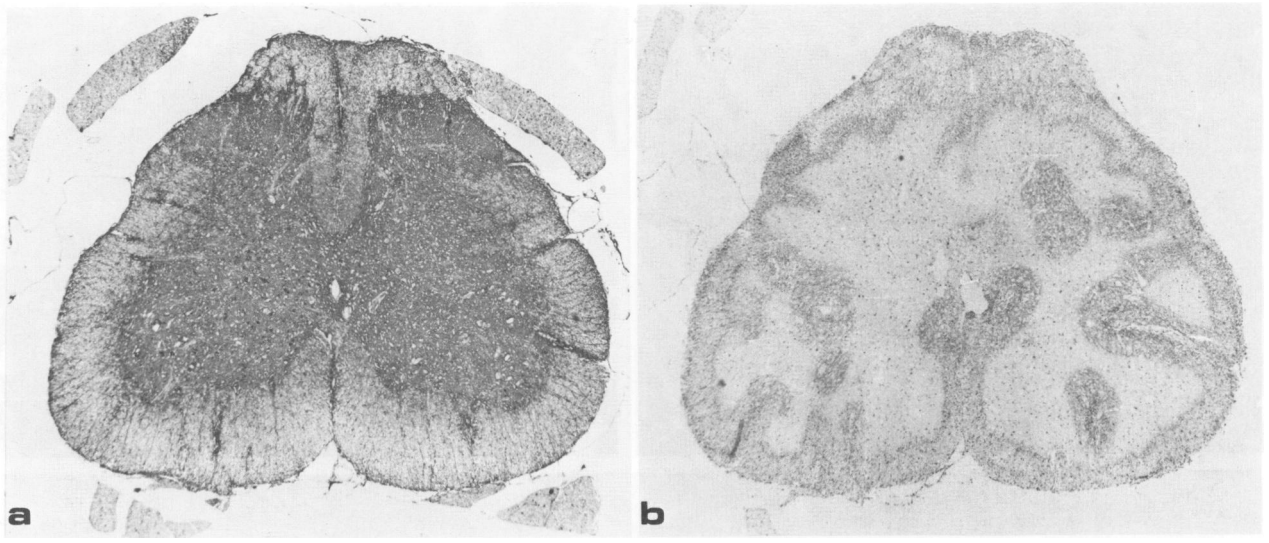


**Figure 5**—Lewis rat given 5 × $10^7$ MBP-activated spleen cells followed by intravenous injection of 8-18C5 antibody; distribution of rat and mouse immunoglobulins in the spinal cord 24 hours after antibody injection. **a**—Immunostaining for rat immunoglobulins shows reactivity throughout the spinal cord, accentuated in the gray matter and in perivascular areas. (×50) **b**—Adjacent serial section immunostained for mouse immunoglobulins; reactivity on the subpial surface of the cord and in perivenous areas. (×50)

the absence of anti-myelin glycolipid antibodies,[15,43,56] indicating that other myelin antigens may be important targets for antibody-mediated demyelination.

For antibody responses to mediate demyelination several criteria have to be fulfilled, the most important of which is the requirement that the target epitope is accessible to the humoral immune response. This means that not only must the antigenic determinant be exposed on the surface of the myelin sheath or oligodendrocyte, but the blood–brain barrier must either be breached, or intrathecal synthesis of the antibody must occur, so that the antibody can reach its target.

These criteria are met in the models we have chosen in this study. The blood–brain barrier is disrupted in the course of EAE,[38–40] allowing circulating serum components to enter the CNS. Moreover, the immunocytochemical data on the localization of MOG and, more importantly, the epitope recognized by the 8-18C5 antibody demonstrate that it is exposed on the myelin surface of the rat. On the basis of biochemistry and immunocytochemistry, this antigen is distinct from the other well-characterized myelin proteins MBP,[57] PLP,[58,59] MAG,[60,61] and cyclic nucleotide phosphodiesterase.[62] It is of further interest that this antigen is exclusively present on CNS myelin and oligodendrocytes but absent from peripheral myelin and Schwann cells. This antigen thus fulfills the requirements for a target antigen in CNS-specific demyelination.

Interestingly, the initiation of demyelination by the antibody led to a slight increase in the numbers of inflammatory infiltrates in the CNS and increased the severity and duration of disease. This suggests a synergistic effect of antibody-mediated demyelination and T-cell–mediated inflammation in the clinical response to EAE. This view is further supported by the fact that in spite of equal antibody doses, the total extent of demyelination was different in the various experimental groups (see Tables 1–3). Thus, the final extent of demyelination may depend on the balance between T-cell effects (degree of inflammation and activation of monocytes/macrophages) and the concentration of circulating anti-myelin antibodies.

Augmentation of demyelination by 8-18C5 antibody was also found when EAE was started by injection of MBP-activated spleen cells from sensitized donors. These induced a milder form of EAE as compared with T-line cells in the present series of experiments. This is believed to be due to the heterogenous nature of the spleen cell preparation, which includes macrophages, secreting factors suppressive to T-cell activation. To avoid these problems, we have thus used in most of our present study a monospecific T-cell line that allows a more accurate standardization of the experiments.

At present, we may only speculate on whether a similar mechanism plays a role in the pathogenesis of multiple sclerosis. However, it is of interest to note that the epitope of MOG recognized by the 8-18C5 antibody is highly conserved across a number of different species and is also present and exposed at the surface of the human myelin membrane. This sug-

gests that appropriate anti-MOG responses could play a role in the development of demyelinating diseases in man.

In conclusion, in this study we were able to show for the first time that a circulating antibody response to a minor myelin component can, in combination with an inflammatory CNS disease, lead to extensive primary demyelination. In a more general perspective, these results suggest that circulating antibodies specific for epitopes exposed on the surface of CNS elements pose a potential hazard, complicating the clinical course of inflammatory CNS disease.

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