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The Demyelinating Potential of Antibodies to Myelin Oligodendrocyte Glycoprotein Is Related to Their Ability to Fix Complement

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A panel of 13 monoclonal antibodies (mAbs) has been raised to the central nervous system-specific glycoprotein, myelin oligodendrocyte glycoprotein; five of these mAbs recognize a carbohydrate epitope on the molecule. Although all of the mAbs recognized surface epitopes on cultured oligodendrocytes and stained central nervous system tissue sections in a similar manner, marked differences were seen in their ability to induce demyelination in experimental allergic encephalomyelitis in the Lewis rat. This variation in pathogenic potential was not related to the specificity of a given mAb for carbohydrate or peptide epitopes of myelin oligodendrocyte glycoprotein, but correlated with its ability to fix complement. (Am J Pathol 1993, 143:555–564)

Experimental allergic encephalomyelitis (EAE) is an autoimmune-mediated inflammatory demyelinating disease of the central nervous system (CNS) originally induced in susceptible species following immunization with CNS tissue in adjuvant.[1] In EAE extensive demyelination is associated with meningeal and perivascular infiltrates of inflammatory cells, parenchymal edema, and blood-brain barrier (BBB) dysfunction.[2,3] The development of this demyelinating pathology is dependent on synergy between T-cell and B-cell responses to myelin autoantigens,[4,5] the effector mechanisms most clearly analyzed in the Lewis rat. In this species the inflammatory aspect of EAE is initiated by an autoimmune T-cell response to myelin antigens, in particular myelin basic protein (MBP).[6] The passive transfer of MBP-specific T cells induces an acute inflammatory response in the CNS, associated with an increased permeability of the BBB to serum proteins, but minimal demyelination.[7] Extensive primary demyelination in this model is completely dependent on the presence of an antibody response to determinants exposed at the surface of the myelin sheath at the time that the inflammatory response disturbs BBB function. This was formally demonstrated in a cotransfer model in which rats with T-cell-mediated EAE were injected intravenously with the myelin oligodendrocyte glycoprotein (MOG)-specific monoclonal antibody (mAb) 8-18C5.[8] This mAb recognizes an epitope of MOG that is exposed at the outermost surface of the myelin sheath[8] and exhibits complement-dependent demyelinating activity *in vitro*.[9] Normally, circulating antibody is excluded from the CNS by the BBB and is therefore not pathogenic. However, in rats with EAE the mAb can penetrate into the CNS and initiate extensive demyelination, producing lesions similar to those seen in multiple sclerosis.

The involvement of complement-dependent mechanisms in the pathogenesis of both MBP-mediated EAE and in antibody-mediated demyelination has been noted in several studies,[10–13] and antibody-mediated demyelination has been associated with the deposition of granular deposits of C9, indicative of membrane attack complex (MAC) formation.[14] However, *in vivo* complement depletion studies suggest that the MAC is not essential to the pathogenesis of demyelination;[10] the major effector mechanism responsible for antibody-mediated demyelination *in vivo* is a macrophage-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) response [15,16] This re-

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sponse is enhanced by pro-inflammatory mediators formed during the antibody-mediated activation of the early complement components in the lesions[10] and by opsonization of the myelin sheath by complement.[17]

In the present study we have utilized a panel of MOG-specific mAbs to further investigate the relationship between the ability of anti-MOG mAbs to fix complement and their pathogenicity *in vivo*.

Materials and Methods

Myelin Isolation

Myelin was isolated from human, rat, and bovine brain, lyophilized, and stored at −20 C.[18]

Preparation of Lentil Lectin-Binding Glycoproteins

Myelin lentil lectin-binding glycoproteins (LLBG) were isolated by affinity chromatography of the deoxycholate-soluble fraction of CNS myelin. Briefly, CNS myelin was homogenized at 2 mg protein/ml in 10 mmol/L Tris-HCl (pH 8) containing 2% sodium deoxycholate (DOC) and 0.05% leupeptin, stirred overnight at 4 C, and then centrifuged for 30 minutes at 15,000 × *g*. The supernatant was depleted of MBP on a 20-ml Bio-Rad Affi-Gel Blue column previously equilibrated in 100 mmol/L Tris-HCl (pH 9.3) containing 0.05% DOC. The MBP-depleted run-through was dialyzed against 10 mmol/L Tris-HCl (pH 8) containing 0.05% DOC and then applied to a 20-ml column of lentil lectin Sepharose 4B (Pharmacia-LKB, Milton Keynes, England) previously equilibrated in the same buffer. Myelin LLBG were eluted in buffer containing 0.3 mol/L methyl-α-D-mannopyrannoside. LLBG-containing fractions were pooled, concentrated by ultrafiltration in a stirred cell (Amicon, Danvers, MA), and stored at −20 C.

Purification of MOG

Bovine MOG was purified on an affinity column of 8-18C5 anti-MOG mAb coupled to CNBr-activated Sepharose 4B (Pharmacia). Bovine white matter was extracted in 10 mmol/L Tris (pH 8) containing 4% DOC and 0.05% leupeptin as described for LLBG. The DOC-soluble fraction was cycled three times over an 8-18C5 affinity column previously equilibrated in the same buffer.

The column was washed with 10 column volumes of 10 mmol/L Tris (pH 8) containing 1.0% DOC, 0.01% sodium dodecyl sulfate (SDS), and 0.15% NaCl, followed by 10 column volumes of 10 mmol/L Tris (pH 8) containing 0.05% DOC. MOG was eluted in 50 mmol/L diethylamine in 10 mmol/L Tris (pH 11.2) containing 0.05% DOC and immediately brought to pH 8 with 5 mol/L HCl. The eluate was dialyzed against 10 mmol/L Tris (pH 8) containing 0.5% DOC, concentrated by ultrafiltration, (Amicon) and stored at −20 C.

Analysis of this material by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting revealed that the only contaminants were traces of mouse IgG derived from the affinity column, together with variable amounts of myelin proteolipid protein.[19]

Immunization Protocol for the Generation of mAbs

Two groups of three BALB/c mice were immunized using standard protocols to provide lymphocytes for fusion: Group 1 was immunized with bovine lentil lectin-binding proteins (LLBG); Group 2 was immunized with partially purified bovine MOG.

Spleen lymphocytes were fused with AG8 myeloma cells by a modification of the method of Kohler and Milstein,[20] and the resulting hybridomas were cloned by limiting dilution. Clones were screened for reactivity against partially purified MOG by enzyme-linked immunosorbent assay, and any positive clones were subsequently screened for specific anti-MOG reactivity by Western blot. The isotypes of all of the antibodies generated were tested using a Serotec isotyping kit (Kidlington, Oxford, England).

Ascitic fluid was raised in pristane-primed BALB/c mice, and immunoglobulins were purified by affinity chromatography on ProSep A (Bioprocessing, Ltd., Consett, England).

Antibodies raised in response to MOG were given the prefix Y; those raised in response to LLBG were given the prefix Z.

Screening of Hybridoma Culture Supernatants by Enzyme-Linked Immunosorbent Assay

Microtiter plates were coated with LLBG at 10 μg/ml and then incubated with undiluted culture supernatants, and bound mAb was detected with a

peroxidase-conjugated second antibody (Jackson Immunoresearch Laboratories, Inc., West Grove, Pennsylvania).

Western Blot

Human, bovine, and rat LLBG were separated by electrophoresis on 12% acrylamide gels as described previously.[21] Proteins thus separated by SDS-PAGE were transferred onto Immobilon polyvinylidine difluoride transfer membrane (Millipore, Bedford, MA) at 5 mA/cm^2 for 20 minutes in 48 mmol/L Tris, 39 mmol/L glycine, 13 mmol/L SDS, and 20% methanol. Polyvinylidine difluoride sheets were cut into strips, each strip containing one lane each of human, bovine, and rat LLBG, and incubated in 2.5% low-fat dried milk in phosphate-buffered saline (PBS) for 20 minutes at room temperature, then sequentially were 1) incubated for 30 minutes at room temperature with each of the mAbs diluted in PBS (one mAb per strip); 2) washed for 15 minutes in 0.5% dried milk in PBS; 3) incubated with peroxidase-conjugated anti-mouse antibody (Jackson); 4) washed for 15 minutes in 0.5% dried milk in PBS; and 5) developed with 4-chloronaphthol.[22]

Isoelectric Focusing

Ten µl of each hybridoma supernatant were analyzed by isoelectric focusing on an agarose gel containing Pharmalytes 3-10 and 5-8 (Pharmacia-LKB). The gel was press-blotted onto nitrocellulose, and the mAbs were detected as for Western blot.

Deglycosylation of MOG and Western Blot with mAbs

To determine whether the Y and Z mAbs recognize carbohydrate epitopes, MOG was deglycosylated with a mixture of endoglycosidase F, glycopeptidase F, neuraminidase, and *O*-glycosidase (all enzymes from Boehringer-Mannheim, East Lewes, Sussex, England) to remove both *O*-linked and *N*-linked carbohydrate chains.

LLBG (30 µg) were incubated with 56 µl (2.8 U) endoglycosidase F containing glycopeptidase F, 12.5 µl (6.25 mU) *O*-glycosidase, 0.7 µl (7 mU) neuraminidase, and 315 µl buffer (20 mmol/L sodium phosphate, pH 7.2; 20 mmol/L sodium azide; 50 mmol/L EDTA; 0.5% v/v Nonidet P-40) for 24 hours at 37 C, as recommended by the manufacturer.

Intact and deglycosylated LLBG were separated by SDS-PAGE on a 15% gel. To determine whether all of the MOG had been deglycosylated, a Western blot was made as described above, with the modification that the incubation periods in mAb and conjugate were increased from 1 to 4 hours. The mAb used was Y10.

To detemine whether mAbs recognized carbohydrate epitopes of MOG, 2-µl drops of doubling dilutions (from 2.3 mg/ml) of intact and deglycosylated LLBG were spotted onto strips of nitrocellulose and incubated in 2.5% low-fat dried milk in PBS for 20 minutes at room temperature, then (sequentially) incubated with each of the mAbs at 10 µg/ml (one mAb per strip), washed, incubated with peroxidase-conjugated anti-mouse antibody, washed, and developed with 4-chloronaphthol as described above under Western Blot; again incubation periods in mAbs and conjugate were increased from 1 to 4 hours.

Staining of Rat Oligodendrocytes

Neonatal (7 day) rat oligodendrocytes were prepared by the method of Raff et al[23] and grown on glass coverslips. Oligodendrocytes were stained after 7 days of culture *in vitro*; cells of this age have been shown to be relatively mature, expressing galactocerebroside, MBP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase, and MOG.[24] Oligodendrocytes were stained live to determine whether mAbs recognized surface epitopes and then were fixed with acid-alcohol to allow staining of intracellular astrocyte glial fibrillary acidic protein (GFAP). Each coverslip was mounted on a pedestal and incubated with 150 µl of mAb at 50 µg/ml: all incubations were allowed to proceed for 30 minutes at 37 C and 5% CO_2 in a humidified atmosphere. Coverslips were washed by dipping serially into two containers of Earle's buffered salts (Gibco, Uxbridge, England) and draining of excess fluid on a tissue. Bound mAbs were detected by incubation with 150 µl of a 1/50 dilution of fluorescein isothiocyanate-conjugated anti-mouse IgG (Jackson). Cells were fixed with acid-alcohol for 15 minutes at 0 C, then incubated with 150 µl of a 1/200 dilution of anti-GFAP serum, followed by a further incubation with 150 µl of a 1/50 dilution of rhodamine-conjugated anti-rabbit IgG (Jackson). Coverslips were mounted face down in Citifluor (City University, London). Cells were identified as oligodendrocytes by their morphology and lack of reactivity with antisera to GFAP, a specific astrocyte marker.[25] Antiserum to

GFAP was a generous gift from Dr. M. L. Cuzner (Multiple Sclerosis Laboratories, London).

Staining of CNS and Peripheral Nervous System Tissue Sections for Light Microscopy

Paraffin-embedded sections of paraformaldehyde-fixed normal bovine, human, and rat tissue were stained with Y and Z mAb at 2 μg/ml by a previously described biotin-avidin peroxidase method,[26] in preparation for examining the distribution of MOG within the nervous system.

Staining of CNS Tissue Sections for Immuno-Electron Microscopy

Paraformaldehyde-fixed rat cortex was sectioned and immunostained with each of the Y and Z mAbs to determine whether the epitopes recognized were present on the surface of the myelin sheath and hence were potential targets for demyelination. The method used was as for light microscopy, with the modifications that incubation times were extended to 8 hours, washing was carried out overnight at 4 C, and the hydrogen peroxide/methanol step was omitted. After developing with diaminobenzidine, the material was osmicated and embedded in Epon.

Measurement of Ability of Y and Z mAbs to Fix Complement in Vitro

The abilities of the mAbs to fix complement was measured by a modification of the method of Mayer.[27] Sheep erythrocytes sensitized with Amboceptor antibody (Hoechst-Behring, Marburg, Germany) were used as targets, and rat serum was used as a source of complement. Rat serum was chosen in preference to mouse to mimic the situation in the EAE model.

Rat LLBG (10 μg) (as a source of MOG) was incubated with 1 or 2 μg mAb plus 100 μl 1/60 rat serum for 30 minutes at 37 C. One hundred μl of a 1% suspension of sensitized sheep erythrocytes in veronal-buffered saline (tablets from Oxoid, Ltd., Basingstoke, Hampshire, England) plus 0.1% gelatin were added and incubated for a further 30 minutes at 37 C. The mixture was centrifuged for 1 minute at 5000 × *g*, and the absorbance of the supernatant at 414 nm was measured. Equal amounts of distilled water and 1% sheep erythrocytes were mixed, incubated, and centrifuged, and the absorbance of the resulting supernatant at 414 nm was taken as 100% lysis. Dilutions of this supernatant were made and used as a standard curve against which all other lysis was measured; the lysis occurring in each tube was thus expressed as a percentage of distilled water lysis.

Pathogenicity of Y and Z mAbs in the EAE Model

The ability of the Y and Z mAbs to enhance disease severity and induce demyelination in EAE was examined in Lewis rats injected with MBP-specific T cells. Male Lewis rats weighing 180–200 g were each injected intravenously with 5×10^5 MBP-specific T cells (T-cell line LMBP 9,[8]). Three days later, when the rats began to show clinical signs of disease, groups of three rats were each injected intravenously with 3 mg of one of the Y or Z mAbs. Further groups of rats were also injected with 8-18C5 mAb, polyclonal mouse IgG (pcIgG) or PBS as controls. Animals were weighed and monitored daily for clinical signs of disease and were sacrificed and perfused with 4% paraformaldehyde for histological examination 48 hours after mAb injection. Paraffin-embedded sections were treated with Kluver stain and anti-C9 immunostaining[14] to show, respectively, demyelination and granular complement deposition indicative of MAC formation.

Results

Characterization of the MOG-Specific mAbs

The fusion using spleen cells from a mouse immunized with LLBG, generated eight stable clones designated Z1, Z2, Z3, Z4, Z6, Z7, Z8, and Z12. The second fusion, using spleen cells from a mouse immunized with MOG, generated 11 clones, designated Y1–Y11; of these, Y3 and Y5 ceased to produce antibody after a few weeks and were not stable to freeze-thawing. All clones were found to be of isotype IgG1 κ, with the exceptions of Z2, Z4, and Z12, which were IgG2α κ.

Each of the antibodies gave a different isoelectric focusing pattern, showing that each was the product of a different clone. Screening of hybridoma supernatants against bovine LLPG by Western blot demonstrated that (with the exceptions of Z1, Z3, Z6, and Z7) all of the clones recognized MOG as defined by the pattern obtained by Western blotting using the mAb 8-18C5. Those clones that were not

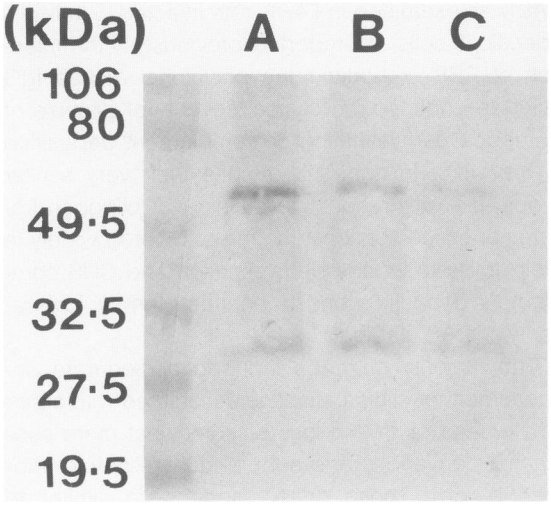

Figure 1. *Western blot demonstrating recognition of human* (Lane A), *bovine* (Lane B), *and rat* (Lane C) *MOG by mAb Z12.*

specific for MOG recognized a protein with properties similar to those of bovine MAG (Z3, Z6 and Z7) or an unidentified glycoprotein with an apparent molecular weight of approximately 80,000 (Z1).

All of the MOG-specific mAbs recognized epitopes present on rat, human, and bovine myelin, suggesting that the immunodominant B cell epitopes are highly conserved. A representative sample of the staining pattern obtained for a single clone, Z12, is shown in Figure 1.

A Western blot showed that no intact MOG remained after deglycosylation (Figure 2a); subsequent dot-blots showed that all 13 mAbs recognized both intact and deglycosylated MOG (Figure 2b). The size of the carbohydrate moiety removed was estimated to be approximately 1.5 kd.

The staining pattern obtained for each of the MOG-specific mAbs on both live oligodendrocytes and paraffin sections was indistinguishable from that given by 8-18C5, the only exception being Z8, which consistently gave a higher background staining. A particulate surface staining of oligodendrocyte cell bodies, processes, and membranous extensions was seen (Figure 3). Staining of paraffin sections of spinal cord demonstrated that the MOG-specific mAbs were specific for CNS myelin, and no staining of adjoining peripheral nervous system tissue was observed; the most intense staining was given by mAb Y10 (Figure 4a).

These results indicate that the epitopes recognized by these new anti-MOG mAbs have a distribution similar to that of 8-18C5, in that the epitopes recognized are exposed at the surface of the CNS myelin sheath. This was confirmed by

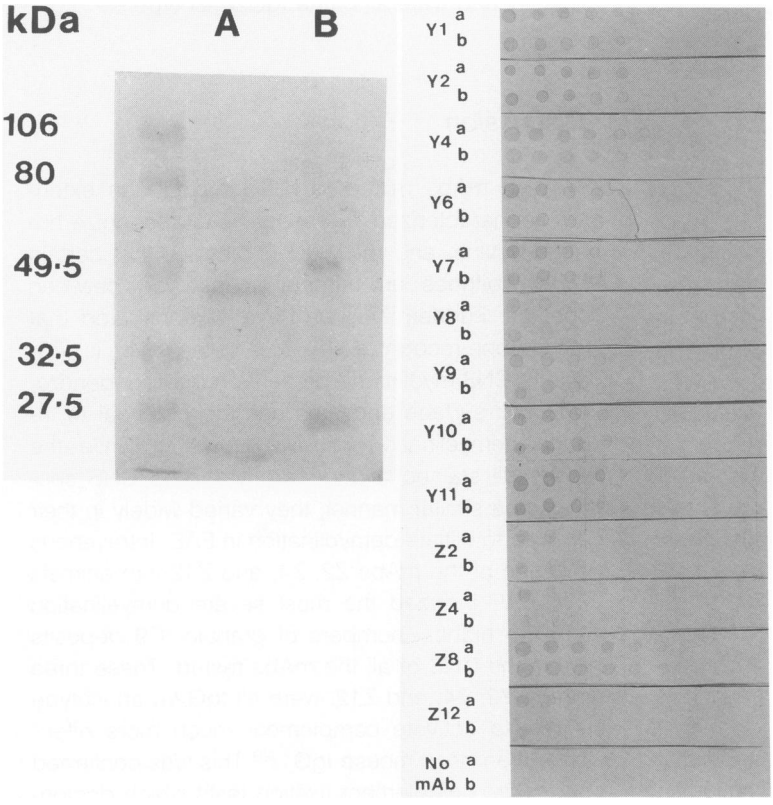

Figure 2. (a) *Western blot of deglycosylated* (Lane A) *and intact* (Lane B) *LLBG detected with mAb Y10, demonstrating that no intact MOG remained after deglycosylation.* (b) *Dot blot of each of the mAbs against intact* (Lane A) *and deglycosylated* (Lane B) *LLBG, demonstrating that all mAbs recognized the peptide moiety of MOG.*

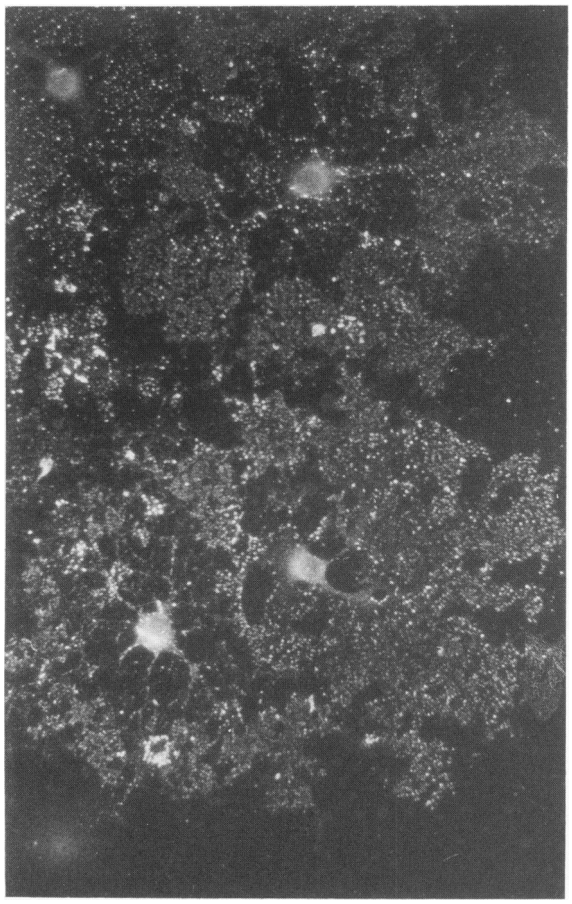

Figure 3. *Rat oligodendrocytes after 7 days in culture, stained with mAb Y10 and fluorescein isothiocyanate-conjugated second antibody (×40).*

immunoelectron microscopy in the cases of mAbs Y1, Y4, Y8, Y9, Y10, Z2, Z4, Z8, and Z12 (although staining with Y8 and Y9 was relatively weak). As described previously[28] MOG immunoreactivity was low and distributed over the surface of the myelin sheath (Figure 4b, 4c). The inability of several of the anti-MOG mAbs (Y2, Y6, Y7, and Y11) to stain myelin with this technique, in contrast to the positive results obtained by Western blotting and the staining of paraffin-embedded sections, suggests that the epitopes involved are lost during the extended preparation of the sections.

All of the mAbs tested were capable of complement fixation. Some, however, fixed complement much more strongly than others, notably the three IgG2α mAbs, Z2, Z4, and Z12 (Table 1).

Pathogenicity of the mAbs in Vivo *Correlates with Their Ability to Fix Complement* in Vitro

The abilities of the Y and Z mAbs to enhance disease severity and the extent of demyelination in EAE was examined in Lewis rats injected with MBP-specific T cells. As reported previously[10] the injection of PBS or nonimmune polyclonal mouse IgG failed to enhance the clinical severity of disease or to induce demyelination or complement deposition in the CNS. In contrast, despite their very similar properties with respect to staining of oligodendrocytes or tissue sections, the mAbs differed widely in their ability to induce demyelination and CNS complement deposition and to enhance the clinical severity of EAE (Table 1).

The majority of mAbs induced a moderate enhancement of clinical disease associated with either small areas of myelin loss or somewhat more substantial perivenous and subpial demyelination (Figure 5b, 5c). These observations were similar to those previously reported for a similar dose of 8-18C5 mAb.[10] However, much more extensive demyelination, often involving the entire subpial region of the spinal cord as well as large perivenous lesions, was seen in those rats injected with Z2, Z4, and Z12 mAbs, each of which induced severe or lethal EAE (Figure 5e, 5f). A clear correlation was seen between the levels of demyelination and complement deposition determined *in vivo* (Figures 5 and 6): furthermore, it was noted that Z2, Z4, and Z12, the mAbs that were most pathogenic *in vivo*, were also the mAbs that fixed complement most efficiently and were all of the IgG2α isotype.

Discussion

Thirteen mAbs raised to MOG have been extensively characterized. These mAbs all recognize human, bovine, and rat MOG, indicating that certain MOG epitopes are strongly conserved between species. Furthermore, we have demonstrated that the epitope recognized by each of the mAbs is specific to CNS myelin and present on the oligodendrocyte cell surface and thus has the potential to induce demyelination *in vivo*. However, while the mAbs all stained oligodendrocytes and CNS sections in a similar manner, they varied widely in their abilities to initiate demyelination in EAE. Intravenous injection of the mAbs Z2, Z4, and Z12 into animals with EAE induced the most severe demyelination and the highest numbers of granular C9 deposits within the CNS of all the mAbs tested. These three mAbs, Z2, Z4, and Z12, were all IgG2α, an isotype known to activate complement much more effectively than does mouse IgG1.[29] This was confirmed by *in vitro* complement fixation tests which demon-

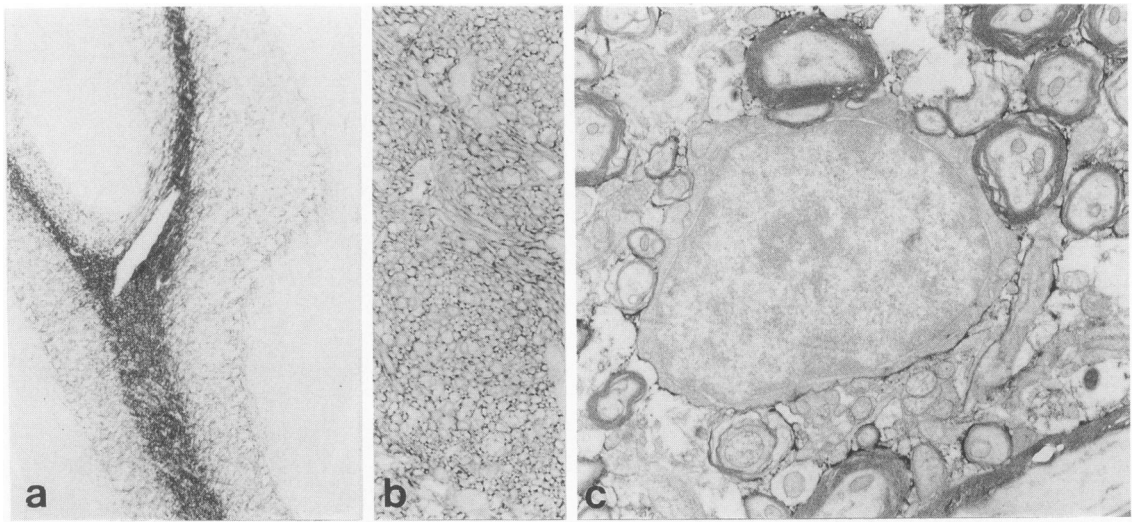

Figure 4. *Immunocytochemical staining for MOG with mAb Y10.* **a:** *Staining of myelin in cerebellar white matter (paraffin section; ×160).* **b:** *Staining of myelin sheath in periventricular white matter of rat forebrain (pre-embedding immunoelectron microscopy; × 640).* **c:** *Electron micrograph from area shown in* **(b)** *demonstrating staining of surface of myelin sheath and an oligodendrocyte (×11,200).*

Table 1. *Pathogenicity of Y and Z mAbs in the EAE model*

Pathogenicity	mAb	*n*	Granular C9 deposits/mm²	Demyelination* (mean ± SD)	Complement fixation† (mean ± SD)
High	Z2	4	13.4 ± 1.8	37.0 ± 4.3	89 ± 4
	Z4	6	11.4 ± 4.9	29.5 ± 9.8	89 ± 2
	Z12	5	11.4 ± 3.2	34.3 ± 9.2	89 ± 1
Low	Y1	4	7.5 ± 4.6	14.9 ± 8.8	74 ± 5
	Y2	3	3.9 ± 1.7	12.5 ± 4.3	73 ± 0
	Y4	4	5.7 ± 3.8	10.2 ± 4.2	66 ± 1
	Y7	1	5.8	14.0	ND‡
	Y9	1	7.5	13.0	43 ± 13
	Y10	4	7.0 ± 5.1	19.6 ± 10.6	62 ± 19
	Y11	5	6.0 ± 5.5	15.7 ± 14.1	67 ± 1
	8-18C5	3	5.9 ± 2.6	5.2 ± 1.0	69 ± 4
Not pathogenic	Y6	3	0 ± 0	0 ± 0	73 ± 10
	Y8	5	1.0 ± 1.9	2.6 ± 4.9	59 ± 1
	Z8	3	0 ± 0	0 ± 0	74 ± 4
	pcIgG	2	0 ± 0	0 ± 0	ND
	No Ab	4	0 ± 0	0 ± 0	14

* Demyelination measured as the number of points of a 100-point grid overlying demyelinated areas.
† Complement fixation measured in terms of the ability of a mAb to inhibit the lysis of sensitized erythrocytes of heterologous serum, that lysis being expressed as a percentage of the lysis of a similar number of erythrocytes caused by distilled water. Measured at two antibody concentrations and the mean token.
‡ ND, not done.

strated that these IgG2α MOG-specific mAbs fixed rat complement more efficiently than did any of the IgG1 mAbs. The high numbers of C9 deposits seen in animals treated with Z2, Z4, and Z12 are therefore probably due to the enhanced ability of these mAbs to activate complement. It would appear that this enhanced complement fixation can lead to more severe demyelination, via either the release of increased levels of inflammatory mediators or subsequent heightening of the inflammatory response, or to more direct effects of MAC damage to the myelin sheath.

Several mAbs (Y2, Y7, and Y11) seen to induce demyelination were not detected on the cell surface by immuno-electron microscopy; however, the finding of C9 deposits in the CNS of these animals would tend to confirm the findings of the oligodendrocyte staining study, ie, that the epitope recognized by these mAbs was present at the cell surface. More difficult to explain is the observation that mAbs Y8 and Z8 apparently both stain the oligodendrocyte cell surface and fix complement *in vitro* but caused little or no demyelination and no complement deposition *in vivo*. Of all the mAbs

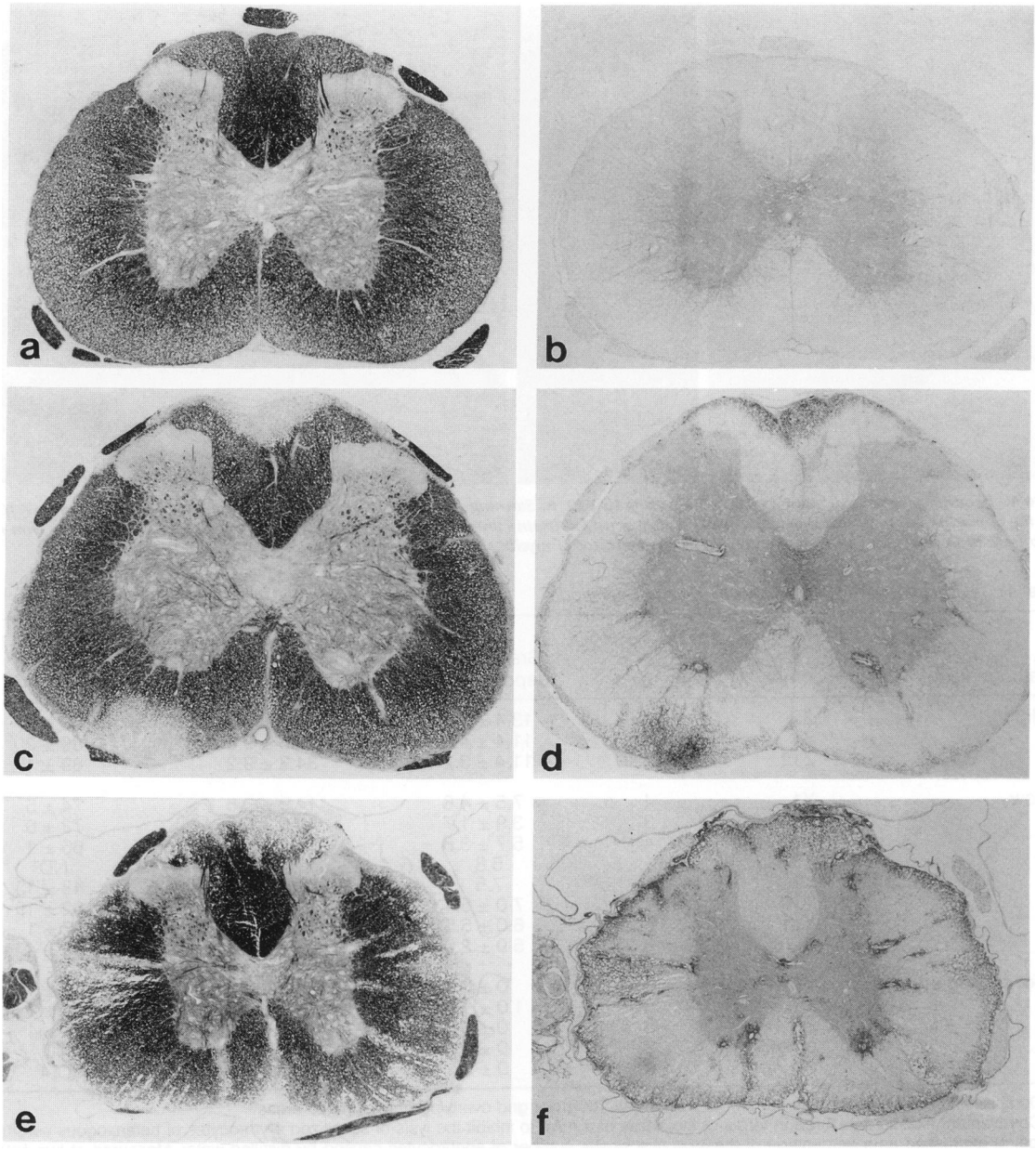

Figure 5. *Demyelination and complement deposition in animals treated with polyclonal mouse IgG* (**a** *and* **b**), *mAb Y2* (**c** *and* **d**) *and mAb Z12* (**e** *and* **f**). Treatment with polyclonal mouse IgG caused no demyelination (**a**) *or complement deposition* (**b**); *mAb Y2 elicited low levels of demyelination* (**c**) *associated with isolated complement deposits* (**d**), *whereas Z12 induced severe demyelination both subpially and perivascularly* (**e**) *and heavy complement deposition in the same areas. All figures show spinal cord stained with Kluver myelin stain* (**a**, **c**, *and* **e**) *or immunocytochemical stain* (**b**, **d**, *and* **f**) *for C9. Magnification,* ×*25.*

tested, Y8 showed relatively poor complement fixation; if its inability to cause demyelination is indeed due to its failure to fix complement, this indicates a vital role for complement in mediating demyelinating disease.

Z8, although apparently specific for oligodendrocytes when tested on cultures of those cells, was seen to lose this specificity on tissue sections, suggesting that the staining of oligodendrocytes may have been due to the presence of Fc receptors; such binding *in vivo* would not be expected to induce complement activation, which is also an Fc-mediated phenomenon. Alternatively, specific or nonspecific binding of Z8 to a variety of different

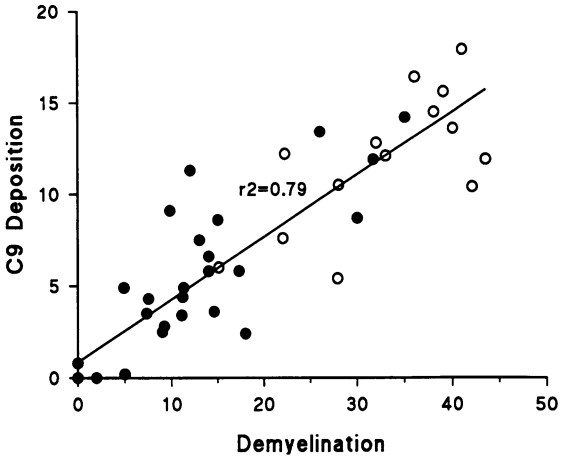

Figure 6. *Correlation of levels of demyelination and C9 deposition in spinal cord after disease induction with anti-MOG mAbs.* ○, *IgG2α mAbs;* ●, *IgG1 mAbs.*

CNS elements *in vivo* may reduce the effective concentration of bound mAb on the surface of myelin and oligodendrocytes beyond the level necessary for the induction of demyelination.

The differing levels of demyelination caused by the IgG1 mAbs may have been due to differences in either their affinities for MOG or their abilities to interact with macrophage Fc receptors. Higher affinity of a mAb for MOG would lead to greater persistance of the mAb on the myelin sheath and consequently greater duration of complement fixation/macrophage activation.

The anti-MOG mAbs were thus shown to vary in their abilities to stain oligodendrocytes and CNS tissue sections and to mediate demyelination *in vivo*. This variation in pathogenic potential was related to the ability of any given mAb to fix complement and suggests that antibody isotype plays an important role in determining the pathogenicity of an autoantibody response to this antigen.

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