

# Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology

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**Antibodies to myelin components are routinely detected in multiple sclerosis patients. However, their presence in some control subjects has made it difficult to determine their contribution to disease pathogenesis. Immunization of C57BL/6 mice with either rat or human myelin oligodendrocyte glycoprotein (MOG) leads to experimental autoimmune encephalomyelitis (EAE) and comparable titers of anti-MOG antibodies as detected by ELISA. However, only immunization with human (but not rat) MOG results in a B cell-dependent EAE. In this study, we demonstrate that these pathogenic and nonpathogenic anti-MOG antibodies have a consistent array of differences in their recognition of antigenic determinants and biological effects. Specifically, substituting proline at position 42 with serine in human MOG (as in rat MOG) eliminates the B cell requirement for EAE. All MOG proteins analyzed induced high titers of anti-MOG (tested by ELISA), but only antisera from mice immunized with unmodified human MOG were encephalitogenic in primed B cell-deficient mice. Nonpathogenic IgGs bound recombinant mouse MOG and deglycosylated MOG in myelin (tested by Western blot), but only pathogenic IgGs bound glycosylated MOG. Only purified IgG to human MOG bound to live rodent oligodendrocytes in culture and, after cross-linking, induced repartitioning of MOG into lipid rafts, followed by dramatic changes in cell morphology. The data provide a strong link between *in vivo* and *in vitro* observations regarding demyelinating disease, further indicate a biochemical mechanism for anti-MOG-induced demyelination, and suggest *in vitro* tools for determining autoimmune antibody pathogenicity in multiple sclerosis patients.**

multiple sclerosis | experimental autoimmune encephalomyelitis | lipid rafts | B cell-deficient mice | encephalitogenicity

**M**ultiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) in which both T cells and antibodies against myelin antigens are routinely detected (1, 2). B cell responses in MS pathogenesis are implicated by the presence of Ig deposits and myelin debris in demyelinating lesions (3–8), and the observation that plasma exchange dramatically reduces clinical disease in a subset of patients (9). Of particular interest to the present study, antibodies to myelin oligodendrocyte glycoprotein (MOG) are detected in the sera and plaques of MS patients (10), and thus are possible predictors of disease progression (11). However, because some control subjects can also harbor anti-myelin antibodies (1, 2, 12), their contribution to MS pathogenesis has been controversial and difficult to identify in individual patients. Further complicating the issue, MS may be several diseases of differing etiologies (5), whereby anti-myelin antibodies may be pathogenic in some forms of MS but merely a reflection of tissue damage in others. Thus, an understanding of whether anti-myelin antibodies are in fact pathogenic, and if so, by what mechanisms they operate, could provide important information for novel diagnostic tools and therapeutic interventions.

The mechanism of pathogenesis of experimental autoimmune encephalomyelitis (EAE), a commonly used animal model for

MS, can involve either or both T cells and B cells, depending on the antigen used (13). T cells specific for an encephalitogenic MOG peptide can induce clinical signs and CNS inflammation and demyelination in EAE (14–16). A monoclonal antibody to MOG induces demyelination *in vitro* (17) and exacerbates T cell-mediated disease in mice and rats (18, 19). We have previously demonstrated that immunization of C57BL/6 mice with either rat MOG protein or rat MOG35–55 peptide results in a B cell-independent disease (16); in contrast, immunization with human MOG protein generates a B cell-dependent disease (20, 21), whereas immunization with human MOG 35–55 peptide leads to only minimal clinical signs of EAE (21). *In vitro* assays have demonstrated that the predominant T cell response in C57BL/6 mice to the extracellular domain of both human and rat MOG proteins is directed to their 35–55 regions (21, 22). Despite the fact that the amino acid at position 42 is neither a T cell receptor nor MHC contact residue (23), it is critical for T cell-mediated disease; the strongly encephalitogenic rodent peptide contains a serine at position 42, whereas the weakly encephalitogenic human analog differs by a proline at that site (24). Consistent with this, substitution of serine with proline at position 42 of rat MOG protein severely attenuates its encephalitogenicity (21).

Attempts have been made to distinguish between pathogenic and nonpathogenic antibodies against MOG antigens in MS and EAE (6). ELISA assays of the antibodies generated by immunization with human or rat MOG do not readily distinguish among different determinants (25). Antibodies generated in H-2<sup>s</sup>, but not H-2<sup>b</sup>, mice can bind to MOG cDNA-transfected fibroblasts (26), suggesting a potential method to discriminate between pathogenic and nonpathogenic antibodies. We have previously shown that antibodies generated by immunization of C57BL/6 mice with human or rat MOG demonstrate comparable titers by ELISA, despite the differences in the B cell dependence of the diseases (21). We postulated that these antisera might recognize different determinants, and that these might reflect differential pathogenicity. In addition, we have shown that a demyelinating monoclonal antibody against MOG binds to the surface of live oligodendrocytes (OLs) in culture, and upon cross-linking, rapidly and sequentially induces the repartitioning of MOG into detergent insoluble microdomains characteristic of lipid rafts, alterations in the phosphorylation state of key proteins, and dramatic changes in cell morphology (27, 28). These observations provided a potential mechanism for B cell-driven disease and suggested that these properties might be predictive of antibody encephalitogenicity.

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Abbreviations: MS, multiple sclerosis; OL, oligodendrocyte; MOG, myelin oligodendrocyte glycoprotein; EAE, experimental autoimmune encephalomyelitis.

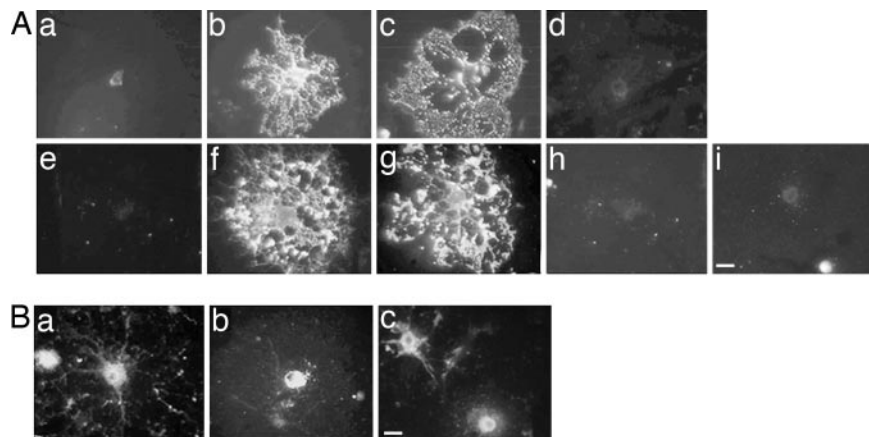
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**Fig. 3.** IgGs from mice immunized with mAb 8-18C5 or human, but not rat or huP42S, MOG protein bind to live OLs. (A) Mouse (a–d) and rat (e–i) OL cultures were incubated with preimmune IgG (a and e), 8-18C5 (b and f), or IgG purified from mice immunized with human (c and g), rat (d and h), or huP42S (i) MOG protein. (Bar, 5  $\mu$ m.) (B) IgGs from mice immunized with human (a), rat (b), or huP42S (c) MOG protein bind intracellular MOG in fixed/permeabilized OLs. (Bar, 5  $\mu$ m.) Results are representative of three independent experiments.

myelin first treated with *N*-glycosidase (PNGase) to remove N-linked carbohydrate chains (Fig. 2*B*). The efficacy of the deglycosylation was demonstrated by treatment of another highly glycosylated myelin glycoprotein, myelin-associated glycoprotein (MAG); upon enzymatic treatment, the  $\approx$ 100-kDa MAG was reduced to two nonglycosylated forms of 72 and 67 kDa, L- and S-MAG, respectively (35) (Fig. 2*C*). The positive control for these studies was anti-MOG mAb 8-18C5, which reacted at the predicted molecular mass of 25 kDa with MOG from untreated mouse myelin (no MOG dimer of 50 kDa was detected) and with recombinant mouse MOG. Anti-human MOG IgG bound both recombinant MOG and myelin MOG; in contrast, anti-rat MOG and -huP42S bound the former, but reacted only weakly with the latter (similar results were obtained with whole serum; data not shown). However, all three IgGs recognized the deglycosylated form of MOG (Fig. 2*B*). We conclude that of the IgGs examined, only the pathogenic monoclonal and anti-human MOG antibodies react with glycosylated MOG, the form most likely to resemble MOG on the surface of OLs and myelin.

Anti-human MOG, but not anti-rat MOG, anti-huP42S, or IgG from nonimmunized mice, immunostained the surface of both mouse and rat live OLs in culture in a manner indistinguishable from that of mAb 8-18C5 (Fig. 3*A*). However, upon fixation and permeabilization of the cells, anti-human, -rat, and -huP42S MOG protein all stained internal antigens in the cell bodies of OLs (Fig. 3*B*). We conclude that purified IgG from mice immunized with human MOG, but not rat or huP42S MOG protein, binds to the surface of differentiated OLs in culture. The internal binding may represent a form that is not yet glycosylated.

**Cross-Linking of MOG on the Surface of Cultured OLs with Anti-Human MOG IgG Induces MOG Repartitioning into a Detergent-Insoluble Fraction and Morphological Alterations.** Antibody binding *per se* does not necessarily indicate biological function. Conversely, the absence of binding as measured by immunoblotting and immunohistochemistry, although strongly arguing against high affinity antigen-antibody interactions, does not entirely preclude the possibility of transient, but biologically significant, roles. On this basis, we postulated that a demonstration of a direct correlation between antibody encephalitogenicity, binding capacity, and physiologically relevant effects on cells would be an important step in relating these data to antibody relevance in disease pathology.

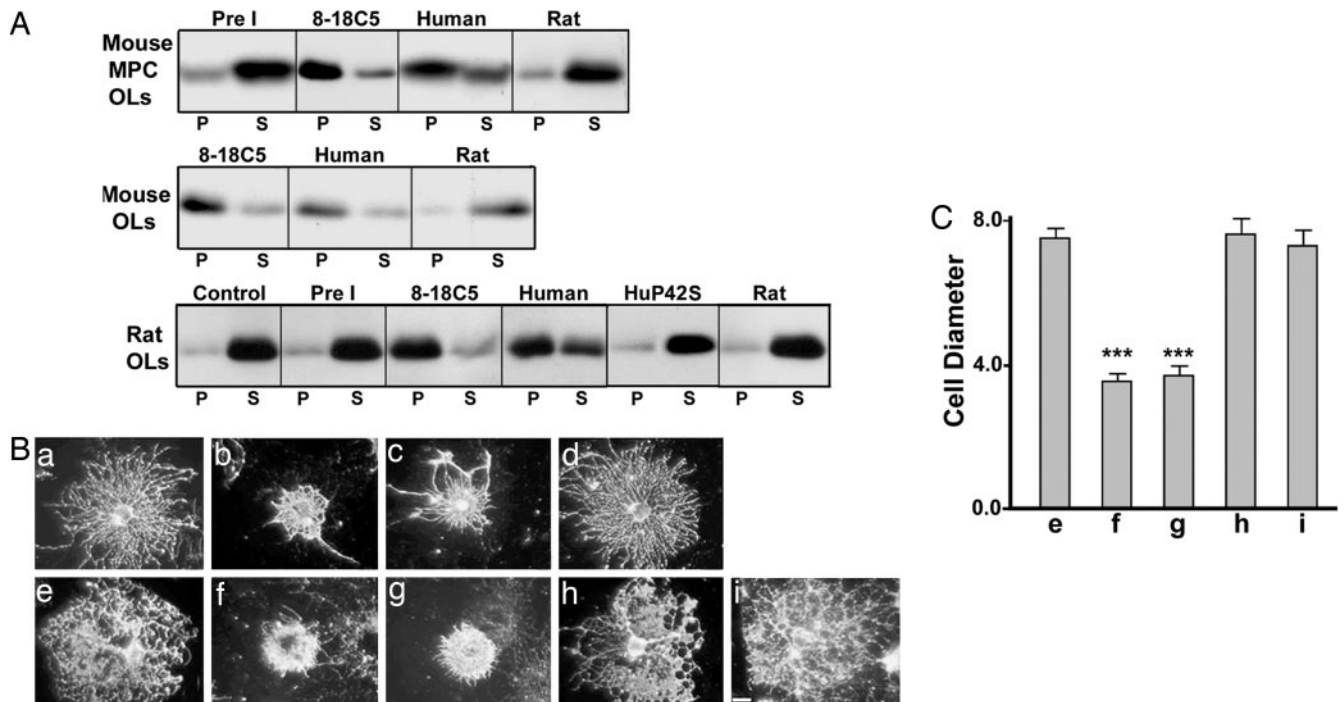
We have shown that, when mAb 8-18C5 bound to the surface

of OLs in culture is cross-linked with a secondary antibody, MOG is rapidly repartitioned from a detergent-soluble to an insoluble fraction with the biochemical characteristics of lipid rafts; this is quickly followed by changes in the phosphorylation status of at least 10 proteins, culminating in dramatic changes in OL cytoarchitecture (27, 28). Here, we extended these observations to the polyclonal IgGs from mice immunized with various forms of human or rat MOG. We found that IgG from mice immunized with human MOG produced effects similar to those previously observed with mAb 8-18C5 (Fig. 4), including repartitioning of MOG into a detergent-insoluble fraction consistent with lipid rafts, and retraction of OL processes thought to correlate with changes in cytoskeletal stability (28). In contrast, no such changes in MOG repartitioning or morphological changes were seen with control IgG, or with IgG from mice immunized with either rat or huP42S MOG (Fig. 4).

In summary, we conclude that the data strongly support the hypothesis that pathogenic (i.e., anti-human MOG), but not nonpathogenic (i.e., anti-rat or -huP42S MOG), antibodies bind to OL cell surfaces and induce membrane protein redistribution and dramatic changes in cell morphology that, based on our previous data obtained with a monoclonal antibody, reflect a striking change in the physiology of the cells. We propose that these considerations are at the heart of the varying B cell dependence in EAE induced by human vs. rat MOG.

## Discussion

The data presented here support four principal conclusions based on antibodies generated in EAE (as summarized in Table 2). First, a single amino acid substitution in human MOG at position 42 changes the mechanism of encephalitogenicity from B cell dependent to independent. Second, although immunization with rat, human, or huP42S MOG generates antibodies against recombinant mouse MOG that are detectable by ELISA, only antibodies directed against human MOG, but not anti-rat or anti-huP42S MOG, are encephalitogenic *in vivo*. Third, antibodies to human MOG bind to glycosylated MOG in immunoblots and to live OLs in culture; anti-rat and anti-huP42S MOG antibodies require deglycosylation or permeabilized cells to be detectable by immunoblot or immunofluorescent microscopy, respectively. Fourth, upon cross-linking, antibodies to human MOG, but not rat or huP42S MOG, induce repartitioning of MOG into subdomains of OL



**Fig. 4.** MOG cross-linking with IgGs from mice immunized with human, but not rat or huP42S, MOG protein induces MOG repartitioning into a detergent insoluble fraction and morphological alterations in OLs. (A) MOG immunoblot of detergent soluble (S) or insoluble pellet (P) fractions from mixed primary or purified OL cultures incubated with media alone (Control), IgG from naive mice (Pre I), mAb 8-18C5, or IgG from mice immunized with either human, huP42S, or rat MOG protein, followed by cross-linking with secondary antibodies. Treatment with mAb 8-18C5 or IgG raised against human MOG, but not huP42S or rat, MOG protein induces repartitioning of MOG into the detergent insoluble fraction. (B) Mouse (a–d) or rat (e–i) OLs were incubated with preimmune IgG (a and e), 8-18C5 (b and f), or IgG from mice immunized with human (c and g), rat (d and h), or huP42S (i) MOG protein, followed by cross-linking with anti-mouse IgG and stained with mAb O4 to visualize OL morphology. (Bar, 5  $\mu$ m.) (C) Diameter (arbitrary units; mean  $\pm$  SEM) of randomly chosen cells. IgG raised against human, but not rat or huP42S, MOG protein induces retraction of OL processes similar to 8-18C5 (\*\*\*,  $P < 0.0001$ ). Results are representative of three independent experiments.

membranes, a crucial target of MS, which is followed by dramatic morphological changes.

Previous authors using transfected fibroblasts have suggested that encephalitogenic antibodies recognize conformation-dependent epitopes (1, 26), although in none of these studies was the encephalitogenicity of the antibodies actually demonstrated, except in the case of mAb 8-18C5. The data presented here indicate that the epitopes previously recognized in transfected fibroblasts may be glycosylation dependent. We show that encephalitogenic anti-MOG mAb 8-18C5 and the encephalitogenic anti-human MOG antibodies bind to glycosylated MOG on immunoblots and surface MOG on live OLs in culture, and activate changes in OLs. It has previously

been established that mAb 8-18C5 binds to discontinuous (i.e., conformation-dependent) determinants but not to linear MOG peptides (36, 37). The conclusion that encephalitogenic antibodies bind to nonlinear determinants is also supported by the studies of von Budingen *et al.* (8), who reported that antibodies raised in marmosets against rat MOG peptides could not transfer EAE to myelin basic protein primed animals, whereas antisera that included antibodies recognizing conformational determinants transferred a severe disease with regard to CNS lesions and demyelination. Bourquin *et al.* (26) demonstrated that B10.S, but not C57BL/6, mice developed EAE after immunization with rat MOG in incomplete Freund's adjuvant, arguing that the disease was B cell mediated and suggesting that the MHC dictated whether a pathogenic or nonpathogenic antibody was generated. Our previous data (21), and those provided here, indicate that the situation is considerably more complex. The MHC may censor the ability of H-2b mice to generate a pathogenic antibody to rat MOG; clearly it does not inhibit the ability to generate a pathogenic antibody to human MOG. On the basis of these correlated data, it is reasonable to conclude that nonpathogenic antibodies recognize linear MOG determinants and do not bind to OLs, but pathogenic antibodies recognize conformational, glycosylation-dependent determinants and bind to OLs.

Although anti-myelin antibodies are commonly found in MS, they are generally of limited diagnostic value because they are also found in a significant number of control patients and patients with nondemyelinating diseases (2, 12). Furthermore, it is possible that some MS patients generate antibody responses that reflect, rather than cause, the disease. The correlates we

**Table 2. Encephalitogenic and biologic properties of recombinant MOG proteins**

Result	MOG immunogen					
	Human MOG		Rat MOG		HuP42S	
	Wild type	$\mu$ MT	Wild type	$\mu$ MT	Wild type	$\mu$ MT
EAE	+	-	+	+	+	+
B cell dependence	Yes		No		No	
ELISA	+	NA	+	NA	+	NA
Pathogenic antibodies	+*	+†	-	-	-	-

NA, not applicable.

\*Determined by binding to OL surfaces and induction of MOG repartitioning and OL morphological alterations.

†Determined by serum passive transfer experiments.

have shown here between antibody binding properties and pathogenesis suggest improved protocols for the identification of pathogenic antibodies in MS patients. We suggest three rapid diagnostic tools for the analysis of MS patient sera to distinguish between pathogenic (e.g., anti-human MOG) and nonpathogenic (e.g., anti-rat MOG) antibodies: screening by (i) immunoblot against native (glycosylated) vs. nonglycosylated MOG, (ii) immunofluorescent microscopy against live vs. fixed, permeabilized OLs, and (iii) light microscopic identification of rapid changes in OL morphology of cells treated with cross-linked IgG. These data provide a link between *in vivo* and *in vitro* observations (27, 28) and further strengthen a model for the biochemical

mechanism for antibody-mediated demyelinating disease (27, 28). They also provide *in vitro* tools to determine whether an autoimmune antibody is pathogenic, and may be useful for evaluating the pathogenicity of antibodies in MS patients as an adjunct to diagnosis and treatment.

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