

# Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis

Patrice H. Lalive\*, Til Menge\*, Cecile Delarasse†, Bruno Della Gaspera†, Danielle Pham-Dinh†, Pablo Villoslada‡, H.-C. von Büdingen§, and Claude P. Genain\*¶

\*Department of Neurology, University of California, San Francisco, CA 94143; †Institut National de la Santé et de la Recherche Médicale, U546, 105 Boulevard de l'Hôpital, 75013 Paris, France; ‡Department of Neurology, Clínica Universitaria de Navarra, Pio XII 36, 31008 Pamplona, NA, Spain; and §Department of Neurology, University Hospital, Frauenklinikstrasse 26, 8091 Zurich, Switzerland

Communicated by Jacob N. Israelachvili, University of California, Santa Barbara, CA, December 15, 2005 (received for review July 6, 2005)

**Myelin oligodendrocyte glycoprotein (MOG) is an integral membrane protein expressed in CNS oligodendrocytes and outermost myelin lamellae. Anti-MOG Abs cause myelin destruction (demyelination) in animal models of multiple sclerosis (MS); however, such pathogenic Abs have not yet been characterized in humans. Here, a method that specifically detects IgG binding to human MOG in its native, membrane-embedded conformation on MOG-transfected mammalian cells was used to evaluate the significance of these auto Abs. Compared with healthy controls, native MOG-specific IgGs were most frequently found in serum of clinically isolated syndromes ( $P < 0.001$ ) and relapsing-remitting MS ( $P < 0.01$ ), only marginally in secondary progressive MS ( $P < 0.05$ ), and not at all in primary progressive MS. We demonstrate that epitopes exposed in this cell-based assay are different from those exposed on the refolded, extracellular domain of human recombinant MOG tested by solid-phase ELISA. In marmoset monkeys induced to develop MS-like CNS inflammatory demyelination, IgG reactivity against the native membrane-bound MOG is always detected before clinical onset of disease ( $P < 0.0001$ ), unlike that against other myelin constituents. We conclude that (i) epitopes displayed on native, glycosylated MOG expressed *in vivo* are early targets for pathogenic Abs; (ii) these Abs, which are not detected in solid-phase assays, might be the ones to play a pathogenic role in early MS with predominant inflammatory activity; and (iii) the cell-based assay provides a practical serologic marker for early detection of CNS autoimmune demyelination including its preclinical stage at least in the primate MS model.**

allergic encephalomyelitis | autoimmunity | clinically isolated syndrome | demyelination

**M**ultiple sclerosis (MS) is an immune-mediated, chronic demyelinating disorder of CNS white matter mediated by CD4<sup>+</sup> myelin-reactive T helper 1 cells (1). Humoral immunity may play a role in MS pathogenesis, as suggested by intrathecal Ab synthesis and oligoclonal B cell expansion, observations of Ab deposition associated with myelin vacuolization in MS plaques (2), and involvement of IgG and complement deposition (3). Myelin oligodendrocyte glycoprotein (MOG) is a target myelin antigen for both humoral and cellular CNS-directed immune responses. The full-length protein contains 218 aa and two predicted transmembrane domains, is posttranscriptionally processed as suggested by apparent electrophoretic mobility, and is a potential site for glycosylation, phosphorylation, isoprenylation, and myristoylation (4). The encephalitogenic properties of MOG are believed to result from the extracellular location of its IgV-like domain on the outermost myelin lamellae, which makes it an exposed target accessible to initial autoimmune attack on compact myelinated axons (5). In both rodent and primate MS models of experimental allergic encephalomyelitis (EAE), Abs against MOG directly induce demyelination (6, 7). Moreover, certain Abs against MOG have been proposed as

predictors of early conversion to clinically definite MS in the context of a first demyelinating event (8).

A common problem encountered in human studies of humoral immunity against MOG is that accurate detection of Ab depends highly on the conformation of the antigens used for detection. Previous studies, either in MS or EAE, have not fully characterized the structural features of the proteins used as Ab targets *in vitro* and have reported conflicting findings, in part because of the different folded forms of this poorly water-soluble antigen. The existing assays detecting anti-MOG Abs use various MOG preparations, recombinant, native-purified, or *in vitro*-translated proteins of variable length including short peptides (4, 8–11). None of these methods takes into account the specific tertiary structure of the folded MOG as it is presented to the immune system *in vivo*, e.g., in association with a hydrophobic, lipid-rich bilayer membrane environment. Therefore, these techniques may fail to detect reactivity against epitopes displayed by native MOG expressed *in situ* on myelin sheaths, as it would be upon an initial immune response.

Here, we describe a cell-based assay that specifically measures Abs directed against conformationally folded, cell membrane-expressed human MOG (designated hMOG<sub>cme</sub>), and evaluate the relative incidence of these antinative MOG Abs in serum of humans and *Callithrix jacchus* marmosets with MS-like EAE. We find that hMOG<sub>cme</sub> Abs predominate during clinically isolated syndrome (CIS) and relapsing-remitting MS (RRMS) and can be detected during the preclinical stage of EAE. These findings underscore the potential value of antinative MOG Abs as a practical candidate biomarker for detecting MS at its early, inflammatory stage.

## Results

**Chinese Hamster Ovary (CHO)-MOG Assay (MOG<sub>cme</sub>) Validation.** Fig. 1 shows high levels of MOG<sub>cme</sub> expression, as demonstrated by staining of MOG-transfected CHO cells with the monoclonal anti-MOG Ab 8-18C5. Detection of hMOG<sub>cme</sub>-specific Abs with this cell-based assay was sensitive because a concentration of <1 ng/ml of 8-18C5 produced a binding ratio (BR) >1.5 (data not shown). Staining with a positive control serum (patient 1158) is shown in Fig. 1C. This control was used in each assay to

Conflict of interest statement: P.H.L., T.M., H.-C.v.B., and C.P.G. are coinventors of an international patent application that contains claims to methods of diagnostics and prognosis of multiple sclerosis. Such methods may be ultimately commercialized.

Abbreviations: MOG, myelin oligodendrocyte glycoprotein; rMOG<sub>125</sub>, recombinant rat MOG<sub>aa1–125</sub>; hMOG<sub>125</sub>, recombinant human MOG<sub>aa1–125</sub>; hMOG<sub>cme</sub>, cell-membrane expressed human MOG; CHO, Chinese hamster ovary; ntCHO, nontransfected CHO; EAE, experimental allergic encephalomyelitis; CIS, clinically isolated syndrome; MS, multiple sclerosis; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; HC, healthy control; BR, binding ratio; Gmean, geometric mean intensity.

¶To whom correspondence should be addressed. E-mail: claudeg@itsa.ucsf.edu.

© 2006 by The National Academy of Sciences of the USA



**Table 1. Time course of *C. jacchus* marmoset EAE and serum IgG binding against hMOG<sub>cme</sub>**

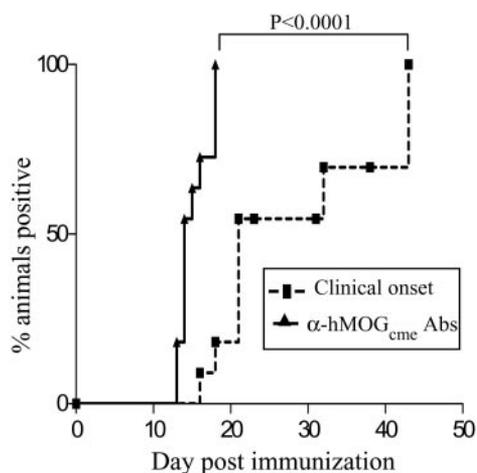
Animal no.	Clinical onset dpi	Death dpi	Maximal clinical score (dpi)	$\alpha$ -hMOG <sub>cme</sub> Ab dpi
UO62-02	43	97	3 (84)	18
UO50-01	21	86	2 (57)	14
UO50-00	21	78	3 (38)	14
UO52-01	21	52	3 (40)	18
UO25-00	21	61	2 (57)	14
UO57-02	32	82	1.5 (73)	18
UO21-99	18	23	1.5 (23)	13
UO23-00	16	31	2 (28)	15
UO30-00	Preclinical	23	0	13
UO61-02	Preclinical	31	0	14
UO53-01	Preclinical	38	0	16
Median $\pm$ SD	21 $\pm$ 9	52 $\pm$ 28	2 $\pm$ 1 (49 $\pm$ 22)	14 $\pm$ 2

dpi, day postimmunization.

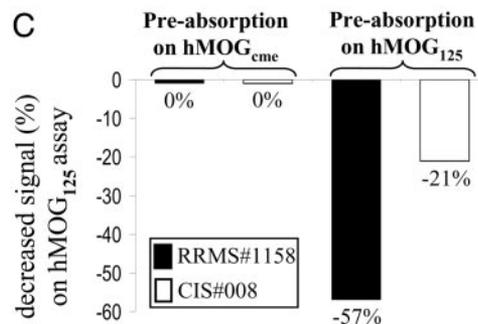
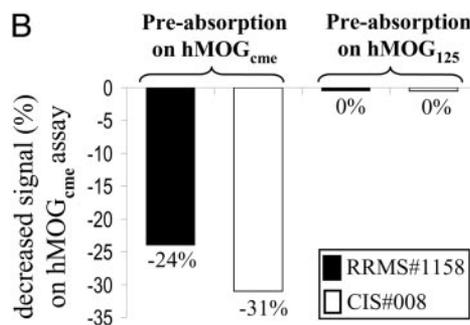
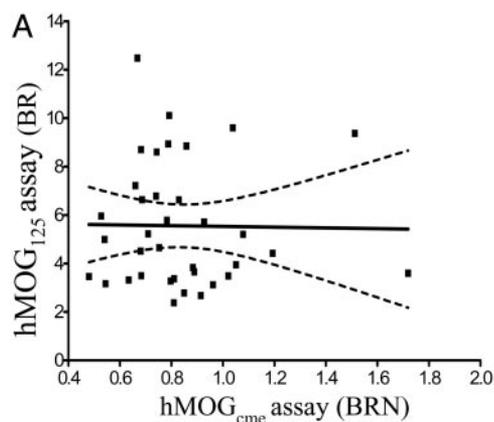
ance of clinical signs was highly significant ( $P < 0.0001$ ) (Fig. 4). Reactivity was not detected in preimmune plasma.

**Comparison of Human IgG Binding on hMOG<sub>125</sub> and hMOG<sub>cme</sub>.** The serum binding characteristics of the CIS cohort ( $n = 36$ ) were tested by ELISA using recombinant human MOG<sub>aa1-125</sub> (hMOG<sub>125</sub>) and compared with hMOG<sub>cme</sub> reactivity by FACS on the MOG-transfected CHO cells. Although some CIS patients displayed high reactivity against hMOG<sub>125</sub>, unlike for hMOG<sub>cme</sub> reactivity, there was no statistical significant difference between CIS and HCs (data not shown). By linear regression and comparison test, results from these two assays showed no correlation ( $P$  not significant), indeed suggesting that different epitopes are detected by both assays (Fig. 5A).

**Specificity of MOG and Differential MOG-Epitope Binding in Human Serum.** To discriminate the epitopes displayed by hMOG<sub>125</sub> from those displayed on hMOG<sub>cme</sub> on MOG-transfected CHO cells, we performed a series of preabsorption experiments with two sera, both representative of early and inflammatory forms of MS: the positive control used in our cell-based assay (RRMS 1158) and a CIS patient displaying a high reactivity to both



**Fig. 4.** Time course of serum IgG directed against hMOG<sub>cme</sub> in marmoset EAE. Results are from 11 EAE *C. jacchus* marmosets immunized with human white matter, 3 of which were killed before onset of clinical disease. First occurrence of serum IgG directed against hMOG<sub>cme</sub> is compared with time of clinical onset of EAE in a Kaplan–Meier survival plot. Please see text and Table 1 for details.



**Fig. 5.** Selective epitope presentation on hMOG<sub>cme</sub>. (A) Binding to hMOG<sub>125</sub> by ELISA compared with binding to hMOG<sub>cme</sub> by FACS in the CIS cohort ( $n = 36$ ). By linear regression analysis, there is no correlation between the results of these two methods ( $P$  not significant,  $r^2 = 0.00023$ , Spearman  $r$ ; straight line is the linear regression curve; dotted line indicates 95% confidence interval) even when clear serum reactivity is present in both assays. (B and C) Preabsorption of serum on either hMOG<sub>cme</sub> (Left) or hMOG<sub>125</sub> (Right), followed by testing by FACS (B, hMOG<sub>cme</sub>) or ELISA (C, hMOG<sub>125</sub>). Preabsorption on hMOG<sub>125</sub> or hMOG<sub>cme</sub> only altered the reactivity in the corresponding system of detection. See *Materials and Methods* for additional details.

hMOG<sub>cme</sub> and hMOG<sub>125</sub> (CIS 008). Preabsorption against ntCHO cells served as control in the hMOG<sub>cme</sub> assay (FACS of MOG-transfected CHO cells), and preabsorption against 1% BSA served as a control for the hMOG<sub>125</sub> ELISA assay. Compared with these controls, preabsorption of either sample on hMOG<sub>125</sub> did not affect hMOG<sub>cme</sub> reactivity. Preabsorption on hMOG<sub>cme</sub> resulted in a decrease in BR (31% for CIS 008 and 24% for RRMS 1158), when tested with hMOG<sub>cme</sub> expressed on the MOG-transfected cells (Fig. 5B). Similarly, when tested on hMOG<sub>125</sub>, no change in reactivity occurred when samples were preabsorbed on the MOG-transfected cells (hMOG<sub>cme</sub>). On the contrary, the samples preabsorbed on hMOG<sub>125</sub> displayed a decrease in BR, by 21% and 57%, respectively, when tested on hMOG<sub>125</sub> (Fig. 5C). These experiments unequivocally demon-

strate that hMOG<sub>cme</sub> and hMOG<sub>125</sub> display separate epitopes of the MOG protein.

## Discussion

Methods to study Ab reactivity to MOG (ELISA, Western blot, liquid-phase assay) commonly use linear peptides, polypeptide, or partially refolded glycosylated MOG. The exact conformation of MOG displayed in these assay systems is difficult to assess and control and may result in the display of some, or partially, aberrant MOG epitopes that are not exposed under physiological conditions *in vivo*. The disease relevance of these Abs is therefore uncertain, as apparent from somewhat conflicting results in previous reports (2, 8–11, 13–16). We show here that Abs against native glycosylated MOG expressed on mammalian cells are commonly detected in MS serum with high sensitivity (<1 ng). Specificity is established by the selective binding of three of five monoclonal anti-MOG Abs tested in this system (murine 8-18C5 and marmoset Fabs M3-31 and M26). Thus, as is the case for these mAbs (9, 12, 13) the hMOG<sub>cme</sub> assay measures Abs that bind to conformational epitopes of MOG.

Our results show that there is no correlation in the CIS cohort between serum reactivity against hMOG<sub>125</sub> (solid-phase ELISA) and hMOG<sub>cme</sub> (MOG-transfected CHO cells), and preabsorption demonstrates that there is no cross-reactivity between epitopes of MOG displayed in these two different assays (Fig. 5). This finding also argues, along with the significant signal quenching in the cell-based assay achieved only by preabsorption on hMOG<sub>cme</sub> (MOG-transfected cells), and not by preabsorption on either hMOG<sub>125</sub> or ntCHO cells, against a nonspecific “sticky” effect of MS serum. The hMOG<sub>cme</sub> assay is unique because it allows the testing of IgG reactivity directed against epitopes presented by the native glycosylated and conformational structure of MOG as it is expressed on intact myelin sheath or oligodendrocytes and subject to membrane lipid-protein interactions, which have been shown to be critical for maintenance of myelin structure and epitope exposure (17).

Analysis of reactivity against hMOG<sub>cme</sub> on the MOG-transfected CHO cells in the different MS clinical subtypes showed a very prominent response in CIS, RRMS, and to a lesser degree SPMS, compared with HC and PPMS. There is therefore a humoral immune response specifically directed against intact MOG expressed on myelin oligodendrocytes in those groups of patients. The predominance of hMOG<sub>cme</sub>-specific Abs in CIS suggests that these Abs represent early stages of the immune response against intact (as opposed to degraded) myelin, and thus may represent a marker of inflammatory phases of disease related to blood-brain-barrier opening and/or molecular mimicry. Our results are in partial agreement with a recent report showing an increase of Ig response directed against *ex vivo* glycosylated-native-MOG in first demyelinating events (14). RRMS is the most common MS subtype that includes ≈85% of the patients at initial presentation. The secondary progressive pattern is known to follow RRMS in ≈50% of the cases after 10 years of disease activity (18, 19). Thus, these two subtypes might be considered as a continuous process starting with a common pathophysiological origin. Although we did not include a group of other neurological disorders in the current study, we have compared MS clinical subtypes among themselves and clearly demonstrated that the hMOG<sub>cme</sub>-specific Ab response is restricted to early forms. It is of importance to note that the PPMS cohort does not show elevated serum IgG against hMOG<sub>cme</sub> and that significant differences in antibody status also exist between SPMS and PPMS, which implies that the serum level of these Abs or lack thereof is not solely related to a progressive course of disease. The HC subjects were age-matched with the CIS group, indicative that the heightened response in CIS is disease-specific and not related to a younger age in this cohort. The lack of heightened Ab responses against hMOG<sub>cme</sub> in PPMS is in

contrast with the increased IgG reactivity against recombinant rMOG<sub>125</sub> and against neurons in this disease subtype (unpublished data and ref. 20). Abs against galactocerebroside, the major myelin glycolipid, are not found in PPMS but are associated mostly with established relapsing-remitting and secondary-progressive forms (21). It is thus becoming increasingly apparent that Ab responses against myelin antigens may follow patterns that reflect a combination of underlying cause, antigen exposure, and secondary immune responses. These patterns of humoral reactivity, rather than a classification based on clinical criteria (RRMS, SPMS, or PPMS) can be exploited to refine our understanding of disease stage, cause, and prognosis.

The high prevalence of hMOG<sub>cme</sub>-reactive Abs in CIS, i.e., contemporary of the first clinically apparent event for MS, is in sharp contrast to other antimyelin Abs, such as those directed against glycolipids that predominate in established MS (21). This observation has two important implications: first, it suggests that hMOG<sub>cme</sub>-reactive Abs may be implicated in the early pathogenesis of disease. Engagement of membrane-embedded MOG by the mAb 8-18C5, which as shown in the current study binds hMOG<sub>cme</sub> with a high affinity, has been shown to induce MOG phosphorylation in oligodendrocytes, leading to pronounced morphological changes with potentially demyelinating effects (22). Second, and/or alternatively, our findings also suggest that hMOG<sub>cme</sub>-reactive Abs may be useful to help diagnose MS at its earliest stages. To consolidate this contention, the hMOG<sub>cme</sub> assay was used to study the time course of the Ab response against hMOG<sub>cme</sub> in marmoset EAE induced by immunization with human white matter. In these animals, serum reactivity against hMOG<sub>cme</sub> was always detected before clinical onset, contrary to anti-myelin basic protein and antigalactocerebroside Abs that occur at later stages (21). Because the immunizing antigen contained native MOG similar in conformation to hMOG<sub>cme</sub>, these findings imply that the hMOG<sub>cme</sub>-reactive Abs are the ones that initiate and/or first result from active demyelination. Regardless of whether they are causative or not, hMOG<sub>cme</sub>-reactive Abs clearly represent a valuable biomarker for disease activity and, at least in the MS model, subclinical disease.

It is of great interest to note that the marmoset Fabs M3-31 and M26, which were obtained from an animal with overt clinical signs of EAE immunized with rMOG<sub>125</sub> and had an established anti-MOG Ab response, are the only ones among those tested that recognize hMOG<sub>cme</sub>. In our previous studies of human MS, using a highly specific competition assay between human serum IgG and marmoset Fabs we found that Fabs M3-24 and M3-8 can compete with serum IgG from patients with established MS, but we have so far not been able to demonstrate any competition between human IgGs and either M3-31 or M26 (12). Although further studies are needed to examine whether IgG purified from patients with a CIS does compete with Fabs M3-31 and M26, these data suggest that the epitopes defined by these two Fabs are the ones targeted by early humoral responses in MS, whereas the ones defined by M3-8 and M3-24 may be part of the Ab response at a later stage.

Taken together, our results strongly suggest that analogous to certain serologic markers that are predictive of type I diabetes (23) anti-hMOG<sub>cme</sub> Abs could be used in humans as a biomarker to diagnose MS or MS risk. Further studies are needed to validate this biomarker and understand the benefits and information that in combination with other Ab profiling techniques (8, 21) it could provide to scientists, treating neurologists, and individuals with suspected or established MS.

## Materials and Methods

**Patients.** Ninety-two patients with clinically definite MS (Poser criteria) (24), 36 patients with CIS, and 37 HCs were recruited from the University of California, San Francisco MS Center, and

**Table 2. Clinical characteristics of patients and HCs**

Patients	No. of cases	Female/male ratio	Median age, yr (range)	Median disease duration, yr (range)	Median EDSS (range)
CIS	36	24/12	32 (18–49)	0.3 (0.1–3)	2 (0–4)
RRMS	35	21/14	41 (19–61)	2.4 (0.6–22.2)	2.5 (0–6.5)
SPMS	33	19/14	46 (36–60)	11 (2–32)	6 (2.5–8.5)
PPMS	24	16/08	54 (40–65)	6.3 (0.8–18.8)	4.5 (2.5–8)
HC	37	24/13	35 (21–63)	—	—

EDSS, expanded disability status scale (25).

the MS Center of Pamplona, Spain (CIS). All investigations were conducted according to the Declaration of Helsinki. Blood was obtained by venipuncture after informed consent in full compliance with the Institutional Review Board, and clotted serum was stored at  $-40^{\circ}\text{C}$  until use. Patients were classified as RRMS ( $n = 35$ ), SPMS ( $n = 33$ ), and PPMS ( $n = 24$ ) MS by clinical history (24). A CIS was defined by a first clinical event indicative of demyelination with no history of previous neurological symptom. Age, gender, disease duration, and disability state [Expanded Disability Status Score (EDSS) (25)] were recorded at time of sampling. HCs were chosen to match sex and age of the CIS group. The median age, disease duration, and EDSS were higher for the SPMS group than for the RRMS and CIS groups (Table 2). All RRMS and 19/33 SPMS patients were treated with IFN- $\beta$ . Two of the PPMS patients were treated with mitoxantrone and monthly pulsed steroids.

**Animals.** *C. jacchus* marmosets were cared for in accordance with the guidelines of the Institutional Animal Care and Usage Committee. EAE was induced by immunization with 100 mg of human white matter, which contains native, membrane-embedded MOG homogenized in complete Freund's adjuvant as described (26). Plasma was obtained from EDTA-anticoagulated blood at baseline and 2- to 4-week intervals and stored at  $-40^{\circ}\text{C}$ . The animals were scored every other day for the development of clinical signs by using a published scale (27).

**Preparation of MOG-Transfected Cells.** CHO cells were transfected with a full-length construct corresponding to the major  $\alpha$ -1 form of human MOG as described (28). CHO cells were cultured in T-225 flasks (Costar), in RPMI medium 1640 supplemented with 10% FCS,  $1\times$  Glutamax, 1 mM sodium pyruvate, and 50  $\mu\text{g}/\text{ml}$  gentamycin. G418 (500  $\mu\text{g}/\text{ml}$ , GIBCO) was added to the medium of transfected cells. Stable surface expression of MOG was verified by immunofluorescence and FACS on transfected cells after multiple passes and washes; cells were used for flow cytometry (FACS) assay when a confluence of 80–90% was reached. To check for surface expression of hMOG<sub>cme</sub>,  $3 \times 10^4$  MOG-transfected CHO cells were deposited on a slide and fixed with 100% methanol for 5 min at  $-20^{\circ}\text{C}$ . After blocking with PBS containing 2% BSA and 2% FCS for 30 min, cells were incubated 1 h at  $37^{\circ}\text{C}$  with the mouse monoclonal anti-MOG Ab 8-18C5 (5  $\mu\text{g}/\text{ml}$ , gift of C. Linington, University of Aberdeen, Aberdeen, Scotland). Fluorescence was revealed after 1-h incubation at  $37^{\circ}\text{C}$  by a goat anti-mouse IgG FITC Ab (Sigma) and examined under a fluorescence microscope (Nikon Eclipse E600). Negative controls were done with secondary Ab alone.

**Serum IgG Reactivity.** Cells were trypsinized, diluted in FACS buffer (PBS, 0.1% Na azide, and 2% FCS), and plated in a 96-well plate (Costar) at a density of 200,000 per well. After blocking in FACS buffer containing 10% FCS for 15 min at  $4^{\circ}\text{C}$ , cells were washed and human serum (1:10) was added for 1-h incubation at  $4^{\circ}\text{C}$ . After washing, cells were incubated with a goat anti-human IgG FITC (Caltag, South San Francisco, CA)

at the recommended concentration for 30 min at  $4^{\circ}\text{C}$ . After a final wash, cells were resuspended in FACS buffer containing propidium iodide (Molecular Probes) at 2  $\mu\text{g}/\text{ml}$  and gently shaken. Samples were kept on ice and analyzed by gating the selected live cell population ( $10^4$  cells) within 1 h of harvesting by trypsinization. Each FACS experiment included an internal positive control consisting of the monoclonal anti-MOG 8-18C5 Ab (0.5  $\mu\text{g}/\text{ml}$ ) and rabbit anti-mouse FITC (DAKO) as secondary Ab. For each sample, the geometric mean intensity (Gmean) of FITC (WINMDI 2.8 software) was measured for MOG-transfected CHO cells and compared with that of ntCHO cells. The BR was calculated as the Gmean for MOG-transfected CHO cells divided by the Gmean for ntCHO. To compare different assays, for each sample the BR was normalized to that of a human positive control (RRMS 1158) included in each experiment.

For studies in marmosets, MOG-transfected CHO cells were incubated for 1 h at  $4^{\circ}\text{C}$  with marmoset serum diluted 1:100. FITC-conjugated Ab against whole monkey IgG (Sigma) diluted at 1:100 was used as secondary Ab and incubated 30 min at  $4^{\circ}\text{C}$ . FACS analysis was performed as described above. IgG binding against hMOG<sub>cme</sub> was considered positive when the BR (Gmean preimmunization/Gmean time point tested) was  $>1.5$ .

**Differential Reactivity of Monoclonal Fab Fragments.** Recombinant Fabs were derived from a *C. jacchus* marmoset immunized with rMOG<sub>1–125</sub> produced in *Escherichia coli* (rMOG<sub>125</sub>) (12). Fabs were diluted in FACS buffer at 0.5  $\mu\text{g}/\text{ml}$  and added to ntCHO or MOG-transfected CHO cells. FITC-conjugated Ab against whole monkey IgG (Sigma) diluted at 1:100 was used as secondary Ab for 30 min at  $4^{\circ}\text{C}$ . FACS was performed as described above.

**ELISA Assay.** hMOG<sub>125</sub> expressed in *E. coli* was coated overnight on polystyrene microtiter plates at 0.5  $\mu\text{g}$  per well (Maxisorb, Nunc). After washing and blocking with 1% BSA in PBS + 0.05% Tween (BSA-PBS-T) for 2 h at room temperature, sera (1:200) were diluted in BSA-PBS-T and added to the plate. Ab binding was detected by an alkaline phosphatase AP-labeled goat-anti-human IgG (Sigma) for 1 h at room temperature. Plates were developed with *para*-nitrophenyl phosphate (Moss, Pasadena, MD) for 30 min in the dark at room temperature and read at 405 nm in a microplate reader (SpectraMax, Molecular Devices). Results were expressed as BR, i.e., signal over BSA background.

**Preabsorption of Sera on hMOG<sub>cme</sub> and hMOG<sub>125</sub>.** To further validate the cell-based assay and eliminate the possibility of nonspecific binding effects from either MS serum or transfection procedures, we conducted binding experiments after preincubation of serum with the respective antigens. For hMOG<sub>cme</sub> preabsorption,  $5 \times 10^6$  MOG-transfected CHO cells and ntCHO cells were separately incubated with serum diluted 1:10 for 1 h at room temperature with gentle agitation. After centrifugation at  $900 \times g$  for 2 min, supernatant were collected and preabsorption was

