Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. Although the cause of MS is still uncertain, many findings point toward an ongoing autoimmune response to myelin antigens. Because of its location on the outer surface of the myelin sheath and its pathogenicity in the experimental autoimmune encephalomyelitis model, myelin oligodendrocyte glycoprotein (MOG) is one of the potential disease-causing self antigens in MS. However, the role of MOG in the pathogenesis of MS has remained controversial. In this study we addressed the occurrence of autoantibodies to native MOG and its implication for demyelination and axonal loss in MS. We applied a high-sensitivity bioassay, which allowed detecting autoantibodies that bind to the extracellular part of native MOG. Antibodies, mostly IgG, were found in sera that bound with high affinity to strictly conformational epitopes of the extracellular domain of MOG. IgG but not IgM antibody titers to native MOG were significantly higher in MS patients compared with different control groups with the highest prevalence in primary progressive MS patients. Serum autoantibodies to native MOG induced death of MOG-expressing target cells *in vitro*. Serum from MS patients with high anti-MOG antibody titers stained white matter myelin in rat brain and enhanced demyelination and axonal damage when transferred to autoimmune encephalomyelitis animals. Overall these findings suggest a pathogenic antibody response to native MOG in a subgroup of MS patients.

antibodies | axonal damage | demyelination | lentiviral expression

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by inflammation, demyelination, gliosis, and neurodegeneration (1). Inflammatory infiltrates composed of macrophages/microglia cells, T cells, and B cells are found in MS lesions (2). In a significant proportion of patients, demyelination appears to be antibody-mediated and complement-dependent, with loss of oligodendrocytes and axonal damage (3). Despite intensive studies, the etiology of disease still remains uncertain (4). It is believed that MS results from an autoimmune response to proteins expressed in oligodendrocytes or the myelin sheath (5). Myelin oligodendrocyte glycoprotein (MOG) is one candidate target self-antigen. MOG expression is confined to the CNS and sequestered at the outermost surface of the myelin sheath (6, 7). This allows easy access by antibodies from the extracellular space.

MOG induces experimental autoimmune encephalomyelitis (EAE) in a variety of species (8–12). In contrast to other models, MOG protein elicited EAE is characterized by a pathogenic antibody response. Although anti-MOG antibodies cannot induce EAE on their own, they strongly enhance T cell and macrophage-initiated demyelination and may augment disease severity (12, 13). Several studies suggest that the pathogenicity of antibodies resides in their ability to recognize native MOG protein with proper glycosylation and to fix complement, while the significance of antibodies to linear epitopes is still controversial (14–17). The role of a specific immune response to MOG in MS patients is less clear. Previous studies have demonstrated that MOG-specific antibodies and T cells are not only detected in MS patients but also in healthy donors (18). In a recent study, the occurrence of serum anti-MOG and to lesser extent anti-myelin basic protein-specific IgM antibodies seemed to predict the incidence of new relapses in early MS patients (19). However, in a second study, a predictive role of the IgM antibody was not confirmed (20).

Anti-MOG antibodies are usually determined by Western blot (WB) with a recombinant fragment (amino acids 1–125) of the MOG protein expressed in *E. coli* ($MOG^{1–125}$). Because these WB are performed under denaturing conditions, they detect primarily antibodies to linear epitopes. The detected antibodies belong to the IgM and IgG isotypes (21) but seem to have low affinity to MOG because they cannot be measured by solution phase assays (22). A recent study indicated that these anti-MOG antibodies were low in serum but enriched in the CNS parenchyma (23). This finding is consistent with another report demonstrating the presence of antibodies to linear MOG peptide in lesions of EAE animals and MS patients (24). Although two studies have demonstrated antibodies to full-length MOG in MS patients (25, 26), none of the studies has characterized antibodies to conformational epitopes of MOG and has assessed their pathogenic role in MS.

Results

Expression of Human MOG in a Human Glioma Line. To obtain MOG in its native form with all posttranslational modifications that may occur in human glial cells, full-length human MOG cDNA was cloned into a lentiviral expression vector and transduced into the human glioblastoma cell line LN18 ($LN18^{MOG}$). The LN18 cell line was also transduced with an empty lentiviral vector to obtain an appropriate control line ($LN18^{Ctr}$), which was grown under the same conditions and solely differed from $LN18^{MOG}$ by the expression of MOG. Expression of MOG was analyzed with the monoclonal antibody (mAb) 8–18C5 (27). MOG was expressed in the $LN18^{MOG}$ line as monomer and dimer (Fig. 1*a*) (28). Surface expression of MOG was confirmed by immunocytochemistry and

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Abbreviations: EAE, experimental autoimmune encephalomyelitis; LN18, a human glioblastoma cell line; $LN18^{Ctr}$, an empty lentiviral vector transduced LN18 cell line; $LN18^{MOG}$, a human MOG gene containing lentiviral expression vector transduced LN18 cell line; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; OIND, other inflammatory disease of the CNS.

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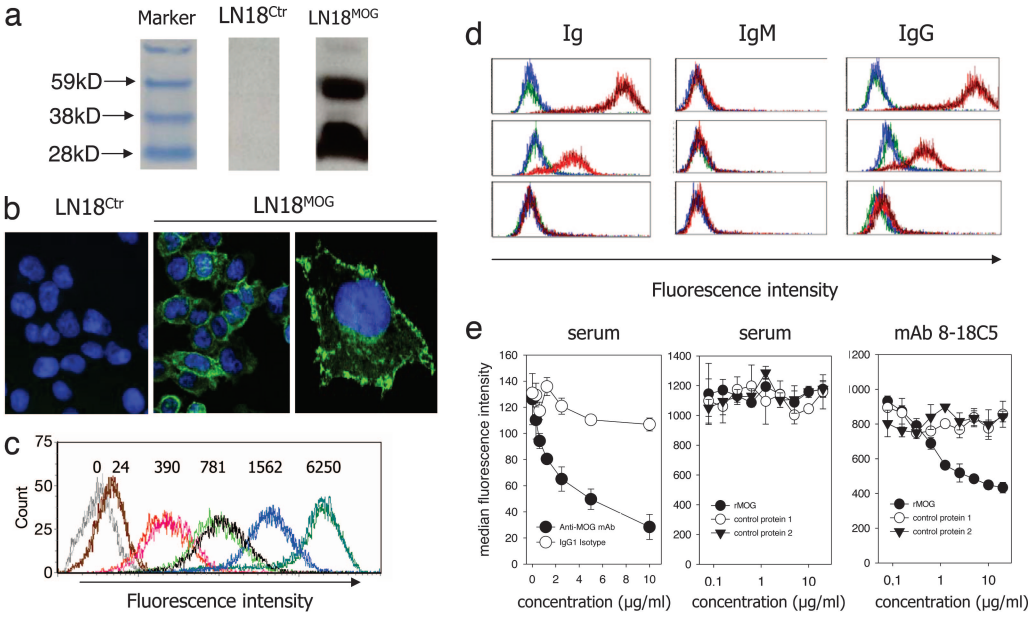

Fig. 1. Recombinant expression of native MOG to determine antibody binding. (*a*) Western blot analysis of MOG expression in cell lysates from LN18^{MOG} and LN18^{Ctr} cells in nonreducing condition. The 8–18C5 mAb stains two proteins corresponding to monomeric and dimeric MOG. (*b*) Immunocytochemistry of LN18^{ctr} cells [*Left* (×200)] and LN18^{MOG} [*Center* (×200) and *Right* (×600)] by 8–18C5 mAb. (*c*) Expression of MOG on LN18^{MOG} cells analyzed by flow cytometry at different concentrations of the 8–18C5 mAb. The antibody concentration is displayed on top of each curve in nanograms per milliliter. Duplicates are shown for each concentration. (*d*) Staining of LN18^{Ctr} (blue line) and LN18^{MOG} (red line) with sera of three MS patients. Serum antibody binding to MOG was detected by anti-human Ig (*Left*), IgM (*Center*), and IgG (*Right*) secondary antibodies and quantified by flow cytometry. (e) Competition assay for serum antibody binding to native MOG. Antibody-positive serum was applied at a 1:100 dilution to the LN18^{MOG} cells in the presence of different concentrations of 8–18C5 (filled circles) or an irrelevant IgG1 isotype antibody (open circles) (*Left*). Competition assay for antibody binding to recombinant MOG$^{1–125}$. Antibody-positive serum (*Center*) or 8–18C5 mAb (*Right*) was applied to the LN18^{MOG} cells in the presence of different concentrations of recombinant MOG$^{1–125}$ (filled circles) or two irrelevant recombinant proteins (open circles and filled triangles). Binding of antibodies was determined by secondary anti-human (*Left* and *Center*) or -mouse (*Right*) IgG antibodies and quantified by flow cytometry.

flow cytometry (Fig. 1 *b* and *c*). A highly reproducible surface staining was observed at different concentrations of the mAb demonstrating that the LN18^{MOG} cell line can be used to quantify antibody responses to native MOG (Fig. 1*c*).

Detection of Human Serum Antibodies to Native MOG. Serum samples were analyzed for antibody staining of LN18^{MOG} and LN18^{Ctr} cells. We observed large differences in the extent of antibody binding to the LN18^{MOG} line in human sera (Fig. 1*d*). While some sera stained the LN18^{MOG} cells similarly to the mAb 8–18C5 (even at a dilution of 1:10,000), others did not show any specific binding to the LN18^{MOG} compared with the LN18^{Ctr} cells. Immunocytochemistry of LN18^{MOG} cells with anti-MOG antibody-positive sera revealed a membrane staining similar to the staining obtained with the mAb 8–18C5. Antibodies to native MOG in serum were predominantly IgG (Fig. 1*d*).

The MOG specificity of the serum antibodies was investigated by competition assays with the mAb 8–18C5. While an irrelevant IgG1 isotype antibody did not compete with the binding of serum antibody, 8–18C5 mAb decreased serum staining in a dose-dependent manner (Fig. 1*e*). Recombinant MOG$^{1–125}$ did not compete with the binding of serum antibodies, suggesting that the human antibodies, in contrast to 8–18C5 mAb, recognize a strictly conformational epitope not comprised in the recombinant MOG$^{1–125}$ (13). This assumption was further supported by the lack of serum antibody reactivity with recombinant MOG$^{1–125}$ in WB performed under denaturating conditions or immunocytochemistry on paraformaldehyde fixed LN18^{MOG} cells (data not shown).

Determining Antibodies to Native MOG in MS Patients. The transduced cell lines were then used to quantify antibody responses in serum. We found an increased antibody reactivity to native MOG in MS patients compared with patients with other inflammatory disease of the CNS (OIND; Fig. 2*a*) or healthy age-matched control donors (HD) (Fig. 2*b*). Further stratification of the isotype by secondary antibodies revealed that MS patients differed from controls with respect to IgG but not IgM antibodies to native MOG (data not shown). The same result was obtained in three additional independent patient-control groups, one of them with blinded samples from a multicenter study comparing different methods to detect anti-MOG antibodies. When patients were stratified for their disease course, all MS patient groups had higher antibody levels in serum compared with controls. The number of patients with antibodies against native MOG was highest in primary progressive MS (PP-MS) patients (Fig. 2*b*).

Biological Consequences of Human Antinative MOG Antibodies *in Vitro*. LN18^{Ctr} and LN18^{MOG} cell were incubated with 1:100 diluted anti-MOG antibody-positive or -negative sera and cell survival determined after 20 h. 8–18C5 mAb mixed with anti-MOG antibody-negative serum was used as positive control. 8–18C5 mAb strongly decreased the cell number of the LN18^{MOG} but had no effect on LN18^{Ctr} cells. While antibody-negative sera had no differential effect on LN18^{MOG} and LN18^{Ctr} cells, all antibody-positive sera reduced cell numbers of the LN18^{MOG} compared with the LN18^{Ctr} cells (Fig. 3). Complement activity in anti-MOG antibody-negative and -positive sera did not differ significantly excluding unspecific complement activation as the cause of cytotoxicity (Fig. 3).

Demyelinating Property of Human Antinative MOG Antibodies in EAE. Rat brain sections were incubated with either anti-MOG antibody-positive or -negative sera. 8–18C5 mAb was used as

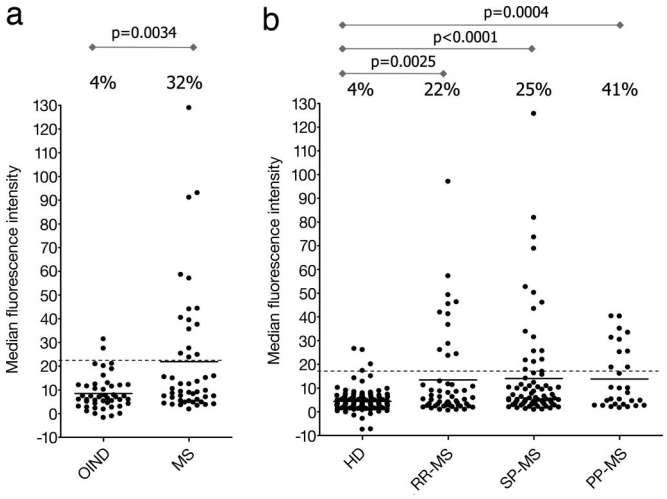

Fig. 2. Increased IgG antibody titers to native MOG in MS patients. (*a*) Comparative analysis for serum IgG antibody titers to native MOG in MS ($n = 47$) and OIND ($n = 47$) patients. (*b*) Comparative analysis for IgG antibody titers to MOG in HD ($n = 140$) and a second group of MS patients stratified for disease course (54 patients with RR-MS, 80 patients with SP-MS, and 29 patients with PP-MS). Antibody binding to $LN18^{MOG}$ and $LN18^{Ctr}$ cells was determined in each patient by secondary anti-human IgG antibodies and quantified by flow cytometry. The MOG-specific antibody response was calculated by subtracting median fluorescence intensities obtained with $LN18^{Ctr}$ from the one obtained with $LN18^{MOG}$ cells. Titers were compared by the Kruskal-Wallis nonparametric analysis. The *P* values are shown for the comparison of different patient groups. The number of patients with titers exceeding the mean of OIND (*a*) and HD (*b*) by two standard deviations is shown.

positive control. 8–18C5 strongly stained myelin sheaths in the white matter of the rat brain. A similar staining was observed with sera that contained high titers of antibodies to native MOG, but not with antibody-negative control sera (Fig. 4).

To test the demyelinating properties of the human anti-MOG antibody, serum was concentrated to obtain an antibody concentration comparable to the amount of the 8–18C5 mAb necessary to produce demyelination in the EAE model (7 fold concentrated). Active EAE was induced by immunization of Lewis rats with guinea pig MBP peptide 72–85. At onset of EAE, animals were divided in groups with a similar disease score of 1–2. The animals received 7-fold concentrated serum of an antibody-negative or an antibody-positive patient by i.v. injection. In addition, concentrated anti-MOG antibody-positive serum was depleted from IgG and also injected in rats. The 8–18C5 mAb was administered at a high concentration, ≈10-fold higher than the expected anti-MOG antibodies in the concentrated human serum.

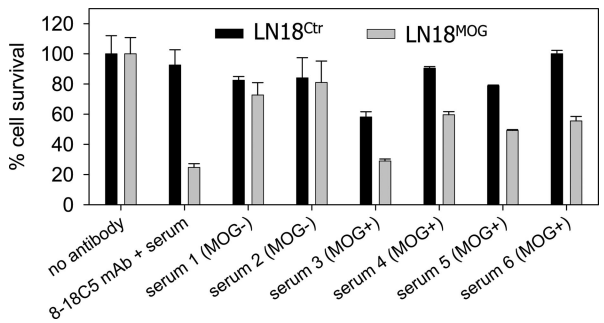

Fig. 3. Human antibodies to native MOG induce cell death of MOG-expressing target cells. $LN18^{MOG}$ (gray bars) and $LN18^{Ctr}$ (black bars) cells were incubated with anti-MOG antibody-positive and -negative MS sera. 8–18C5 mAb supplemented by serum from an antibody-negative patient was used as control. The cell numbers were determined after 20 h and normalized with the negative control sample (equals 100%). The experiment was performed in duplicate; mean and standard deviation are shown. The complement activity of all of the sera ranged between 50 and 55 CAE unit, without significant difference between anti-MOG antibody-positive and -negative sera. One representative experiment of three is shown.

While administration of the human sera had no impact on the EAE score, demyelination and axonal loss were significantly increased in animals injected with antibody-positive serum (Fig. 5*a*). The occurrence of demyelination in rats receiving antibody-positive serum was confirmed by electron microscopy (EM) showing demyelinated axons in the perivascular area (data not shown). Similarly, amyloid precursor protein (APP) staining, which strongly correlated with demyelination, was significantly enhanced in the rats receiving the anti-MOG antibody-positive human serum. The APP staining, which probably relates to the susceptibility of demyelinated axons to neurotoxicity, turned out to be better quantifiable than the LFB/PAS staining and thus a more reliable and distinct marker for the antibody effect. Depletion of IgG from the anti-MOG antibody-positive serum significantly reduced APP staining to the level observed with anti-MOG negative serum (Fig. 5*b*). These differences were not due to differences in the extent of inflammation. Because the anti-MOG antibody-negative serum contained more complement activity than the antibody-positive serum, unspecific complement activation can be excluded as the cause of the observed pathogenic effects.

Discussion

Despite accumulating evidence that adaptive immune responses play a critical role in the pathogenesis of MS, it has been challenging to identify pathogenic T, B cells, or antibodies. The pathogenic potency of myelin-specific T cells derived from MS patients was formally demonstrated in TCR transgenic mice, but this required a highly artificial setup and active immunization to reliably induce disease (29). No pathogenic and pathognomonic human B cell clonotype or antibody have been identified in MS so far, although a number of findings support the existence of pathogenic antibodies in this disease (30, 31). This is different from other human autoimmune diseases such as myasthenia gravis, where pathogenic antibodies have been identified and characterized (32).

According to the Rose–Witebsky postulates, three criteria need to be met to define an aberrant immune response as autoimmune (33). The first level of evidence is the least stringent, and only requires the presence of autoantibodies or associations with other autoimmune diseases. The next level calls for indirect evidence, such as induced or spontaneous autoimmune disease reproduced in an experimental animal. These animal models may allow passive transfer of disease. The most convincing evidence for an autoimmune etiology of a human disorder is achieved when the disease can be transferred from person to person.

These criteria have not been met in MS. While elevated antibody titers have been described for a number of self and foreign antigens in MS patients, none of them has proven biological activity. Among all of the autoantibodies in MS that are under investigation, MOG seems to be a promising target. In our study we applied a new strategy to characterize the human antibody response to native MOG. In contrast to previous studies, we expressed the human protein in a human glia cell line to reflect MOG expressed in the human brain as close as possible (25, 26). The transduced cell line, which stably expresses high levels of MOG on the surface, and a cell line transduced with an empty vector were used to screen for antibodies. We identified serum antibodies against a strictly conformational epitope of MOG. The IgG, but not the IgM, antibody response to native MOG was significantly higher in MS patients compared with

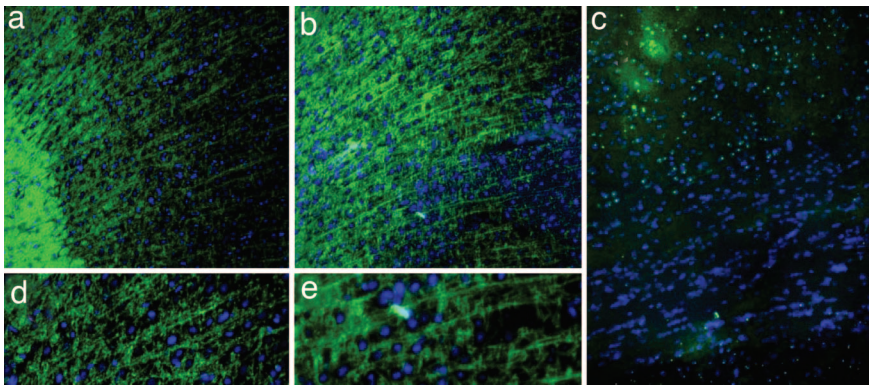

Fig. 4. Human antibodies to native MOG bind to intact myelin. Rat brain slices were stained with 8–18C5 mAb (*a* and *d*), anti-MOG antibody-positive and -negative sera. Staining was visualized by an anti-IgG antibody labeled with Alexa Fluor 488. Stainings of one representative anti-MOG antibody-positive serum of five (*b* and *e*) and one of four negative sera (*c*) are shown. (Magnification: *a*–*c*, ×100; *d* and *e*, ×200.)

different control groups. In contrast to a previous report, these antibodies were more prevalent in PP-MS than relapsing remitting (RR) MS (26). The antibodies seem to be directed against the same epitope that elicits a pathogenic antibody response in EAE. Serum with high anti-MOG antibody titers promoted cell death of MOG expressing target cells *in vitro*. Furthermore, these human anti-MOG antibodies not only stained MOG expressing target cells but also myelin in rat brain comparable to the staining pattern observed with the mAb 8–18C5. The transfer of the human serum to EAE rats precipitated demyelination and consequently damage to demyelinated axons. Although the serum had to be concentrated to generate these effects, the level of the human anti-MOG antibody after injection in the rats was still considerable lower than in the patient's serum. After a single injection, the antibody reached a maximal concentration of less than 40% compared with the concentration in the serum of patients with high titers (0.5 ml 7-fold concentrated human serum injected into rats with a blood volume of ≈10 ml). Given the presence of antibody over months in the patients' serum, it is conceivable that this antibody contributes to demyelination and axonal damage in the inflammatory lesion.

The results are in line with pathogenic concepts originating from animal models. While antibodies to linear and conformational epitopes of MOG are generated by immunization with spinal cord homogenate, only those antibodies that target conformational epitopes seem to be pathogenic. These findings parallel the results in human disease. While antibodies to linear epitopes or recombinant fragments of MOG can be detected in MS patients, these antibodies do not bind the native protein nor do they induce pathogenic changes of MOG positive target cells.

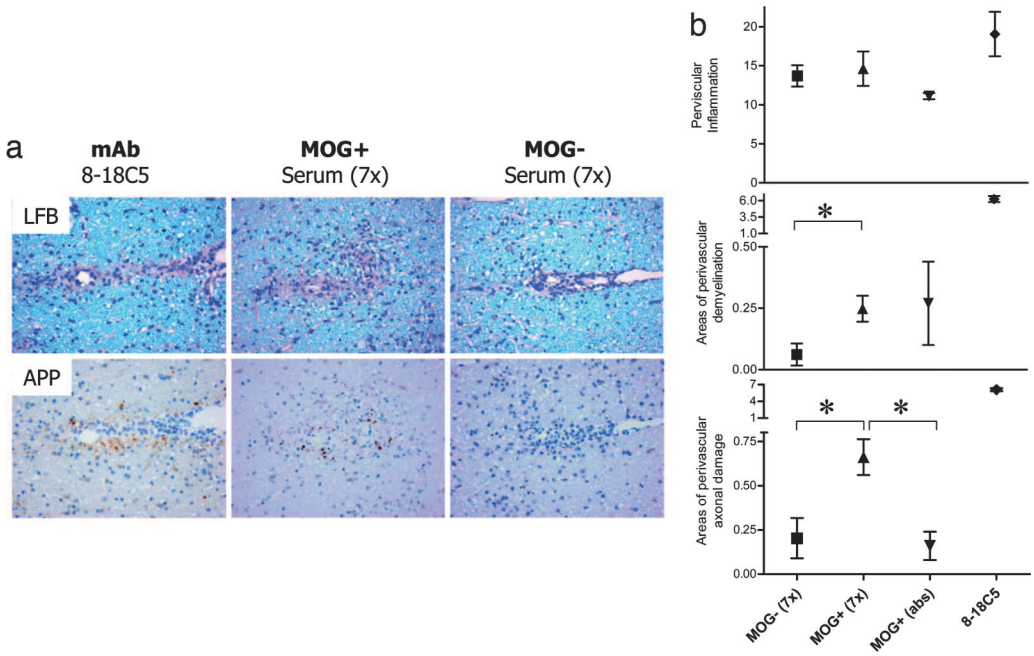

Fig. 5. Human antinative MOG antibodies induce demyelination and axonal damage in rat EAE. (*a*) EAE was induced in Lewis rats, and different sera or 8–18C5 mAb were injected intravenously. Demyelination was determined by LFB/PAS staining, and axonal damage was determined by APP staining on spinal cord sections. Representative perivascular areas are shown for animals treated with 8–18C5 mAb, anti-MOG antibody-positive or -negative sera. (*b*) Comparative analysis of demyelination, axonal damage, and perivascular infiltrates in EAE animals injected with 7-fold concentrated anti-MOG antibody negative [MOG− (7x), four animals], 7-fold concentrated anti-MOG antibody positive before [MOG+ (7x), four animals] and after IgG absorption [MOG+ (abs), two animals], and the control mAb of 1 mg of 8–18C5 mAb. *, $P < 0.05$ (*t* test). One representative experiment of three is shown. The mean EAE scores were 2 (8–18C5), 1 (MOG+), 1.25 (MOG−), and 0.75 [MOG+ (abs)]. Complement activity was 67 for MOG+ and 77 CAE units for MOG− serum.

Their role as biomarkers has remained controversial (19, 20). In contrast IgG antibodies to native MOG are elevated in a subgroup of MS patients and are pathogenic for MOG expressing cells resulting in demyelination and axonal damage. While not all criteria of the Rose–Witebsky postulates were met, our study provides additional evidence for the autoimmune pathogenesis of MS, and it demonstrates the importance of investigating antibody responses to native autoantigens in autoimmune diseases. Because anti-MOG antibodies are only elevated in a subgroup of patients, further studies have to address how this autoantibody relates to clinical and pathological parameters and whether antibody responses to other native autoantigens play a role in MS. The occurrence of antibodies with demyelinating properties further supports the pathogenic role of the humoral immune system in MS and calls for the development of B cell directed therapies not only for RR but also PP-MS (34, 35).

Materials and Methods

Patients and Controls. Patients and controls were recruited at the Departments of Neurology in Düsseldorf and Marburg. MS was diagnosed according to the McDonald criteria. Control groups consisted of aged-matched HD or patients with OIND (e.g., bacterial or viral meningitis, viral encephalitis, neurosyphilis, HIV infection). The ethics committees of the universities of Marburg and Düsseldorf approved the study.

Cloning and Expression of MOG. A human brain total RNA (BD Biosciences) was used to synthesize cDNA. For expression of human full-length MOG (247 aa) using a lentivirus expression system (Invitrogen), the primers 5′-ATTGAGATCTGAGATGGCAAG-3′ and 5′-GAGATCTCAGAAGGGATTTCG-3′ were used to add BglII restriction sites at 5′ and 3′ ends of the MOG cDNA, respectively. The PCR product was cloned into the plasmid pLenti6/V5 (Invitrogen). pLenti6/V5-MOG and the packaging mix were used to transfect a 293FT cell line by Lipofectamine 2000 (Invitrogen). Virus-containing supernatant was used to transduce the human LN18 glioblastoma cell line (36). As a control, we transduced the LN18 line with an empty vector pLenti6/V5. This stably transduced cell line was maintained under the same conditions as the $LN18^{MOG}$ line and used as control throughout the experiments.

Flow Cytometry Analysis. The surface expression of MOG on LN18 was verified by flow cytometry with anti-MOG monoclonal antibody (8–18C5) in combination with secondary FITC-labeled goat anti-mouse antibody (BD Biosciences). $LN18^{MOG}$ cells were used to measure anti-MOG antibodies. The feasibility and sensitivity of the assay were studied by titration experiments of the mAb 8–18C5. $LN18^{MOG}$ cells (20,000) in 20 μl of RPMI medium 1640 growth medium were added to each well of 96-well plates in duplicates containing 20 μl of diluted 8–18C5 mAb. The plates were placed on ice and shaken for 20 min. Cells were then washed twice with washing buffer (PBS plus 1% FBS). FITC-labeled goat anti-mouse antibody (diluted 1:50) was added on ice. After 20 min, cells were washed twice and resuspended in 150 μl of washing buffer. Cells were analyzed on a FACSCalibur machine (BD Biosciences). Human serum was diluted 1:18 in growth medium and added to the cells yielding a final dilution of 1:36 (37). Anti-MOG antibodies were determined as described above, except that the secondary antibody was replaced by FITC-labeled anti-human Ig, IgG, or IgM (Serotec).

Competition Assay. Human anti-MOG antibody-positive sera were diluted 1:100 and mixed with different concentrations of the 8–18C5 mAb. $LN18^{MOG}$ cells (20,000) in 20 μl were incubated with 20 μl of serum/mAb mixture. The staining procedure was described above. FITC-labeled anti-human IgG was used as secondary antibody. An IgG1 isotype antibody (HHF35; Serotec) was used as control in the competition experiment.

Preabsorption Assay. A total of 1 μg/ml anti-MOG mAb or 1:40 diluted anti-MOG antibody-positive human sera were preabsorbed with recombinant human MOG (1–125) at different concentrations. $LN18^{MOG}$ cells (20,000) in 20 μl of medium were incubated with 20 μl of serum or mAb and recombinant MOG protein. The staining procedure was described above. FITC-labeled anti-mouse Ig or anti-human IgG was used as secondary antibody, respectively. Two irrelevant recombinant proteins were used as control in the competition experiment.

Western Blot Analysis. $LN18^{MOG}$ or $LN18^{Ctr}$ were lysed with RIPA buffer (Sigma). Lysate or recombinant MOG (1–125) was separated by 4–15% SDS/PAGE (Invitrogen). The blots were incubated with 1:100 diluted serum or 0.2 μg/ml anti-MOG mAb. An HRP-conjugated goat anti-human Ig or anti-mouse Ig (Serotec) was used as secondary antibody. Antibody binding was detected by ECL system (Amersham Biosciences).

Immunocytochemistry and Image Analysis. The immunocytochemistry of $LN18^{MOG}$ or $LN18^{Ctr}$ was performed by using standard protocols. The experiment was performed by using 0.3 μg/ml anti-MOG mAb or 100-fold-diluted anti-MOG antibody-positive human serum as primary antibody source and an Alexa 488-conjugated goat anti-human Ig or goat anti-mouse Ig (Invitrogen) as secondary antibody. DAPI (Invitrogen) was used for the nuclear staining. Images were captured and analyzed by an Olympus IX71 microscope system.

***In Vitro* Cytotoxicity Assay.** A total of 50,000 $LN18^{MOG}$ or $LN18^{Ctr}$ cells in 200 μl of growth medium were seeded into a 96-well plate. Two microliters of sera from anti-MOG antibody-positive or -negative patients was added in duplicate to the plate. Four micrograms of mAb per milliliter mixed with 100-fold-diluted anti-MOG antibody-negative serum was used as control. After incubation at 37°C for 20 h, cells were washed, resuspended in 200 μl of washing buffer, and transferred to Falcon tubes. The cell number in each well was determined by using a cell counter.

Serum Staining of Rat Brain Sections. Frozen rat brain tissue was sectioned (12-μm slices) on a cryostat at −20°C (Leica). After acetone fixation and blocking of sections, 0.3 μg/ml anti-MOG mAb or 100-fold-diluted sera were used as primary antibody, and Alexa 488-conjugated goat anti-human IgG or goat anti-mouse IgG (Invitrogen) was used as secondary antibody. Images were captured and analyzed as described.

Concentration of Human Serum. Anti-MOG positive and negative sera were concentrated by using Amicon Ultra-15 centrifugal filter devices (Millipore). Twenty-two milliliters of serum was concentrated to a final volume of 3 ml. IgG absorption by protein G Sepharose column (Amersham Biosciences) was performed on 1 ml of the serum. The nonconcentrated, concentrated serum, flow-through, and IgG absorbed concentrated sera were analyzed by flow cytometry for anti-MOG antibody titers.

Animal Experiments. Female Lewis rats (Charles River Laboratories) were obtained and kept according to the local animal guidelines. All animals weighed 180 g at immunization and were 6–8 weeks of age. All procedures were performed according to an animal experimentation protocol that was approved by the institutional animal care and use committee at the Georg August University and the Bezirksregierung Braunschweig, Germany.

For EAE induction, animals were immunized s.c. with 100 μg of guinea pig MBP72–85 (LPQKSQRSQDENPV, purity >80%; Jerini) emulgated in incomplete Freund's adjuvant (IFA) sub-

stituted with 5 mg/ml inactivated *Mycobacterium tuberculosis* H37 Ra (both from Difco) (38). Clinical signs of EAE were rated and were cross-checked by independent observers. Five hundred microliters of human serum or 8–18C5 (100 μg per animal diluted in anti-MOG antibody-negative serum) was injected into the retrobulbar venous plexus of the animals at a disease score between 1 and 1.5. Thirty hours after serum injection, animals were anesthetized and perfused transcardially with PBS and 4% PFA.

Histology and Immunohistochemistry. Brains and spinal cords were dissected, cut, and embedded in paraffin. Inflammation, demyelination, and axonal damage were assessed by hematoxylin/eosin staining, Luxol fast blue staining, and immunohistochemistry for APP, a marker for axonal damage (Clone 2C11; Chemicon). Bound antibody was visualized by using an avidin–biotin technique with 3,3′-diaminobenzidine as chromogen. The extent of inflammation is given as the number of inflammatory infiltrates/rat spinal cord cross-section. For the assessment of perivascular demyelination and axonal damage in transferred EAE rats, the number of vessels with perivascular demyelination and axonal damage per spinal cord cross-section was determined. All analyses were performed by a blinded investigator (C.S.). At least 20 stained spinal cord cross-sections per rat were examined.

Statistical Analysis. Kruskal–Wallis nonparametric analysis was performed for the comparison of antibody titer in patients and controls (normality was not passed for *t* test). A *t* test was used for comparison of LFB or APP score in rats treated with different sera. The analysis was performed by SigmaStat 3.0 (Systat).

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