# Pathogenicity of Human Antibodies against Myelin Oligodendrocyte Glycoprotein

Melania Spadaro, PhD,[1] Stephan Winklmeier, MSc,[1] Eduardo Beltrán, PhD,[1] Caterina Macrini, MSc,[1] Romana Höftberger, MD,[2] Elisabeth Schuh, MD, PhD,[1] Franziska S. Thaler, MD,[1] Lisa Ann Gerdes, MD,[1] Sarah Laurent, MD, PhD,[1] Ramona Gerhards, MSc,[1] Simone Brändle, PhD,[1] Klaus Dornmair, PhD,[1] Constanze Breithaupt, PhD,[3] Markus Krumbholz, MD,[4] Markus Moser, PhD,[5] Gurumoorthy Krishnamoorthy, PhD,[5] Frits Kamp, PhD,[6] Dieter Jenne, MD,[7] Reinhard Hohlfeld, MD,[1,8] Tania Kümpfel, MD,[1] Hans Lassmann, MD,[9] Naoto Kawakami, PhD,[1]* and Edgar Meinl, MD[1]*

**Objective:** Autoantibodies against myelin oligodendrocyte glycoprotein (MOG) occur in a proportion of patients with inflammatory demyelinating diseases of the central nervous system (CNS). We analyzed their pathogenic activity by affinity-purifying these antibodies (Abs) from patients and transferring them to experimental animals.
**Methods:** Patients with Abs to MOG were identified by cell-based assay. We determined the cross-reactivity to rodent MOG and the recognized MOG epitopes. We produced the correctly folded extracellular domain of MOG and affinity-purified MOG-specific Abs from the blood of patients. These purified Abs were used to stain CNS tissue and transferred in 2 models of experimental autoimmune encephalomyelitis. Animals were analyzed histopathologically.
**Results:** We identified 17 patients with MOG Abs from our outpatient clinic and selected 2 with a cross-reactivity to rodent MOG; both had recurrent optic neuritis. Affinity-purified Abs recognized MOG on transfected cells and stained myelin in tissue sections. The Abs from the 2 patients recognized different epitopes on MOG, the CC′ and the FG loop. In both patients, these Abs persisted during our observation period of 2 to 3 years. The anti-MOG Abs from both patients were pathogenic upon intrathecal injection in 2 different rat models. Together with cognate MOG-specific T cells, these Abs enhanced T-cell infiltration; together with myelin basic protein–specific T cells, they induced demyelination associated with deposition of C9neo, resembling a multiple sclerosis type II pathology.
**Interpretation:** MOG-specific Abs affinity purified from patients with inflammatory demyelinating disease induce pathological changes in vivo upon cotransfer with myelin-reactive T cells, suggesting that these Abs are similarly pathogenic in patients. ANN NEUROL 2018;00:000–000

**ANN NEUROL 2018;84:315–328**

High levels of antibodies (Abs) to conformationally intact myelin oligodendrocyte glycoprotein (MOG) have initially been detected in pediatric patients,[1] then also in a proportion of patients with different

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.25291

Received Feb 28, 2018, and in revised form May 15, 2018. Accepted for publication Jul 1, 2018.

Address correspondence to: Dr Meinl, Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Großhaderner Str 9, 82152 Planegg-Martinsried, Germany. E-mail: edgar.meinl@med.uni-muenchen.de
*Naoto Kawakami and Edgar Meinl contributed equally

From the [1]Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Munich, Germany; [2]Institute of Neurology, Medical University of Vienna, Vienna, Austria; [3]Department of Physical Biotechnology, Martin Luther University of Halle-Wittenberg, Halle, Germany; [4]Department of Neurology and Hertie Institute for Clinical Brain Research, Eberhard Karl University, Tübingen, Germany; [5]Max Planck Institute of Biochemistry, Martinsried, Germany; [6]Department of Biophysics, Biomedical Center, Ludwig Maximilian University of Munich, Munich, Germany; [7]Comprehensive Pneumology Center (CPC), Institute of Lung Biology and Disease, Helmholtz Zentrum München, Munich, and Max Planck Institute of Neurobiology, Planegg-Martinsried, Germany; [8]Munich Cluster for Systems Neurology, Munich, Germany; and [9]Center for Brain Research, Medical University of Vienna, Austria.

 

demyelinating diseases such as optic neuritis, myelitis, encephalomyelitis, brainstem encephalitis, acute disseminated encephalomyelitis (ADEM), and anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis, and in a few patients with multiple sclerosis (MS).[2–6] Patients with autoantibodies to MOG have distinct brain magnetic resonance imaging (MRI) characteristics.[7,8] It is debated whether anti-MOG disease constitutes a separate entity.[9]

In animal models, some monoclonal Abs (mAbs) to MOG induce demyelination provided the blood–brain barrier is breached giving the Abs access to the CNS (reviewed in Hohlfeld et al,[5] Mayer and Meinl[10]). Only a proportion of anti-MOG Abs are able to induce demyelination in vivo, related to complement activation[11] and recognition of conformationally correct MOG.[12,13] In rodents, pathogenic MOG-specific Abs mainly recognize the FG loop of MOG as the prototype mAb 8-18C5,[14] whereas patients with Abs to MOG recognize different loops of MOG, most frequently the CC′ loop around the amino acid P42.[15]

Previous experiments to test the potential pathogenic activity of human anti-MOG Abs in vitro reported that sera of patients with Abs to MOG activated complement,[16] stimulated natural killer cell mediated toxicity,[17] induced cytoskeletal changes in oligodendroglial cells,[18] mediated myelin destruction in slice cultures,[19] and facilitated MOG uptake by macrophages.[20] Peripheral injection of concentrated serum from MS patients in rats with experimental autoimmune encephalomyelitis (EAE) slightly enhanced demyelination and axonal loss.[21] Total IgG preparations pooled from 5 neuromyelitis optica (NMO) patients were injected intracerebrally and induced myelin changes independent of complement, but no inflammation.[22] Intrathecal injection of IgG from a patient with MOG Abs accelerated EAE in mice.[23] Peripheral injection of IgG from MS patients with Abs to MOG exacerbated EAE in mice.[24] Thus, there is evidence that human Abs to MOG are pathogenic, but one has to consider that patients with neuroinflammation may have multiple autoantibodies,[25–27] which complicates the interpretation of transfer experiments with whole IgG preparations. Transfer experiments with human affinity-purified Abs to MOG have not yet been done, and therefore detailed pathogenic mechanisms of human Abs to MOG remain to be elaborated.

Patients with Abs to MOG have a pathology described as MS pattern II,[28–31] characterized by active demyelination along with deposition of C9neo, suggesting an Ab-mediated demyelination.[32,33] Transfer experiments with autoantibodies to MOG from these patients were hampered because only a proportion of MOG Abs from patients cross-react with rodent MOG[15,28]; therefore, the linkage of human MOG Abs to a certain neuropathology is still speculative.

The aim of this study was to analyze which human Abs to MOG are pathogenic, to identify recognized epitopes of pathogenic autoantibodies, to test whether they can mediate MS type II pathology, and to explore their pathogenic mechanisms. To this end, we combined affinity purification of Abs that recognize cell-based MOG, epitope identification with mutants of MOG, staining of tissue sections, and transfer experiments in 2 EAE models. This showed that Abs to MOG were pathogenic by 2 mechanisms; in synergy with myelin basic protein (MBP)-specific T cells they mediate MS type II pathology, and together with MOG-specific T cells they enhance T-cell infiltration.

## Patients and Methods

### *Standard Protocol Approvals, Registrations, and Patient Consents*

We analyzed sera from 260 patients with inflammatory CNS diseases for anti-MOG reactivity. The clinical characteristics of patients who scored positive in our cell-based assay detecting Abs to MOG are summarized in the Table. All MOG Ab–positive patients were followed longitudinally. Informed consent was obtained from each donor according to the Declaration of Helsinki and the ethical committee of the medical faculty of Ludwig-Maximilians-Universität München approved this study.

### *Determination of anti-MOG Reactivity and Epitope Recognition*

Patients positive for Abs to MOG were identified with a cell-based flow cytometry assay using viable cells and a serum dilution of 1:50, as described.[28,34] Isotype-specific secondary Abs were obtained from Southern Biotech (Birmingham, AL). To identify the recognized epitopes, mutant variants of MOG were applied and the percentage binding compared to human MOG was calculated as described.[15] In some experiments, we used a recombinant variant of the mAb 8-18C5 (designated r8-18C5), which has the same antigen recognition site, but a human IgG1 Fc part.[35]

### *Production and Validation of Recombinant Human MOG*

We aimed to produce a recombinant version of the extracellular domain (ECD) of human MOG that comes as close as possible to the conformation of MOG displayed in transfected cells. To this end, we produced the ECD of human MOG in HEK293-EBNA cells and added at the C-terminus instead of the first transmembranous region a HisTag and an AviTag using the pTT5 vector.[36] MOG

was biotinylated by using the BirA biotin ligase Kit (Avidity, Aurora, CO). Folding of the purified protein (0.2mg/ml) was analyzed by circular dichroism using a Jasco J-810 spectropolarimeter (JASCO Corporation, Tokyo, Japan). To further validate the anti-MOG binding activity of our recombinant MOG, we tested whether this MOG was bound by B cells from mice with a knock-in of the heavy chain of the anti-MOG 8-18C5.[37] To this end, we formed MOG tetramers with our biotinylated MOG and fluorescently labeled streptavidin (Jackson ImmunoResearch, West Grove, PA).

### Affinity Purification of Anti-MOG Abs

Biotinylated MOG was bound to a HiTrap Streptavidin HP column (GE Healthcare, Munich, Germany). Ig from plasma (obtained from ethylenediaminetetraacetic acid [EDTA]–blood) was first enriched by ammonium sulfate precipitation and then loaded on this column. Bound Ig was eluted (100mM glycin, 150mM NaCl, pH 2.5) and immediately neutralized with 1M Tris-HCl, pH 8.8. The eluates from both patients were separated by reducing and nonreducing sodium dodecyl sulfate gel electrophoresis and stained by Coomassie. The excised gel bands were in-gel digested essentially as described.[35] Peptides were analyzed by matrix-assisted laser desorption/ionization time of flight/time of flight using a 4800 Analyzer (Applied Biosystems, Foster City, CA). The eluates were tested by enzyme-linked immunosorbent assay (ELISA) for streptavidin reactivity using streptavidin-coated plates.

### Staining of Tissue with Patient Abs

Rat brains were fixed in 4% paraformaldehyde (PFA) for 1 hour, cryoprotected with 40% sucrose, and snap frozen. Seven-micrometer-thick sagittal sections were incubated with 0.3% hydrogen peroxide for 20 minutes and with 10% donkey serum in phosphate-buffered saline (PBS) for 1 hour, and then labeled with the Abs at 4°C overnight. The next day, sections were labeled with a donkey–antihuman IgG (H+L) secondary Ab (Jackson ImmunoResearch) and visualized with an avidin–biotin–diaminobenzidine reaction.

### Transfer EAE and Rat T-Cell Lines

Antigen specific T cells were established from Lewis rats immunized with antigen emulsified in complete Freund adjuvant as described previously.[38] The following antigens were used: recombinant MOG (amino acid 1–125), MBP purified from guinea pig brain, and ovalbumin (OVA) purchased from Sigma-Aldrich (St Louis, MO). To induce mild EAE, freshly restimulated $15 \times 10^6$ MOG-specific T cells or $1.2 \times 10^6$ MBP-specific T cells were injected intravenously in Lewis rats. Clinical scores were evaluated as follows: 0 = normal; 0.5 = loss of tail tonus; 1 = tail paralysis; 2 = gait disturbance; 3 = hindlimb paralysis. Two days after injection of T cells, 100 μg of the indicated Ab preparations was injected intrathecally into the cisterna magna to animals anesthetized by fentanyl/midazolam/medetomidine. For the monitoring of clinical score, animals were followed until full recovery and were then sacrificed. For histopathological analysis, 72 hours after Ab injection, animals were perfused with PBS and 4% PFA in PBS under terminal anesthesia with fentanyl/midazolam/medetomidine; the spinal cord and brain were then postfixed with 4% PFA in PBS at 4°C. The procedures are approved by the government of Upper Bavaria.

### Histological Examination of the EAE Rats

Brain, spinal cord, and optic nerves were dissected and embedded in paraffin. Serial sections of all tissues were stained with hematoxylin/eosin, Luxol fast blue (LFB) myelin stain, and Bielschowsky silver impregnation for axons. Immunocytochemistry was performed on paraffin sections after antigen retrieval in a food steamer with EDTA buffer, pH 8.5. Primary Abs against the following targets were used in the following dilutions: CD3 (T cells; rabbit monoclonal; Neomarkers, Fremont, CA; RM-9107-5; 1:2,000), ED1 (phagocytic macrophages and microglia; mouse monoclonal; Serotec, Raleigh, NC; MCA341R, 1:10,000), Iba 1 (pan microglia and macrophages; rabbit polyclonal; Wako, Osaka, Japan; 019-19741; 1:3,000), cyclic nucleotide phosphodiesterase (oligodendrocytes; mouse monoclonal; Sternberger Monoclonals, Lutherville, MD/BioLegend, San Diego, CA; SMI 91; 1:2,000), glial fibrillary acidic protein (astrocytes; rabbit polyclonal; Dako, Santa Clara, CA; Z0334; 1:3,000), human Ig (biotinylated species specific antihuman Ig; donkey polyclonal, Jackson ImmunoResearch, 709-065-149; 1:1,000) and activated complement (C9neo antigen, rabbit polyclonal; 1:2,000).[11] Bound primary Abs were visualized with a biotin/avidin/peroxidase system. To quantify the inflammation, $CD3^+$ T cells/$mm^2$ were counted in a zone of 200 μm spanning from the ventral subpial surface into the tissue of the pons. To quantify demyelination, the distance of subpial demyelination from the ventral surface of the pons was measured. To this end, macrophages were stained with ED1 and the distance from the pial surface on which could be seen classical macrophages with degradation products was measured. This also represents the area of macrophages in LFB staining that contain myelin degradation products.

## Results

### Anti-MOG Reactivity in Patients with Inflammatory CNS Diseases and Cross-Reactivity to Rodent MOG

We tested sera from 260 patients with different inflammatory CNS diseases; 17 of them had autoantibodies to

MOG (clinical details in the Table). The highest anti-MOG reactivity was seen in patients with relapsing optic neuritis and NMO phenotype. The vast majority of patients with MS do not have Abs to MOG, but Abs to MOG are detected in special cases with MS.[34] The 5 patients with MOG Abs included in the Table fulfill the diagnostic criteria of MS, including MS-typical cerebrospinal fluid (CSF) and radiological features, but had a clinical phenotype that overlaps with NMO (severe myelitis, brainstem involvement, and optic neuritis). These patients did not have Abs to NMDAR or AQP4. Details of their clinical picture, their MRI, and their anti-MOG reactivity have been described in a previous paper.[34] We determined the cross-reactivity to rodent MOG of these patients. Further analysis of the pathogenic features of Abs to MOG was performed with Patients 7 and 5, who showed a high reactivity toward MOG and cross-reactivity to rodent MOG (Fig 1). Both patients had a recurrent optic neuritis, one of the diseases associated with MOG Abs.[39,40] These patients were followed for periods of about 26 and 35 months and kept recognizing MOG. Their anti-MOG reactivity was so high that a reactivity could still be detected at serum dilutions of 1:3,000 to 1:10,000. Both patients had anti-MOG of isotype IgG1. Patient 5 had in addition to IgG also persisting IgM to MOG.

The applications of mutant variants of MOG showed that the 2 patients recognized different epitopes on MOG (see Fig 1C, D). The binding to MOG of Patient 5 was reduced by the mutation P42S, indicating that this patient's Abs recognize the CC′ loop on MOG; the MOG Abs of IgG and IgM isotype showed similar reactivity to MOG mutants. Patient 7 showed a stronger reactivity to mouse MOG than to human MOG. Such a feature we had previously noted in 12 of 111 patients analyzed.[15] Consistent with the better recognition of mouse MOG, this patient also showed a stronger reactivity to the MOG mutant P42S, in which the serine present in murine MOG replaces the proline of human MOG. Another mutation at the EF loop (H103A, S104E) greatly reduces the MOG binding of this patient. MOG residues important for binding of Abs from Patients 5 and 7 are visualized in Figure 1E.

From Patient 5, we could also analyze CSF and this showed that anti-MOG IgG were present in this compartment, but there was no evidence that the anti-MOG IgG present in the CSF was produced intrathecally; after adjustment to equal IgG concentrations, similar anti-MOG reactivity was seen in CSF and serum (see Fig 1F).

### *Specificity of Affinity-Purified Abs to MOG*

We produced the ECD of human MOG in HEK cells with an AviTag at the C-terminus replacing the transmembranous and intracellular part. Then MOG was enzymatically biotinylated at the AviTag and bound to a streptavidin column, which puts the extracellular part of MOG on the beads in the same orientation as in the membrane. The confirmation with beta-sheet formation was seen by circular dichroism (Fig 2A). To further validate this MOG preparation, we formed MOG tetramers and tested the binding to B cells from mice with a knock-in of the heavy chain of the anti-MOG mAb 8-18C5 and found that this stained about one-third of the B cells from these mice, which is in line with their published MOG-binding activity (data not shown).[37] With this protein, we could affinity purify MOG-specific Abs from both patients (see Fig 2). Starting from > 600ml blood, we eluted from the MOG-column 471 μg of IgG and 55 μg of IgM from Patient 5 and 571 μg IgG but no IgM from Patient 7. Mass spectrometry showed that the eluates from Patient 5 contained IgG, IgM, α-2 macroglobulin, fibrinogen, and albumin, and from Patient 7 IgG and fibrinogen. Importantly, no MOG was detected in the eluates. The eluates did not bind to streptavidin as seen by ELISA using streptavidin-coated plates. We could not obtain Abs that recognize MOG on transfected cells from donors who did not have a strong anti-MOG reactivity in their blood. This excludes that the anti-MOG reactivity we observed in the purified fraction is an artifact due to the purification procedure.

These affinity-purified Abs showed a highly enriched reactivity to human MOG in a cell-based assay; when plasma and affinity-purified Abs were adjusted to the same concentration of 12 μg/ml, we noted the following mean channel fluorescence (MCF) ratios, which were calculated as described above: Patient 5: plasma 14.9, purified 190.3, flow through 8.1; Patient 7: plasma 8.6, purified 207.5, flow through 3.5 (see Fig 2). We noted that in both patients the reactivity to our mutated variants was the same in the anti-MOG Abs from the starting material and the eluates. We also compared the affinity-purified MOG Abs from both patients with the prototype anti-MOG 8-18C5. For this comparison we used a recombinant variant of 8-18C5 with a human Fc-IgG1, so the same detection Ab could be used. Our dose responses show that these purified MOG Abs recognized MOG in a cell-based assay still in the ng/ml range and came quite close to the intensity of MOG binding of the 8-18C5. The isotype of the anti-MOG response of the affinity-purified Abs of both patients was IgG1. We also analyzed the cross-reactivity of the patient-derived MOG Abs to rat MOG, because their pathogenicity will be tested in a rat model (see below). We noted that Patient 7 recognized rat MOG more strongly than human MOG, which is consistent with our observation that this patient also recognizes mouse MOG more strongly than human MOG (see Fig 1D); mouse and rat MOG are very similar although not identical.

The flow through of the column used for affinity purification of MOG Abs from these 2 patients still contained

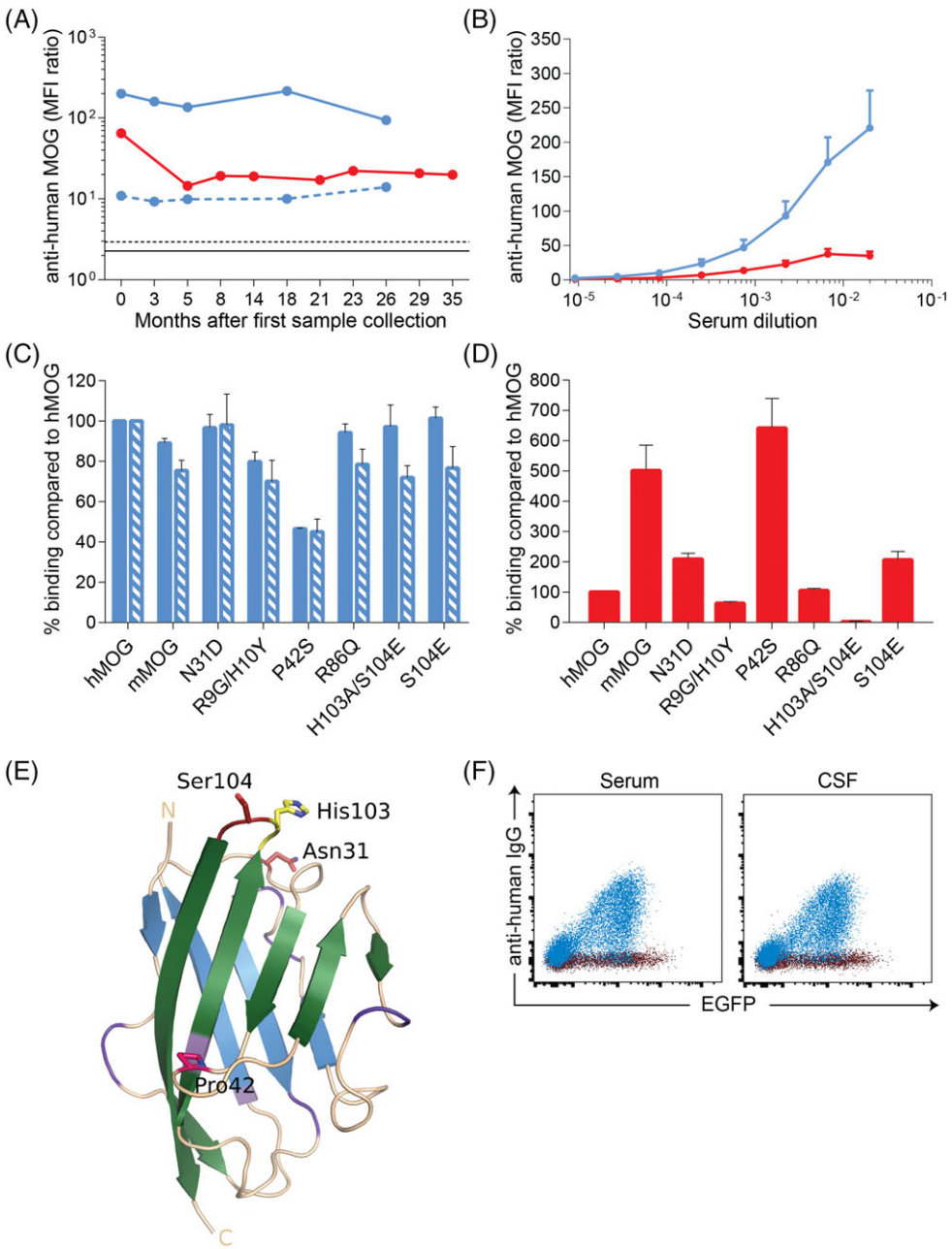


**FIGURE 1: Anti–myelin oligodendrocyte glycoprotein (MOG) reactivity in the 2 patients selected for transfer experiments. The anti-MOG reactivity in serum and plasma of Patient 5 (blue) and Patient 7 (red) was determined with transfected cells as described in Patients and Methods. (A) Longitudinal analysis. Solid lines indicate anti-MOG IgG; the dotted bluish line shows persisting anti-MOG IgM in Patient 5. The solid black line shows the cutoff for anti-MOG IgG, the dotted black line the cutoff for anti-MOG IgM. (B) Anti-MOG reactivity in serum dilutions. (C, D) Reactivity to human MOG (hMOG), mouse MOG (mMOG), and the indicated mutations of MOG. The IgG responses are indicated in solid bars, the anti-MOG IgM response from Patient 5 in hatched bars. (E) The structure of the human MOG model[15] is shown as a ribbon representation with residues influencing antibody binding depicted as stick models. In addition, residues that differ between mouse and human MOG are colored pink (Pro 42), light violet (2 conservatively mutated interior 13-strand residues), and violet (remaining nonidentical residues). N and C indicate the N-terminal and C-terminal part of the extracellular domain of MOG. (F) Anti-MOG in cerebrospinal fluid (CSF) of Patient 5. CSF (IgG 0.022g/l) was used undiluted and serum was diluted 1:377 to obtain the same IgG concentration as in the CSF. The calculated mean fluorescence intensity (MFI) ratio (MOG-enhanced green fluorescent protein [EGFP]/EGFP) of the CSF was 72.44, whereas that of the serum sample was 86.34. Control EGFP transfectants are shown in gray, the MOG-EGFP transfectants in blue. Error bars indicate the standard error of the mean of 2 to 3 experiments.**

anti-MOG reactivity as seen with MOG transfectants. This was not due to a limited capacity of the column, as it could still bind the mAb 8-18C5. Along this line, from another patient (Patient 14), we could obtain only a small amount of anti-MOG IgG with this column and the flow through still contained a similar reactivity to MOG as the starting

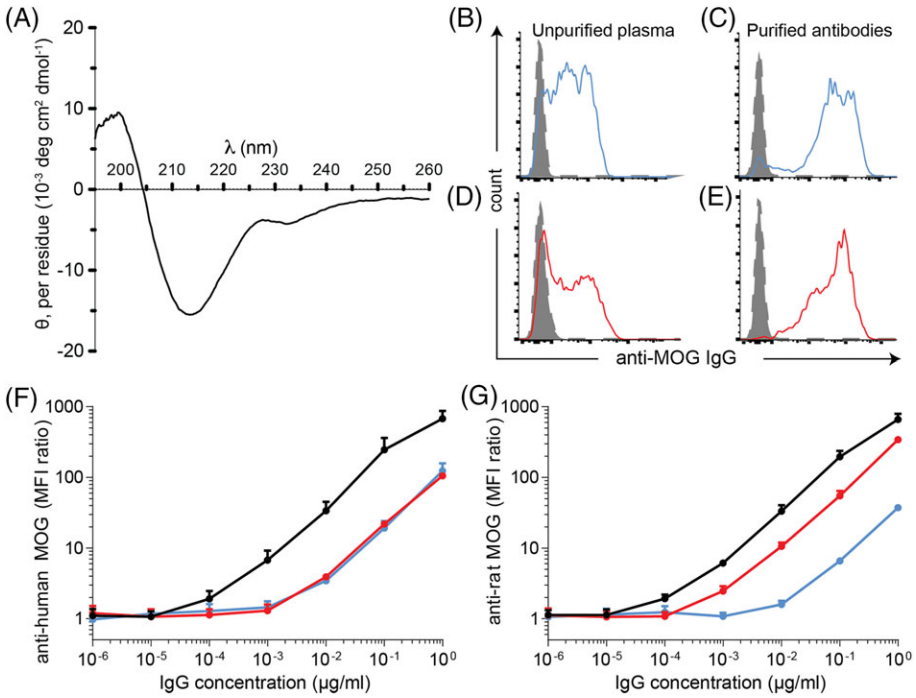


**FIGURE 2: Affinity-purified antibodies (Abs) to myelin oligodendrocyte glycoprotein (MOG). (A) Circular dichroism spectrum of MOG (0.2mg/ml). The beta-sheet formation is indicated by the negative band at 213nm. (B–E) Comparative analysis of plasma and affinity-purified MOG Abs to cells transfected with MOG of Patient 5 (blue) and Patient 7 (red). Plasma and purified Abs were used at an IgG concentration of 12 µg/ml. Closed graphs indicate the recognition of enhanced green fluorescent protein (EGFP)-transfected cells, open graphs of MOG-EGFP transfectants. (F, G) MOG recognition of the affinity-purified Abs from Patients 5 (blue) and 7 (red) in comparison with the recombinant humanized mAb 8-18C5 (black) on transfected cells. Error bars indicate standard error of the mean of 2 to 3 experiments. MFI = mean fluorescence intensity.**

material. Thus, the ECD of human MOG produced in HEK cells binds only a fraction of Abs to MOG.

### *Staining of Brain Tissue with Affinity-Purified Abs to MOG*

The affinity-purified Abs from both patients bound to myelin in tissue sections from the rat; r8-18C5 was used as a positive control (Fig 3). We noted a stronger binding of the Abs from Patient 7 (see Fig 3C, D) than from Patient 5, which is consistent with the dose response of these preparations to rat MOG on the surface of rat transfectants (see Fig 2G). Because the MOG reactivity of these patients was established by using native cells, while the tissue was fixed with PFA, we compared the recognition of live and PFA-fixed cells after MOG transfection. This showed that Patients 5 and 7 recognized MOG also after PFA fixation of the transfected cells, but the background was much higher with fixed cells (data not shown).

### *Pathogenicity and Histopathological Changes Induced by Patient-Derived Abs to MOG*

We analyzed the pathogenic potential of patient-derived MOG-specific Abs in 2 models of T-cell–mediated EAE in the Lewis rat. In both models, we injected the MOG Abs intrathecally 2 days after the injection of either MOG-specific T cells or MBP-specific T cells. Because the amount of purified Abs from patients was limited, we first established the details of the transfer models with 8-18C5 and the humanized r8-18C5. These experiments showed that EAE can be enhanced, when 8-18C5 or r8-18C5 were injected 2 days later than the T cells. Under these conditions, the peak of disease was reached at day 5; the animals recovered largely until day 10. Therefore, we sacrificed the EAE rats after injection with the patient-derived Abs at day 5.

The MOG-specific T cells alone did not induce a clinical effect in our Lewis rat model. However, when affinity-purified Abs from both Patients 5 and 7 were injected, a clinical disease was induced (Fig 4). As control, we used human ivIg and Ig obtained from a protein G column. This control human Ig did not induce disease, whereas the positive control 8-18C5 enhanced disease. In contrast to the MOG-specific T cells, the MBP-specific T cells induced a clinical disease on their own in the absence of any added Ab, consistent with previous observations with MBP-specific T cells in this rat model.[41] One day after injection of r8-18C5 and the Abs from Patient 7 the clinical disease was enhanced.

All animals shown in Figure 4 were perfused at day 5 and analyzed by histopathology. A quantitative analysis of the T-cell infiltration and of demyelination in all

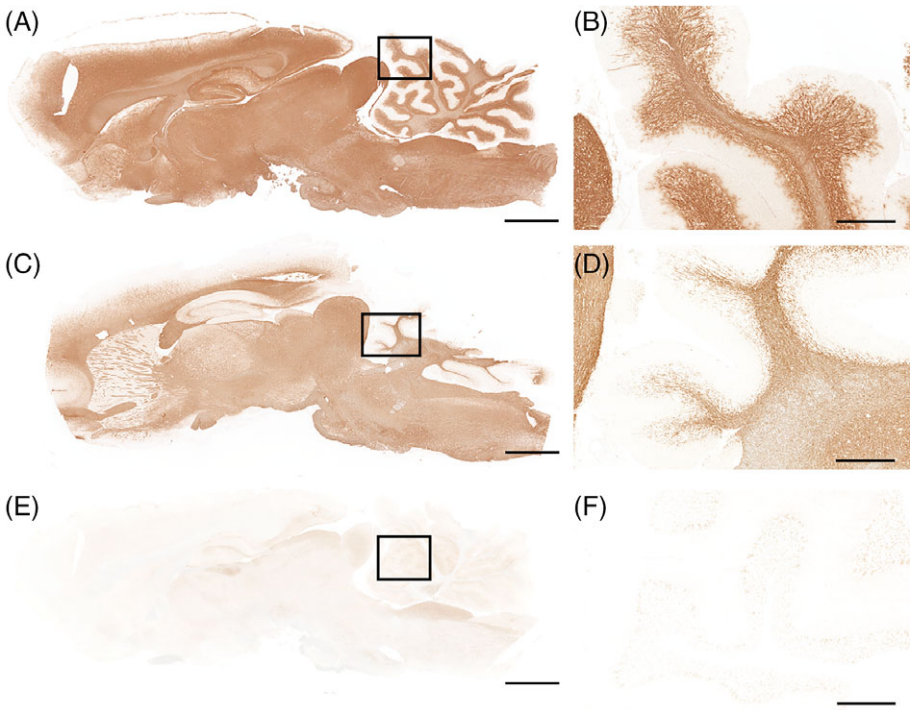


**FIGURE 3: Myelin staining of affinity-purified myelin oligodendrocyte glycoprotein (MOG)-specific antibodies (Abs). Samples were stained on sagittal rat brain sections. The humanized r8-18C5 was used as positive control (A) and showed a specific myelin staining throughout the cerebrum and cerebellum (B; rectangle in A enlarged). The affinity-purified MOG-specific Ab from Patient 7 (C, D) showed a strong binding to myelin; a recombinant human IgG that does not bind MOG on transfected cells (r#7_D7) was negative (E, F). All Abs were used at a concentration of 3 μg/ml. Scale bars = 2mm (A, C, E), 300 μm (B, D, F).**

17 animals revealed the following. The patient-derived MOG-specific Abs massively enhanced the T-cell infiltration in the subpial area of the pons when given together with cognate MOG-specific T cells, but not together with MBP-specific T cells (see Fig 4). Pathological analysis of animals injected with MOG-specific T cells alone or together with control Abs displayed a moderate inflammatory reaction in the spinal cord and less obviously in the brain and optic nerve, consisting of T-cell infiltrates in the meninges and CNS tissue and of $ED1^+$ macrophages, being restricted to the meninges (Fig 5, middle panels).

In combination with the injection of the MOG-specific Abs from Patients 5 and 7, a massively enhanced T-cell and macrophage infiltration in the meninges and the subpial CNS tissue was observed, and this was similar to the pathology observed after injection of the 8-18C5 Ab (see Figs 4 and 5). The enormous enhancement of the infiltration of T cells is already visible at a low magnification displaying cross sections of the whole spinal cord (see Fig 5, first and third rows). Human immunoglobulin reactivity was seen on subpial myelin, but only traces of activated complement (C9neo antigen) and a slight perivascular demyelination were present (data not shown).

Following transfer of MBP-specific T cells alone (which induced with the applied cell number a mild EAE on their own) or in combination with control Abs, a different pathology was seen. It consisted of mild to moderate T-cell infiltration together with the dispersion of $ED1^+$ macrophages throughout the tissue (Fig 6). In combination with patient-derived MOG-specific Abs, human Ig was also seen on subpial myelin, but this was associated with complement C9neo activation. This was accompanied by subpial demyelination (see Fig 4D), which was seen by LFB staining and by immunostaining for cyclic nucleotide phosphodiesterase. Demyelination and complement activation were massive with the Abs from Patient 7, less intense but detectable with the Abs from Patient 5, and absent after control Ab injection (see Figs 4D and 6). Due to injection into the cisterna cerebelli magna, the Abs hardly reached the optic nerve.

Thus, in this model, we see an impressive effect of the MOG Abs on the histopathology, but only a slight enhancement of the clinical disease. There are 2 reasons for this. First, the sensitivity to detect an enhanced clinical disease is lower if the control group is already sick (see Fig 4B) as compared to a model in which the control group is not sick at all (see Fig 4A). Second, the clinical score in this EAE model detects only motor functions. We have quantified the amount of lipopolysaccharide (LPS) in the samples used for in vivo experiments and found that the

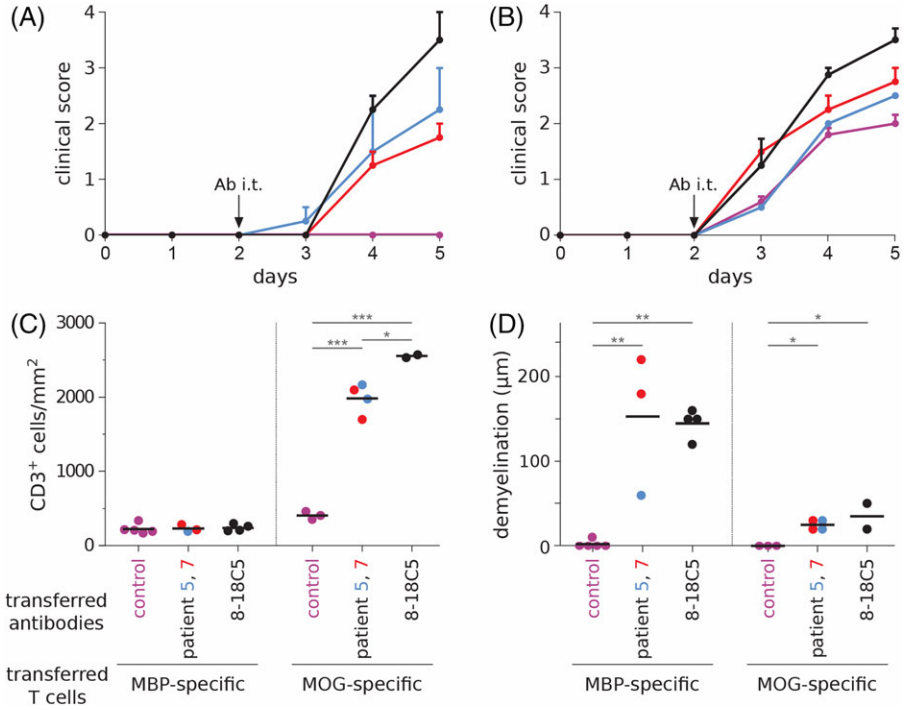


**FIGURE 4: Pathogenicity of affinity-purified patient-derived myelin oligodendrocyte glycoprotein (MOG)-specific antibodies (Abs). Lewis rats were injected with MOG-specific (A) or myelin basic protein (MBP)-specific T cells (B). Two days later, 100 µg of affinity-purified MOG-specific Abs from Patient 5 (blue), Patient 7 (red), control IgG (purple), or 8-18C5 (black) were injected intrathecally (i.t.) into the spinal fluid (cisterna magna). (A) Three animals received human control IgG, 2 Abs from Patient 7 and 2 from Patient 5. Because the animals with the control IgG did not show any clinical disease, the induction of the clinical EAE with MOG-specific Abs from patients (data from the patients pooled) reached statistical significance at day 4 ($p < 0.05$) and day 5 ($p = 0.005$) using the unpaired 2-tailed *t* test. (B) Together with MBP-specific T cells, 1 animal received Abs from Patient 5, 2 animals Abs from Patient 7, 5 control IgG. As positive controls, r8-18C5 (A, B) and 8-18C5 (A) were used. Error bars indicate standard error of the mean. All animals were perfused at the end of the observation period and analyzed for histopathology. (C, D) Quantification of inflammation and demyelination of animals shown in A and B. (C) The T-cell infiltrates in the subpial region at the basis of the pons were counted with a 40 × objective, and the number of $CD3^+$ T cells/$mm^2$ was calculated. (D) The distance of subpial demyelination at the basis of the pons was measured. (C, D) We performed analysis of variance testing followed by Tukey honest significant difference test. * < 0.05; ** < 0.01; *** < 0.001.**

contaminating amount of LPS was similar in control Ig and patient preparations; < 10ng were injected per animal. The same Ig preparations had different effects depending on the antigen specificity of the coinjected T cells; the patient Abs enhanced microglia activation and T-cell infiltration only together with MOG-specific T cells, but not in the context of MBP-specific T cells; a strong activation of terminal complement complex C9neo, conversely, was seen in the context of MBP-specific T cells, but little activation was seen in the context of MOG-specific T cells. We conclude from all this that the effects we describe were induced by the patient-derived Ig and not by LPS.

In this project, we had tested 3 different human Ig control preparations, namely ivIg, human IgG not specific for MOG obtained from a protein G column, and recombinant IgG with human Fc part. None of these human Ig variants recognized MOG, and none of them had any effect on enhancement of the disease. As a further control experiment, we injected OVA-specific T cells in the absence or presence of an intrathecal injection of r8-18C5. In this context, no induction of clinical disease and no demyelination or complement activation was present (data not shown).

## Discussion

Our study shows that Abs to MOG affinity-purified from the blood of patients with inflammatory demyelination are pathogenic in transfer experiments to rodents. We found that these patient-derived MOG-specific Abs mediate damage to the CNS by different mechanisms. In synergy with T cells that induce clinical EAE, associated with profound blood–brain barrier damage and activation of macrophages (MBP-specific T cells in the Lewis rat in our model), human Abs to MOG mediate MS type II–like pathology, characterized by active demyelination (phagocytes containing myelin in the lesion) and local activation of the terminal complement complex, visible as deposition of C9neo.[32,33] We show here that these features are induced by the patient-derived MOG-specific Abs. This suggests that in patients with MOG Abs and MS type II pathology,[28–31] MOG Abs are responsible for this part of the pathology.

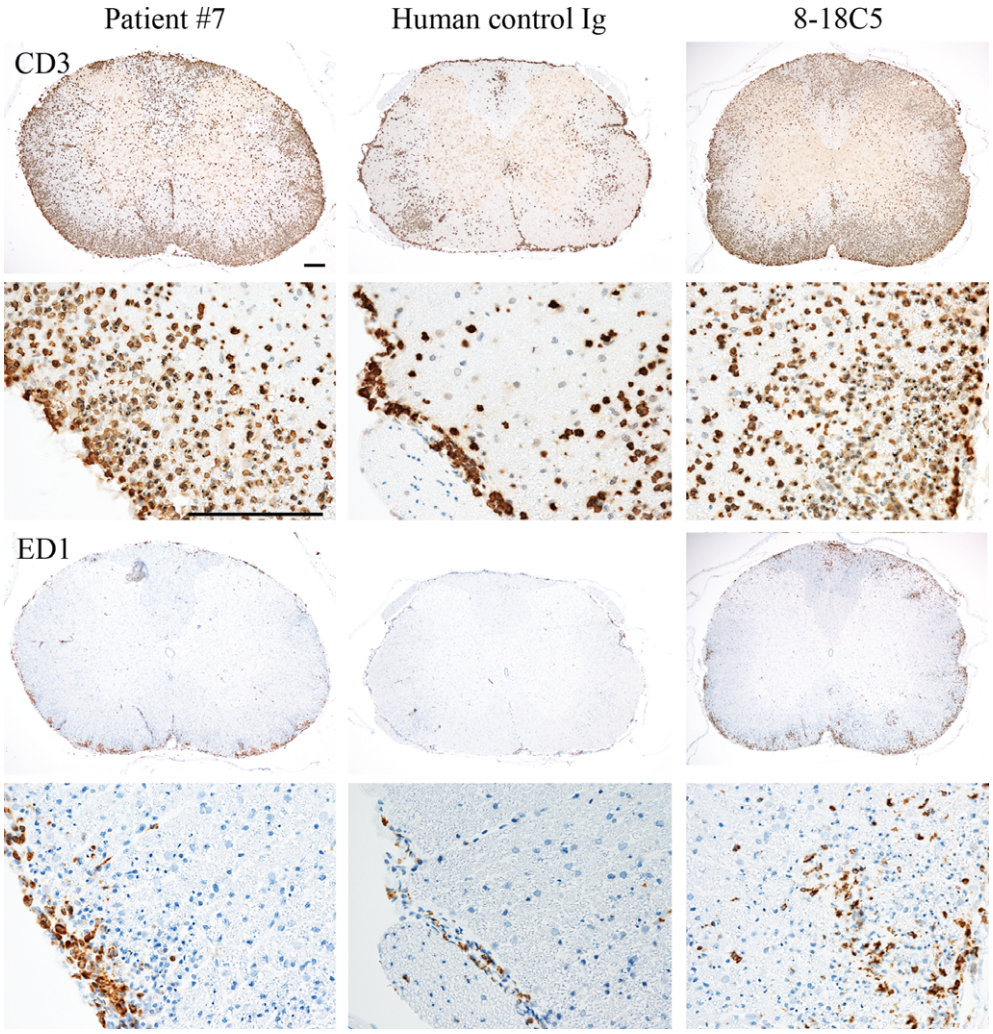


**FIGURE 5: Affinity-purified antibodies (Abs) to myelin oligodendrocyte glycoprotein (MOG) enhance T-cell activation and promote microglia activation in the subpial parenchyma together with MOG-specific T cells. Spinal cord pathology is shown following passive cotransfer of MOG-specific T-cells with control IgG or anti-MOG Abs. Experimental autoimmune encephalomyelitis after injection of control Abs is characterized by T-cell infiltration in the meninges and diffusely in the spinal cord parenchyma, but ED1$^+$ macrophages are largely restricted to the meningeal space (middle panels). After injection of Patient 7 Ab (left panels) or 8-18C5 (right panels), there is a massive enhancement of subpial T-cell infiltration and ED1$^+$ macrophages pass the astrocytic glia limitans and infiltrate the central nervous system parenchyma. Scale bars = 100 μm.**

Remarkably, most patients with MOG Abs and an MS type II pathology described so far do not have a typical MS,[42] but rather an encephalomyelitis overlapping with MS and NMO spectrum disorder. It is discussed whether this should be grouped as MOG Ab disease. Conversely, most patients with clinical MS and an MS type II pathology do not have Abs to MOG,[30,34] suggesting that these patients recognize other not yet identified autoantigens.

In our second model, in synergy with cognate MOG-specific T cells, which by themselves do not induce clinical disease, but only mild, predominantly meningeal inflammation in our rat model, the same affinity-purified Ab preparations induced clinical disease with other pathological features, namely a massively enhanced T-cell infiltration. An enhancement of T-cell activation by mAbs to MOG has been shown in 2 recent studies and suggested to be mediated by opsonization of the antigen.[20,23] We found that the patient-derived anti-MOG Abs not only enhanced T-cell infiltration induced by MOG-specific T cells, but also stimulated microglia/macrophage infiltration in the subpial gray matter. This indicates that human anti-MOG Abs in the CSF might also participate in the development of gray matter pathology together with MOG-specific T cells. MOG-specific T cells have been observed in patients with demyelination, and their recognized epitopes were identified.[43] Further studies are needed to analyze MOG-specific T cells in patients with Abs to MOG.

Our 2 EAE transfer models show that the human Abs to MOG mediate tissue destruction via 2 different

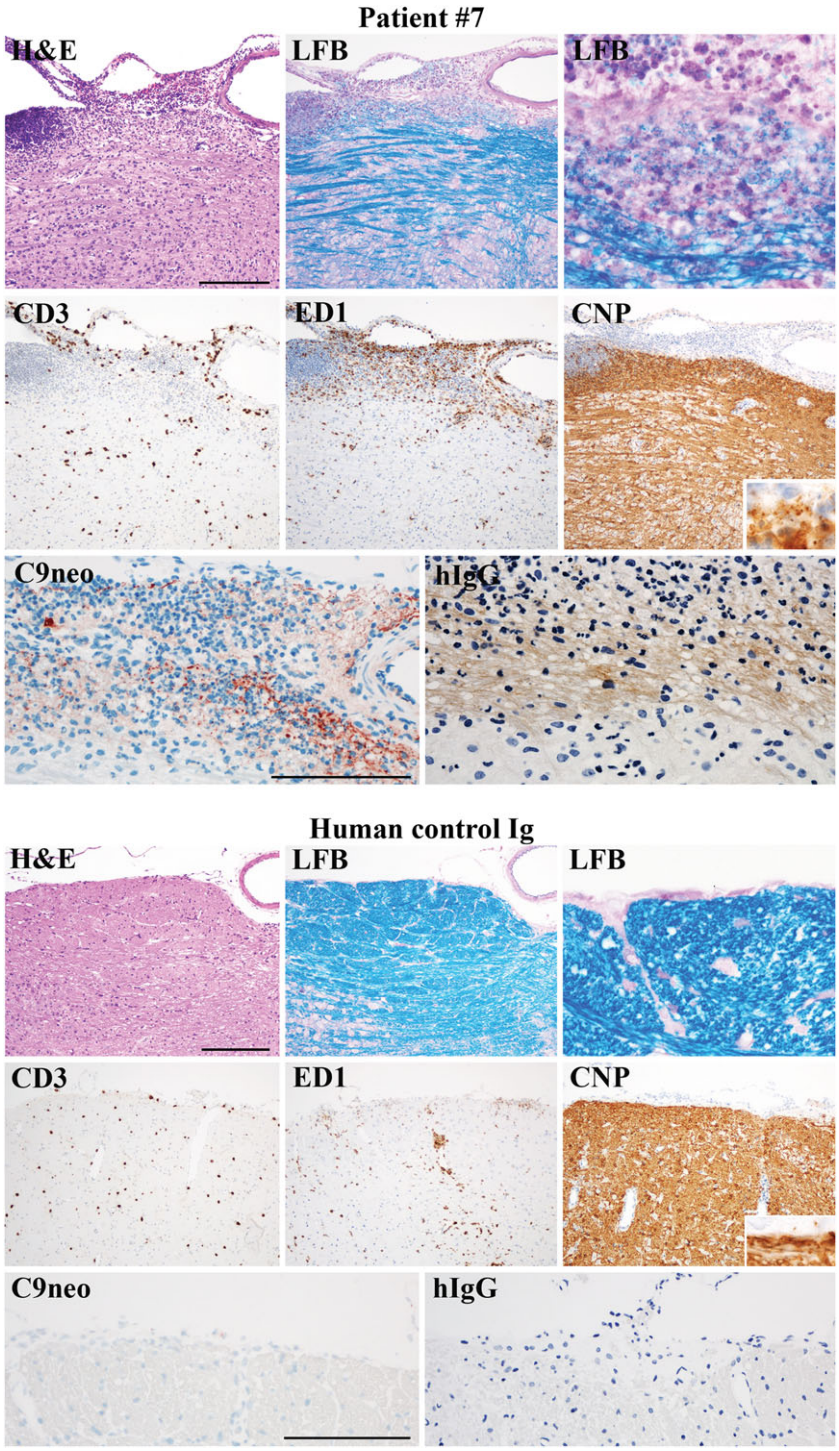


FIGURE 6: Affinity-purified antibodies (Abs) to myelin oligodendrocyte glycoprotein (MOG) induce complement activation and demyelination together with myelin basic protein (MBP)-specific T cells. Experimental autoimmune encephalomyelitis was induced with MBP–specific T cells. After 2 days, either MOG-specific affinity-purified Abs from Patient 7 (upper panels) or human control Ig (lower panels) was injected. When human control Ig was injected, there is a diffuse infiltration of the tissue by $CD3^+$ T cells and $ED1^+$ macrophages, but there is no deposition of human IgG on myelin or activation of complement (C9neo; lower panels). However, when anti-MOG Ig from Patient 7 was cotransferred, inflammation is massively enhanced and $ED1^+$ macrophages are concentrated at sites of active myelin destruction, associated with immunoglobulin deposition on myelin and complement activation (C9neo antigen deposition; lower left of upper panels). Scale bars = 100 μm. CNP, cyclic nucleotide phosphodiesterase; H&E = hematoxylin and eosin; LFB = Luxol fast blue.

**TABLE 1. Features of Patients with Anti-MOG Reactivity**

| ID | Current Diagnosis | Gender | Age at First MOG$^+$ Sample, yr | Reactivity to Human MOG, MFI Ratio | Reactivity to Mouse MOG, MFI Ratio |
|---|---|---|---|---|---|
| 5 | Relapsing bilateral ON | F | 42 | 220.7 | 212.9 |
| 14 | Relapsing bilateral ON | M | 54 | 44.9 | 20.6 |
| 8 | NMOSD | M | 37 | 38.3 | 3.0 |
| 7 | Relapsing unilateral ON | M | 46 | 34.7 | 216.8 |
| 16 | NMOSD | M | 30 | 18.6 | 5.6 |
| 17 | Relapsing bilateral ON | F | 31 | 18.2 | 2.1 |
| 6 | Monophasic encephalitis | F | 31 | 17.7 | 2.3 |
| 10 | RRMS | F | 37 | 11.9 | 8.3 |
| 13 | Relapsing encephalomyelitis | M | 34 | 8.6 | 5.5 |
| 1 | NMOSD | M | 40 | 6.1 | 1.7 |
| 3 | Relapsing encephalomyelitis | M | 26 | 5.4 | 1.8 |
| 4 | RRMS | F | 55 | 4.6 | 7.1 |
| 11 | RRMS | F | 50 | 4.1 | 1.5 |
| 2 | Relapsing encephalomyelitis | F | 66 | 4.0 | 0.9 |
| 9 | RRMS | M | 32 | 3.9 | 3.1 |
| 12 | RRMS | F | 23 | 2.9 | 3.9 |
| 21 | NMOSD | F | 33 | 2.7 | 1.8 |

Details about Patients 4, 9, 10, 11, and 12 are reported in Spadaro et al.[34] and about Patient 2 in Spadaro et al.[28] Patients with MOG antibodies might constitute a condition called MOG antibody disease. The cutoff for recognition of human MOG was 2.27 (mean + 3 standard deviation of controls). The MFI ratio was calculated as the mean of 2 to 5 experiments.

F = female; M = male; MFI = mean fluorescence intensity; MOG = myelin oligodendrocyte glycoprotein; NMOSD = neuromyelitis optica spectrum disorders; ON = optic neuritis; RRMS = relapsing–remitting multiple sclerosis.

mechanisms. This could be revealed because in our models the 2 different T-cell lines showed different intensities of T-cell reactivation in the CNS.[41,44] In the model with MBP-specific T cells, strong T-cell activation in the CNS was associated with blood–brain barrier disruption and the diffuse infiltration of the CNS tissue by recruited ED1$^+$ macrophages. Therefore, the incoming Abs find a good environment to mediate demyelination via Ab-dependent cell-mediated cytotoxicity and complement activation, which results in a pathology similar to MS type II. In the model with MOG-specific T cells, T-cell activation in the CNS is not optimal and recruitment of ED1$^+$ macrophages is sparse and largely restricted to the meninges. Here, the entering MOG-specific Abs massively enhance the T-cell recruitment and activation, because they recognize the same antigen; this then promotes infiltration of ED1$^+$ macrophages, which is associated with clinical disease but may be too low to effectively induce demyelination. Our observation that the patient-derived Abs perform tissue destruction by 2 different mechanisms, demyelination and enhanced inflammation, is consistent with a previous study transferring sera from immunized nonhuman primates.[45]

Our EAE experiments indicate further that the anti-MOG Abs are not pathogenic on their own, as together with irrelevant T cells no pathology was induced. This is consistent with previous observations in other EAE models[37] or after intrathecal injection of the 8-18C5 Ab[46] and supports the concept that the anti-MOG Abs perform a second hit to enhance pathology. Thus, human MOG Abs are pathogenic, but the precise pathological effects depend on their interactions with T cells; the human anti-MOG Abs can mediate MS type II pathology and gray matter injury upon transfer.

Experiences with mAbs in animals have shown that recognition of conformational MOG is required for

pathogenicity.[12,14] The secondary structure of MOG is characterized by 2 antiparallel beta-sheets that form an immunoglobulinlike beta-sandwich fold.[47] In rodents, pathogenic MOG-specific Abs mainly recognize the FG loop of MOG as the prototype mAb 8-18C5.[14] Although the epitope specificity of human anti-MOG Abs was previously dissected by ELISA[48] and transfection of mutated variants of MOG,[15] epitope specificity of pathogenic Abs from patients was unknown. The pathogenic MOG-specific autoantibodies from the 2 patients recognize different epitopes, and both are different from the one recognized by 8-18C5. Patient 5 recognized the CC′ loop, as its binding was reduced by the mutation P42S; this is the most frequently recognized part of human MOG.[15] This patient nevertheless strongly recognized mouse MOG, although the mouse MOG contains P42S. These 2 characteristics of MOG recognition (reduced reactivity to P42S, but strong recognition of mouse MOG) we had observed before in 5 of 111 patients.[15] Patient 7 recognized the FG loop of MOG, as its binding was completely abrogated by the mutation H103A+S104E. This resembles the recognition of 8-18C5, which is also abrogated by the double mutation H103A+S104E. A closer look at the reactivity of Patient 7 to other mutants of MOG points to epitopes that are discontinuous like the one recognized by the mAb 8-18C5,[47] but that differ from the 8-18C5 epitope as they are influenced by P42 positioned in the CC′ loop and/or the glycosylation site at N31 in addition to binding to the FG loop. The observed binding pattern of Patient 7 would therefore be consistent with the recognition of an ensemble of epitopes that include the FG loop and are located at the top, membrane-distal part and/or at the 5-stranded front β-sheet of MOG (see Fig 1E).

Together, this part of our analysis shows that pathogenic MOG Abs from patients recognize different loops on MOG.

The anti-MOG response of the patients with recurrent optic neuritis persisted for the observation periods of 26 and 35 months. This extends our knowledge of kinetic of MOG Abs. In children with ADEM, the Abs to MOG appeared only transiently and were rapidly lost, whereas in children with MS the MOG Abs persisted for years.[49] One of our analyzed patients had the unusual feature of having both an anti-MOG IgG and an anti-MOG IgM response. Both reactivities were directed against the same epitope of MOG. The co-occurrence of anti-IgG and anti-IgM to MOG is rare but was noted in a previous study in 3 of 19 children with ADEM and Abs to MOG.[17] The long-term persistence of an anti-MOG IgM response might be surprising, but it is consistent with recently described human IgM memory B cells that have passed the germinal center.[50] Our study shows that rarely an IgM response to MOG may also persist.

Our study has the following limitations. First, we injected the patient-derived Abs intrathecally, not systemically, although MOG Abs are typically detected in the blood. In pilot experiments with mAbs, we noted that EAE can be enhanced both by peripheral and by intrathecal injection, but that higher amounts of Abs were needed when the Abs were injected systemically. Because the amount of patient-derived Abs was limited, we chose intrathecal injection. We feel this is justified, as we found MOG Abs also in the CSF. From Patient 5, we could analyze CSF and found strong anti-MOG reactivity without evidence for intrathecal production of Abs to MOG. Second, we analyzed the pathology only at 1 time point after injection because we could inject only a limited number of animals with precious patient-derived Ig material. Compared to recombinant Abs, however, patient-derived Abs more closely reflect the human in vivo situation. This is important when evaluating the pathogenic potential of the MOG Abs present in the blood of patients, as the effector function of IgG is regulated by its glycosylation[51] and there is evidence that IgG glycosylation is altered in MS patients.[52] Third, we show that human MOG Abs identified in a cell-based assay include pathogenic Abs, but it remains unclear whether all of the MOG Abs are pathogenic and which features of the human Abs would allow predicting their pathogenicity. Our approach displaying the correctly folded extracellular part of MOG on a column purified only a proportion of MOG Abs. To affinity-purify and subsequently test the pathogenic activity of the other MOG Abs, MOG might have to be displayed in a membrane-bound environment. Our observation that the extracellular part of MOG purifies only part of the MOG Abs is consistent with the previous observation that in a cell-based assay a short construct of MOG lacking the intracellular part is less sensitive to detect anti-MOG Abs than full-length MOG.[53] Possible reasons for the differential reactivity to the 2 MOG variants with the same ECD include oligomerization or yet unidentified effects of the intracellular part of MOG on the conformation of the extracellular part. Furthermore, human MOG Abs are heterogeneous with respect to cross-reactivity to rodents. To address the pathogenicity of MOG Abs not cross-reactive with rodent MOG, mice with a knock-in of human MOG or even transfers to nonhuman primates might have to be used.

Together, we show here that Abs to MOG, which were affinity purified from the blood of patients and recognize different epitopes on MOG, synergize with T cells in transfer experiments to rodents; they induce MS type II pathology and trigger T-cell infiltration with microglia/macrophage activation in the subpial parenchyma. We conclude that MOG Abs contribute to the pathology of

patients with inflammatory demyelinating diseases by these mechanisms.

## Acknowledgment

This work was supported by the German Research Foundation (SFB TR128, KA2651/2-1, KA2651/3-1), the Munich Cluster for Systems Neurology (ExC 1010 SyNergy), the Clinical Competence Network for Multiple Sclerosis, the Nonprofit Hertie Foundation, the Novartis Foundation for Therapeutic Research, the European Union's Horizon 2020 Research and Innovation Program (No. 668036, "RELENT"). Responsibility for the information and views set out in this article lies entirely with the authors, the Werner Reichenberger Foundation, and the Verein Therapieforschung für Multiple Sklerose-Kranke.

We thank H. Rübsamen, V. Pichler, and R. Mentele for expert technical assistance and Drs S. Liebscher and J. Havla for comments on the manuscript. Part of the Flow Cytometry analysis was supported by Dr. L. Richter, Core Facility Flow Cytometry at the Biomedical Center, Ludwig-Maxmilians-Universität München.

## Author Contributions

M.S., S.W., E.B., C.M., R.Hö., R.G., M.M., F.K., H.L., and N.K. conducted experiments, and acquired and analyzed data. E.S., F.S.T., L.-A.G., S.L., G.K., D.J., S.B., K.D., M.K., R.Ho., T.K., S.W., and C.B. analyzed data and contributed to manuscript preparation. M.S., H.L., T.K., R.Ho., N.K., and E.M. designed the study and wrote the manuscript.

## Potential Conflicts of Interest

Nothing to report.

## References

1. O'Connor KC, Chitnis T, Griffin DE, et al. Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal fluid of multiple sclerosis patients are characterized by low-affinity interactions. J Neuroimmunol 2003;136:140–148.
2. Sinmaz N, Nguyen T, Tea F, et al. Mapping autoantigen epitopes: molecular insights into autoantibody-associated disorders of the nervous system. J Neuroinflammation 2016;13:219.
3. Ramanathan S, Dale RC, Brilot F. Anti-MOG antibody: the history, clinical phenotype, and pathogenicity of a serum biomarker for demyelination. Autoimmun Rev 2016;15:307–324.
4. Peschl P, Bradl M, Hoftberger R, et al. Myelin oligodendrocyte glycoprotein: deciphering a target in inflammatory demyelinating diseases. Front Immunol 2017;8:529.
5. Hohlfeld R, Dornmair K, Meinl E, Wekerle H. The search for the target antigens of multiple sclerosis, part 2: CD8 + T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol 2016;15:317–331.
6. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG autoantibody-associated demyelinating diseases. Nat Rev Neurol 2013;9:455–461.
7. Jarius S, Ruprecht K, Kleiter I, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. J Neuroinflammation 2016;13:280.
8. Jurynczyk M, Geraldes R, Probert F, et al. Distinct brain imaging characteristics of autoantibody-mediated CNS conditions and multiple sclerosis. Brain 2017;140:617–627.
9. Zamvil SS, Slavin AJ. Does MOG Ig-positive AQP4-seronegative opticospinal inflammatory disease justify a diagnosis of NMO spectrum disorder? Neurol Neuroimmunol Neuroinflamm 2015;2:e62.
10. Mayer MC, Meinl E. Glycoproteins as targets of autoantibodies in CNS inflammation: MOG and more. Ther Adv Neurol Disord 2012;5:147–159.
11. Piddlesden SJ, Lassmann H, Zimprich F, et al. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. Am J Pathol 1993;143:555–564.
12. Brehm U, Piddlesden SJ, Gardinier MV, Linington C. Epitope specificity of demyelinating monoclonal autoantibodies directed against the human myelin oligodendrocyte glycoprotein (MOG). J Neuroimmunol 1999;97:9–15.
13. von Budingen HC, Hauser SL, Fuhrmann A, et al. Molecular characterization of antibody specificities against myelin/oligodendrocyte glycoprotein in autoimmune demyelination. Proc Natl Acad Sci U S A 2002;99:8207–8212.
14. Breithaupt C, Schafer B, Pellkofer H, et al. Demyelinating myelin oligodendrocyte glycoprotein-specific autoantibody response is focused on one dominant conformational epitope region in rodents. J Immunol 2008;181:1255–1263.
15. Mayer MC, Breithaupt C, Reindl M, et al. Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases. J Immunol 2013;191:3594–3604.
16. Mader S, Gredler V, Schanda K, et al. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. J Neuroinflammation 2011;8:184.
17. Brilot F, Dale RC, Selter RC, et al. Antibodies to native myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. Ann Neurol 2009;66:833–842.
18. Dale RC, Tantsis EM, Merheb V, et al. Antibodies to MOG have a demyelination phenotype and affect oligodendrocyte cytoskeleton. Neurol Neuroimmunol Neuroinflamm 2014;1:e12.
19. Peschl P, Schanda K, Zeka B, et al. Human antibodies against the myelin oligodendrocyte glycoprotein can cause complement-dependent demyelination. J Neuroinflammation 2017;14:208.
20. Kinzel S, Lehmann-Horn K, Torke S, et al. Myelin-reactive antibodies initiate T cell-mediated CNS autoimmune disease by opsonization of endogenous antigen. Acta Neuropathol 2016;132:43–58.
21. Zhou D, Srivastava R, Nessler S, et al. Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. Proc Natl Acad Sci U S A 2006;103:19057–19062.
22. Saadoun S, Waters P, Owens GP, et al. Neuromyelitis optica MOG-IgG causes reversible lesions in mouse brain. Acta Neuropathol Commun 2014;2:35.
23. Flach AC, Litke T, Strauss J, et al. Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease. Proc Natl Acad Sci U S A 2016;113:3323–3328.
24. Khare P, Challa DK, Devanaboyina SC, et al. Myelin oligodendrocyte glycoprotein-specific antibodies from multiple sclerosis patients

exacerbate disease in a humanized mouse model. J Autoimmun 2018;86:104–115.

25. Pittock SJ, Lennon VA, de Seze J, et al. Neuromyelitis optica and non organ-specific autoimmunity. Arch Neurol 2008;65:78–83.
26. Titulaer MJ, Höftberger R, Iizuka T, et al. Overlapping demyelinating syndromes and anti-N-methyl-D-aspartate receptor encephalitis. Ann Neurol 2014;75:411–428.
27. Shimizu F, Schaller KL, Owens GP, et al. Glucose-regulated protein 78 autoantibody associates with blood-brain barrier disruption in neuromyelitis optica. Sci Transl Med 2017;9(397).
28. Spadaro M, Gerdes LA, Mayer MC, et al. Histopathology and clinical course of MOG-antibody associated encephalomyelitis. Ann Clin Transl Neurol 2015;2:295–301.
29. Di Pauli F, Höftberger R, Reindl M, et al. Fulminant demyelinating encephalomyelitis: Insights from antibody studies and neuropathology. Neurol Neuroimmunol Neuroinflamm 2015;2:e175.
30. Jarius S, Metz I, König FB, et al. Screening for MOG-IgG and 27 other anti-glial and anti-neuronal autoantibodies in 'pattern II multiple sclerosis' and brain biopsy findings in a MOG-IgG-positive case. Mult Scler 2016;22:1541–1549.
31. Kortvelyessy P, Breu M, Pawlitzki M, et al. ADEM-like presentation, anti-MOG antibodies, and MS pathology: TWO case reports. Neurol Neuroimmunol Neuroinflamm 2017;4:e335.
32. Lucchinetti C, Brück W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707–717.
33. Kuhlmann T, Ludwin S, Prat A, et al. An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol 2017; 133:13–24.
34. Spadaro M, Gerdes LA, Krumbholz M, et al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2016;3:e257.
35. Brändle SM, Obermeier B, Senel M, et al. Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. Proc Natl Acad Sci U S A 2016;113:7864–7869.
36. Perera NC, Wiesmuller KH, Larsen MT, et al. NSP4 is stored in azurophil granules and released by activated neutrophils as active endoprotease with restricted specificity. J Immunol 2013;191:2700–2707.
37. Litzenburger T, Fässler R, Bauer J, et al. B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. J Exp Med 1998;188:169–180.
38. Flügel A, Willem M, Berkowicz T, Wekerle H. Gene transfer into CD4 + T lymphocytes: green fluorescent protein-engineered, encephalitogenic T cells illuminate brain autoimmune responses. Nat Med 1999;5:843–847.
39. Ramanathan S, Reddel SW, Henderson A, et al. Antibodies to myelin oligodendrocyte glycoprotein in bilateral and recurrent optic neuritis. Neurol Neuroimmunol Neuroinflamm 2014;1:e40.
40. Chang T, Waters P, Woodhall M, Vincent A. Recurrent optic neuritis associated with MOG antibody seropositivity. Neurologist 2017;22: 101–102.
41. Kawakami N, Lassmann S, Li Z, et al. The activation status of neuroantigen-specific T cells in the target organ determines the clinical outcome of autoimmune encephalomyelitis. J Exp Med 2004; 199:185–197.
42. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. N Engl J Med 2018;378:169–180.
43. Varrin-Doyer M, Shetty A, Spencer CM, et al. MOG transmembrane and cytoplasmic domains contain highly stimulatory T-cell epitopes in MS. Neurol Neuroimmunol Neuroinflamm 2014;1:e20.
44. Berger T, Weerth S, Kojima K, et al. Experimental autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. Lab Invest 1997;76:355–364.
45. von Budingen HC, Hauser SL, Ouallet JC, et al. Frontline: epitope recognition on the myelin/oligodendrocyte glycoprotein differentially influences disease phenotype and antibody effector functions in autoimmune demyelination. Eur J Immunol 2004;34:2072–2083.
46. Vass K, Heininger K, Schafer B, et al. Interferon-gamma potentiates antibody-mediated demyelination in vivo. Ann Neurol 1992;32: 198–206.
47. Breithaupt C, Schubart A, Zander H, et al. Structural insights into the antigenicity of myelin oligodendrocyte glycoprotein. Proc Natl Acad Sci U S A 2003;100:9446–9451.
48. Menge T, Lalive PH, von Budingen HC, Genain CP. Conformational epitopes of myelin oligodendrocyte glycoprotein are targets of potentially pathogenic antibody responses in multiple sclerosis. J Neuroinflamm 2011;8:161.
49. Pröbstel AK, Dornmair K, Bittner R, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. Neurology 2011;77:580–588.
50. Weisel F, Shlomchik M. Memory B cells of mice and humans. Annu Rev Immunol 2017;35:255–284.
51. Seeling M, Bruckner C, Nimmerjahn F. Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity? Nat Rev Rheumatol 2017;13:621–630.
52. Wuhrer M, Selman MH, McDonnell LA, et al. Pro-inflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid. J Neuroinflammation 2015;12:235.
53. Waters P, Woodhall M, O'Connor KC, et al. MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. Neurol Neuroimmunol Neuroinflamm 2015;2:e89.