Anti-MOG autoantibodies in Italian multiple sclerosis patients: specificity, sensitivity and clinical association

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Keywords: antibody, antibody index, autoimmunity, multiple sclerosis, myelin oligodendrocyte glycoprotein

Abstract

There is considerable evidence that multiple sclerosis (MS) is an immune-mediated disease characterized by infiltration of inflammatory cells into the CNS and demyelination. Several myelin proteins may be encephalitogenic, including myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein (MOG), the latter being expressed on the external layer of myelin sheaths and hence accessible to antibody attack. We investigated MOG autoreactivity in serum and cerebrospinal fluid (CSF) by ELISA, employing the recombinant extracellular domain of MOG as antigen. We tested serum samples from 262 MS patients (175 relapsing-remitting, 43 primary progressive and 44 secondary progressive), 131 patients with other neurological diseases (OND) and 307 healthy controls. No patients or controls were receiving immunomodulating treatments. We found anti-MOG antibodies in the serum of 13.7% MS patients, mainly in those with secondary progressive MS (25%), in 13.7% of OND patients and in 6.2% of controls. We found a direct correlation (R² = 0.6, P = 0.002) between disease severity and anti-MOG titer only in patients with primary and secondary progressive MS. Anti-MOG antibodies were present in the CSF of 11.4% MS patients and 18.9% OND patients. Intrathecal synthesis of anti-MOG antibodies was demonstrated in four (4.5%) of MS patients and no OND patients. Anti-MOG antibodies are not specific for MS; however, they may characterize a subset of MS patients and this may be revealed by serial assays in relation to changing disease phase.

Introduction

Multiple sclerosis (MS), the most common inflammatory disorder of the central nervous system (CNS), is characterized by an initial inflammatory phase followed by selective demyelination and finally neurodegeneration (1). Several lines of evidence suggest that autoreactive antibodies are important in MS pathogenesis (2–4). Various antigens have been proposed as targets of the autoimmune response (5,6). Myelin oligodendrocyte glycoprotein (MOG) has been suggested as it is confined to the CNS, expressed on the outer surface of the myelin sheath (7,8), and hence accessible to antibody attack. Immunostaining studies have identified anti-MOG antibodies on disrupted myelin and within macrophages present in CNS lesions of MS patients. Anti-MOG antibodies have also been found in the brain lesions of marmosets with experimental allergic encephalomyelitis (EAE) (3). Several papers have reported the presence of anti-MOG antibodies in the serum and cerebrospinal fluid (CSF) of MS patients (9–14). However, such reactivity has not been found consistently (15,16). Morris et al. (17) characterized the production of anti-MOG autoantibodies in MOG-induced EAE in mice and found that the antibodies were more prevalent during relapses of chronic stages than during acute induction.

To further explore the relation between anti-MOG antibodies and MS, we used recombinant techniques to synthesize an extracellular portion of human MOG (ecMOG) and used it as antigen in an enzyme-linked immunosorbent assay (ELISA) we developed to detect antibodies in the serum and CSF of a large number of Italian MS patients.
series of MS patients with different forms and severity of the disease.

Methods

Patients

We studied 262 MS patients, 175 with relapsing-remitting (RR), 44 with secondary progressive (SP) and 43 with primary progressive (PP) disease forms according to Lublin’s criteria (18). Disability was assessed using the expanded disability status scale (EDSS) (19), Data on oligoclonal bands in CSF were available in 250 of 262 MS patients; 130 of 250 MS patients (92%) were positive for oligoclonal bands in CSF were available in 250 of 262 MS patients; 130 of 250 MS patients (92%) were positive for oligoclonal bands in the CSF at the beginning of the disease; 20 MS patients were also negative for oligoclonal banding on repeated analyses. We also studied 59 patients (m/f: 33/26; mean age: 52.6 ± 16.7 years) with non-inflammatory CNS diseases (NI-CNS) [amyotrophic lateral sclerosis (n = 13), cerebellar atrophy (n = 1), Leber’s optic neuropathy (n = 1), mitochondrial encephalopathy (n = 2), brain tumor (n = 2), ischemic stroke (n = 2), Parkinson’s disease (n = 1), cervical cord compression (n = 1), and stroke (n = 36)]; 18 patients (m/f: 9/9; mean age: 52.4 ± 17.3 years) with inflammatory CNS disease (INF-CNS) [encephalitis (n = 15), meningitis (n = 2) and thrombophlebitis of the cavernous sinus (n = 1)]; and 54 patients (m/f: 19/35; mean age: 37.3 ± 17.4 years) with autoimmune condition of the peripheral nervous system (Aut-PNS) [myasthenia gravis (n = 40) and Guillain–Barré syndrome (n = 14)].

For 88 patients with MS, 20 with NI-CNS, and 17 with INF-CNS, paired serum and CSF samples were available. None of the patients were receiving immunosuppressive treatment at the time of blood sampling/lumbar puncture.

Serum samples from 307 healthy blood donors (controls) (m/f: 169/138, mean age: 38.2 ± 11.4 years) were also analyzed.

Serum and CSF sampling

Serum samples were frozen immediately after centrifugation and stored at −20°C pending ELISA. Lumbar punctures were performed according to standard procedures and the CSF stored at −20°C pending assay. All CSF samples were routinely subjected to mononuclear cell count, albumin quotient, isoelectrofocusing and IgG assay (20,21). Written informed consent to sample blood and CSF was obtained from all patients.

Synthesis of recombinant human MOG

cDNA from human brain tissue, obtained during therapeutic surgery, was prepared by standard procedures and subjected to PCR using primers specific for the extracellular domain (amino acids 29–149) of MOG (ecMOG) (22,23). The amplified PCR product (363 bp) was cloned into Escherichia coli pGEX-6p-1 (Amersham-Pharmacia Biotech, Uppsala, Sweden) and sequenced. The recombinant MOG protein was induced, cleaved from GST and purified according to the manufacturer’s instructions (Amersham-Pharmacia Biotech). Recombinant protein concentrations were determined by the Bradford colorimetric method (Pierce, Rockford, IL). Purity was checked by SDS-PAGE and western blot using a mouse anti-MOG monoclonal antibody (clone 8.18-C5, kindly provided by C. Linington) (8). GST was used as control protein.

Enzyme-linked immunosorbent assay

Ninety-six-well cell culture cluster plates (Costar, Corning, NY) were coated with 75 μl/well of ecMOG, diluted in phosphate buffered saline (PBS) at a final concentration of 10 μg/ml, and left in a humidified chamber overnight at 4°C. The wells were then extensively washed with PBS plus 0.05% Tween-20 (wash buffer, PBS-T) and saturated with carbonate buffer (0.1 M, pH 9.2) supplemented with 1% bovine serum albumin (BSA; Sigma, St Louis, MO) for 2 h at 37 °C. The plates were then washed three times and 75 μl/well of diluted serum (1:200 in wash buffer plus 1% BSA) was added for 1 h at room temperature. CSF samples were added undiluted. After repeated washing, the plates were incubated with 75 μl/well of peroxidase-conjugated anti-human IgG antibody (Sigma) for 2 h at room temperature. After washing, the wells were incubated with 100 μl/well of p-phenylenediamine-2-HCl (OPD reagent, Abbott, Wiesbaden-Delkenheim, Germany) for 10 min at room temperature and the reaction stopped by adding 100 μl/well of H2SO4 1 M. The optical density (OD) of samples was read at 450 nm in a microplate reader Titertek Multiskan

Table 1. Characteristics of 262 MS patients

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sex (m/f)</th>
<th>Age at sampling&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EDSS at sampling&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Disease duration at sampling&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n = 262)</td>
<td>103/159</td>
<td>39 ± 11.9</td>
<td>3.3 ± 2.2</td>
<td>9.1 ± 8.2</td>
</tr>
<tr>
<td>RR (n = 175)</td>
<td>60/115</td>
<td>34.7 ± 9.5</td>
<td>2.2 ± 1.4</td>
<td>6.7 ± 6.7</td>
</tr>
<tr>
<td>Relapse (n = 59)</td>
<td>18/41</td>
<td>35.9 ± 8.9</td>
<td>2.3 ± 1.5</td>
<td>6.7 ± 6.6</td>
</tr>
<tr>
<td>Remission (n = 116)</td>
<td>42/74</td>
<td>34.1 ± 9.7</td>
<td>2.1 ± 1.4</td>
<td>6.7 ± 6.8</td>
</tr>
<tr>
<td>Progressive (n = 87)</td>
<td>43/44</td>
<td>47.8 ± 11.4</td>
<td>5.4 ± 1.8</td>
<td>13.8 ± 9.8</td>
</tr>
<tr>
<td>PP (n = 43)</td>
<td>25/18</td>
<td>50.1 ± 10.6</td>
<td>4.8 ± 1.9</td>
<td>9.7 ± 6.9</td>
</tr>
<tr>
<td>SP (n = 44)</td>
<td>18/26</td>
<td>45.5 ± 11.8</td>
<td>6.1 ± 1.5</td>
<td>17.7 ± 8.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean age in years ± SD.
<sup>b</sup>Mean EDSS ± SD.
<sup>c</sup>Mean duration of MS in years ± SD.
MS, multiple sclerosis; PP, primary progressive; SP, secondary progressive; RR, relapsing-remitting.
Flow Laboratories, Lugano, Switzerland). All serum and CSF samples were assayed in duplicate. Background, measured as OD from wells where serum or CSF was omitted, was subtracted from each measurement. Data were presented as means ± SD. Mouse anti-MOG monoclonal antibody 8.18-C5 diluted 1:10 (1600 μg/ml) was used as positive control (mean OD 1.672 ± 0.374). The mean OD plus two standard deviations of the 307 healthy blood donors was chosen as the normal range; serum readings higher than this (OD = 0.802) were considered positive for anti-MOG antibodies (Table 2). For CSF, the mean OD plus two standard deviations of the 20 NI-CNS patients (0.315) was chosen as cut-off; CSF readings higher than this were considered positive for anti-MOG antibodies (Table 3).

Patient serum samples positive for anti-MOG antibodies were tested against the recombinant human MGT-30 protein, as irrelevant antigen, using an ELISA assay routinely used for testing anti-titin antibodies in our MG patients (24).

### Intrathecal synthesis of anti-MOG antibodies

The intrathecal synthesis of anti-MOG antibodies, expressed as the antibody index (AI), was assessed as described (21). The AI is the ratio of the quotient of specific anti-MOG antibody units (Qspec) to the quotient of total IgG (QIgG), where quotients for specific and total IgG are defined as the ratios of CSF to serum concentrations of these entities. The normal range for the AI is 0.7–1.3; values greater than 1.4 indicate intrathecal synthesis (21).

### Immunoblotting

Serum samples from anti-MOG antibody positive patients were tested by western blot for comparison with the ELISA

### Table 2. Anti-MOG antibodies: OD values and frequency of positivity

<table>
<thead>
<tr>
<th>Group</th>
<th>OD$_{450}$ mean ± SD</th>
<th>P value$^a$</th>
<th>No. of patients positive for anti-MOG antibodies (%)</th>
<th>P value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS (n = 262)</td>
<td>0.540 ± 0.271</td>
<td>&lt; 0.0001$^c$</td>
<td>36 (13.7)</td>
<td>0.0015$^c$</td>
</tr>
<tr>
<td>PP (n = 43)</td>
<td>0.514 ± 0.210</td>
<td>0.021$^d$</td>
<td>2 (4.7)</td>
<td>0.014$^d$</td>
</tr>
<tr>
<td>SP (n = 44)</td>
<td>0.647 ± 0.342</td>
<td></td>
<td>11 (25)</td>
<td></td>
</tr>
<tr>
<td>RR (n = 175)</td>
<td>0.520 ± 0.258</td>
<td></td>
<td>23 (13.1)</td>
<td></td>
</tr>
<tr>
<td>Relapse (n = 59)</td>
<td>0.528 ± 0.237</td>
<td>0.027$^d$</td>
<td>5 (8.5)</td>
<td>0.029$^d$</td>
</tr>
<tr>
<td>Remission (n = 116)</td>
<td>0.516 ± 0.269</td>
<td>0.006$^d$</td>
<td>18 (15.5)</td>
<td></td>
</tr>
<tr>
<td>OND (n = 131)</td>
<td>0.543 ± 0.301</td>
<td>&lt; 0.0001$^c$</td>
<td>18 (13.7)</td>
<td>0.014$^d$</td>
</tr>
<tr>
<td>NI-CNS (n = 59)</td>
<td>0.622 ± 0.331</td>
<td>&lt; 0.0001$^c$</td>
<td>11 (18.6)</td>
<td>0.003$^a$</td>
</tr>
<tr>
<td>INF-CNS (n = 18)</td>
<td>0.521 ± 0.248</td>
<td></td>
<td>3 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Aut-PNS (n = 54)</td>
<td>0.463 ± 0.263</td>
<td></td>
<td>4 (7.4)</td>
<td></td>
</tr>
<tr>
<td>Healthy controls (n = 307)</td>
<td>0.409 ± 0.196</td>
<td></td>
<td>19 (6.2)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Fisher’s PLSD.
$^b$Fisher’s exact test.
$^c$Versus healthy controls.
$^d$Versus SP.
$^e$Versus Aut-PNS patients.

MS, multiple sclerosis; PP, primary progressive; SP, secondary progressive; RR, relapsing-remitting; OND, other neurological diseases; NI-CNS, non-inflammatory central nervous system disorders; INF-CNS, inflammatory central nervous system disorders; Aut-PNS, autoimmune disorders of the peripheral nervous system.

### Table 3. Anti-MOG antibodies in CSF of MS patients

<table>
<thead>
<tr>
<th>Group</th>
<th>OD$_{450}$ mean ± SD</th>
<th>No. of cases positive</th>
<th>For anti-MOG Abs (%)$^a$</th>
<th>For total IgG synthesis (%)$^b$</th>
<th>For anti-MOG-Ig synthesis$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MS (n = 88)</td>
<td>0.173 ± 0.158</td>
<td>10 (11.4)</td>
<td>40 (45.5)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PP (n = 19)</td>
<td>0.217 ± 0.264</td>
<td>3 (15.8)</td>
<td>9 (47.4)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SP (n = 10)</td>
<td>0.203 ± 0.115</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>RR (n = 59)</td>
<td>0.154 ± 0.113</td>
<td>5 (8.5)</td>
<td>30 (50.8)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Relapse (n = 23)</td>
<td>0.166 ± 0.123</td>
<td>2 (8.7)</td>
<td>13 (56.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Remission (n = 36)</td>
<td>0.146 ± 0.107</td>
<td>3 (8.3)</td>
<td>17 (47.2)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>NI-CNS (n = 20)</td>
<td>0.121 ± 0.097</td>
<td>2 (10)</td>
<td>4 (20)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>INF-CNS (n = 17)</td>
<td>0.224 ± 0.198$^c$</td>
<td>5 (29.4)</td>
<td>1 (5.9)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Defined as OD above NI-CNS mean + 2 SD.
$^b$Calculated by method of Reiber et al. (21).

MS, multiple sclerosis; PP, primary progressive; SP, secondary progressive; RR, relapsing-remitting; NI-CNS, non-inflammatory CNS disorders; INF-CNS, inflammatory CNS disorders.

(Flow Laboratories, Lugano, Switzerland). All serum and CSF samples were assayed in duplicate. Background, measured as OD from wells where serum or CSF was omitted, was subtracted from each measurement. Data were presented as means ± SD. Mouse anti-MOG monoclonal antibody 8.18-C5 diluted 1:10 (1600 μg/ml) was used as positive control (mean OD 1.672 ± 0.374). The mean OD plus two standard deviations of the 307 healthy blood donors was chosen as the normal range; serum readings higher than this (OD = 0.802) were considered positive for anti-MOG antibodies (Table 2). For CSF, the mean OD plus two standard deviations of the 20 NI-CNS patients (0.315) was chosen as cut-off; CSF readings higher than this were considered positive for anti-MOG antibodies (Table 3).

Patient serum samples positive for anti-MOG antibodies were tested against the recombinant human MGT-30 protein, as irrelevant antigen, using an ELISA assay routinely used for testing anti-titin antibodies in our MG patients (24).
results. Mouse 8.18-C5 anti-MOG monoclonal antibody was used as positive control. One μg/lane of purified recombinant ecMOG was loaded onto 12% SDS-polyacrylamide gel and electrotransferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked with 5% dried low-fat milk in PBS-T and cut into 2 mm strips for incubation with diluted human sera (1:200 in 0.5% milk in PBS-T) or mouse monoclonal anti-MOG antibody (8.18-C5, diluted 1:1000 in 0.5% milk in PBS-T) for 2 h at room temperature. Membranes were incubated with alkaline phosphatase-conjugated anti-human IgG or anti-mouse IgG (Sigma) for 1 h at room temperature and bound antibodies were detected by 5-bromo-4-chloro-3-indolylphosphate (Pierce). Serum samples were also tested against mock-transfected bacterial lysate.

Statistical analysis
All group comparisons were performed using ANOVA test. Significant differences were further analyzed using post hoc Fisher’s PLSD. Fisher’s exact test for 2 × 2 tables was used to test differences between non-parametric variables. P-values < 0.05 were considered significant. The sensitivity of the assay was calculated by dividing the number of patients positive for autoantibodies by the total number of patients; specificity was calculated by dividing the number of controls negative for autoantibodies by the total number of controls. Correlations were investigated by linear regression. All statistical analyses were performed using Statview 5.01 (Sas Institute Inc., Cary, NC).

Results

Anti-MOG antibodies in MS patients and controls
The mean OD (0.540 ± 0.271) of the MS group was significantly higher than that of the controls (0.409 ± 0.196) (P < 0.0001), but did not differ significantly from the mean OD of the OND group (0.543 ± 0.301) (inflammatory, non-inflammatory and autoimmune) (Table 2).

Table 2 also shows the percentages of antibody-positive sera in each patient group. Thirty-six of 262 (13.7%) MS patients were positive for antibodies against ecMOG (P = 0.0015 versus controls).

A similar percentage of positivity (18/131, 13.7%) was found in OND patients (P = 0.014 versus controls); 19/307 (6.2%) controls were positive.

The specificity was high: 93.8% of controls were negative for anti-ecMOG antibodies; however the sensitivity of the test for MS patients was very low (13.7%).

Twenty-seven out of 230 (11.7%) MS patients positive for oligoclonal bands in the CSF had detectable anti-MOG antibodies.

Cases positive for anti-MOG antibody by ELISA were confirmed by western blot using the recombinant ec-MOG employed for the ELISA procedure (Fig. 1). All serum samples tested against the irrelevant recombinant MGT-30 immunodominant domain of titin were negative (data not shown).

Anti-MOG antibody levels in relation to type and clinical severity of MS
Patients with SP MS had a mean OD of 0.647 ± 0.342, significantly higher than that of PP patients (0.514 ± 0.21, P = 0.021), RR patients in remission (0.516 ± 0.269, P = 0.006) and RR patients in relapse (0.528 ± 0.237, P = 0.027) (Table 2).

The frequency of positive titers was higher in SP (25%) than in PP (4.7%) (P = 0.014) and in RR in relapse (8.5%) (P = 0.029); while the difference between SP and RR in remission (15.5%) was not significant. Differences between other groups (Table 2) were not significant either.

We found a linear correlation between EDSS and anti-MOG antibody titer only in MS patients with progressive forms of the disease (SP and PP; R² = 0.6, P = 0.002) (Fig. 2). No correlation between MOG-antibody titer and disease duration or progression rate (EDSS/disease duration) at time of blood sampling was found.
**Anti-MOG antibodies in OND patients in relation to specific disease**

The mean OD of the OND group was significantly higher than that of controls ($P < 0.0001$) (Table 2). NI-CNS patients had a mean OD of $0.622 \pm 0.331$, significantly higher than controls ($P < 0.0001$) and Aut-PNS patients ($0.463 \pm 0.263$, $P = 0.0006$), but not different from INF-CNS patients ($0.521 \pm 0.248$, $P = 0.13$) (Table 2). NI-CNS subgroups with highest ODs were those with amyotrophic lateral sclerosis ($0.571 \pm 0.156$, $P = 0.011$ versus controls) and stroke ($0.663 \pm 0.394$, $P < 0.0001$ versus controls).

With regard to frequency of positivity, nine of 36 (25%) stroke patients were positive for anti-MOG antibodies while only one (7.7%) amyotrophic lateral sclerosis patient was.

**Intrathecal synthesis**

Paired serum and CSF samples were available for 88 MS and 37 OND patients (17 INF-CNS; 20 NI-CNS). Intrathecal IgG synthesis, calculated as according to Reiber et al. (21), was occurring in 40 (45.5%) MS, four (20%) NI-CNS, and one (5.9%) INF-CNS patient (Table 3). The mean titer of anti-MOG antibodies in MS patients’ CSF ($0.173 \pm 0.158$) did not differ significantly from those in the various groups of OND patients. Ten (11.4%) MS, two (10%) NI-CNS and five (29.4%) INF-CNS patients were positive for anti-MOG antibodies in CSF, where positive was defined as more than 2 SD above NI-CNS mean (Table 3). The anti-MOG antibody specific IgG index (21) was greater than 1.4 in four MS patients (one PP and three in remission); two of whom were positive and two negative for total IgG intrathecal synthesis. No OND patient had an Al greater than 1.4.

**Discussion**

Myelin basic protein, proteolipid protein, myelin-associated glycoprotein and MOG are recognized as targets of antibodies in MS (25); however, it is unclear whether an autoimmune process related to recognition of any of these proteins is responsible for the demyelination in MS (14). MOG has been proposed as a target of the immune response in MS as it is expressed on the outermost lamellae of myelin sheaths (8,26–28) and not in thymus (27). In addition, anti-MOG autoantibodies have been shown to mediate demyelination both in vitro (29) and in animal models of MS (8,30,31) and to be directed against the extracellular Ig domain of MOG (32,33). Furthermore, in MS lesions, anti-MOG antibodies localize within myelin debris, suggesting a role in myelin damage (3).

We examined serum and CSF reactivity against MOG in a large cohort of MS patients with disease of varying severity and type. We also studied patients with a variety of other inflammatory and non-inflammatory neurological conditions, including autoimmune conditions of the peripheral nervous system.

We found that mean serum levels of anti-MOG antibodies in MS and NI-CNS patients were significantly higher than in healthy controls ($P < 0.0001$), while levels in INF-CNS patients did not differ significantly from controls ($P = 0.06$). In addition, 13.7–18.6% of patients in these groups were positive for anti-MOG antibodies in serum (Table 2), while the frequency of positivity in patients with autoimmune PNS disorders (MG and Guillain–Barré syndrome) was similar to that of controls (Table 2). These results indicate that anti-MOG antibodies are usually present only in patients with a CNS condition and that anti-MOG reactivity is not specific for MS, or even for inflammatory CNS diseases, since positive titers were also found in a proportion of patients with non-inflammatory diseases.

Within the MS series, the SP subgroup had a significantly higher mean OD than the other MS subgroups (Table 2). Patients with the SP form of MS are characterized by progressively worsening neurological disability after a previously relapsing-remitting course. Anti-MOG antibodies—known to have demyelinating potential (8,29,30)—may be implicated in the degenerative changes (axonal and glial loss) characteristic of the SP form of the disease (33–35).

We also found a direct correlation between disability (EDSS score) and anti-MOG titer (Fig. 2) in progressive forms of the disease. By contrast Karni et al. (34) found no correlations between serum anti-MOG antibody levels and clinical parameters (disease course, EDSS score, and disease duration); the larger number of patients included in our study might explain this difference.

Anti-MOG antibodies have been detected since 1991; most studies [exceptions are those of Reindl et al. (14) and Egg et al. (35)] have been on small groups of MS patients of varying disease severity or phase, so that results may have been influenced by clinical variables associated with the disease, and a clear picture of the role of these antibodies in the pathogenesis of MS has not emerged.

Furthermore, ELISA, western blot and immunohistochemistry have been used to detect these antibodies and different antigens (native MOG, recombinant MOG or synthetic peptides) employed, further contributing to the heterogeneity of the results.

Examination of published studies on anti-MOG antibodies in MS shows that when western blot is used, the proportion of positive cases is much higher than when ELISA is used (14,35,36,38). This large difference in sensitivity is disconcerting and is perhaps due to the fact that the recombinant protein used for ELISA has a conformational structure closer to the native protein, while in western blot the protein is linearized prior to antibody recognition by the electrophoretic step. Thus, epitopes recognized by anti-MOG autoantibodies may be rendered more accessible by denaturation, as also suggested by the recent observation that MOG35–55 peptide-specific IgG ELISA titers were significantly higher than anti-recombinant MOG titers in MS (38). Very recently, Berger et al. (39) have shown that IgM anti-MOG antibodies can be detected by western blot analysis in high frequency among patients with a first acute neurologic event suggestive of MS and positive for oligoclonal bands; these patients are those with an early conversion to clinically definite MS. These findings, however, raise issues concerning pathophysiology of the disease, e.g. the association of anti-MOG antibodies with the different stages of MS, and the evaluation of immunological tests, e.g. the need of a methodological consensus for anti-MOG antibody detection and their clinical and predictive value in MS management.
Stratification of our MS patients with regard to the presence of oligoclonal bands in the CSF and the presence of anti-MOG antibodies does not reveal any significant association.

Natural MOG is highly glycosylated and contains epitopes recognized by B cells. Interestingly, Mazzucco et al. (40) found that MS patients and those with other neurological diseases, but not controls, had serum reactivity against a synthetic glycosylated MOG fragment but not against nonglycosylated fragments. Thus, the low percentage of MS patients with anti-MOG antibodies in our series might be due to our use of non-glycosylated recombinant MOG. It should also be noted that we assayed the IgG response to MOG, while others have reported increased frequencies of IgM or IgA antibodies against MOG (35,38,39). However, our preliminary experiments on the detection of IgM anti-MOG antibodies in serum from patients and controls did not reveal a greater frequency of these autoantibodies in MS patients (data not presented).

Of the 88 MS patients for whom CSF samples were available, 10 (11.4%) were positive. A similar proportion of NI-CNS patients was positive (2/20, 10%) whereas about three times more patients with INF-CNS conditions were positive (5/17, 29.4%). However, only 4/88 (4.5%) MS patients were synthesizing anti-MOG antibodies intrathecally, indicating that these antibodies usually derive from the peripheral blood compartment. The small number of patients with intrathecal synthesis further obscures the potential role of anti-MOG antibodies in MS. To clarify the role of anti-MOG antibodies in MS they should be assayed in serum and CSF over time and in relation to changing disease phase.

Acknowledgements
We thank Dr C. Linton (Max Planck Institute for Neurobiology, Martinsried, Germany) for kindly providing the mouse anti-MOG monoclonal antibody 8.18-C5. We thank Don Ward for help with the English.

Abbreviations
Aut-PNS autoimmune disease of the peripheral nervous system
CSF cerebrospinal fluid
CNS central nervous system
cMoG extracellular myelin oligodendrocyte glycoprotein
EDSS expanded disability status scale
INF-CNS inflammatory central nervous system disease
MOG myelin oligodendrocyte glycoprotein
NI-CNS non-inflammatory central nervous system disease
OND other neurological disease
MS multiple sclerosis
PP primary progressive MS
RR relapsing-remitting MS
SP secondary progressive MS

References
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