Objective: To evaluate the presence and specificity of anti–myelin oligodendrocyte glycoprotein (MOG) antibody in the cerebrospinal fluid and plasma of patients with multiple sclerosis (MS).

Design: Case-control study of patients with clinically definite MS compared with patients with other neurologic diseases (ONDs) of the central nervous system and control subjects.

Setting: Referral center in the Department of Neurology of Hadassah University Hospital, greater Jerusalem area, Israel.

Participants: Consecutive cerebrospinal fluid samples from 31 patients with MS, 31 patients with ONDs, and 28 healthy controls; and plasma samples from 33 patients with MS, 28 patients with ONDs, and 31 healthy controls were taken from the cerebrospinal fluid and plasma bank of the Department of Neurology, Hadassah University Hospital.

Main Outcome Measures: Levels and frequencies of anti-MOG antibody in patients with MS, as defined by enzyme-linked immunosorbent assay.

Results: Cerebrospinal fluid levels of antibodies to MOG and to myelin basic protein were significantly higher in patients with MS (P<.001 and P=.001, respectively) and patients with ONDs (P=.005 and P=.03, respectively) compared with controls; frequency of antibodies to MOG, but not to myelin basic protein, was higher in patients with MS and patients with ONDs (P=.01 and P=.003, respectively, for the frequency of anti-MOG antibody, and P=.65 and P=.41, respectively, for the frequency of anti-myelin basic protein antibody). Plasma levels of antibodies to MOG and to myelin basic protein were higher in patients with MS compared with patients with ONDs (P=.003 for both comparisons) and with controls (P=.03 and P=.04, respectively); however, the frequency of antibodies to MOG and myelin basic protein was similar in patients with MS, patients with ONDs (P=.54 and P=.82, respectively), and controls (P=.50 and P=.14, respectively).

Conclusions: The elevated presence of anti-MOG antibody is not specific for MS because a similar appearance was also demonstrated in patients with ONDs. Therefore, it is not clear whether this antibody is pathogenic in MS or, on the contrary, has a defensive role against further immune-mediated damage after myelin breakdown.

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PATIENTS, MATERIALS, AND METHODS

PATIENTS

Samples of CSF and plasma were obtained from 31 and 33 patients with clinically definite MS, respectively. Clinical and laboratory data are presented in Table 1.

Cerebrospinal fluid samples were also obtained from 31 patients with ONDs of the CNS and from 28 controls who were examined for headache. The OND group included 6 patients with degenerative dementia, 3 patients with primary CNS lymphoma, 3 patients with Behçet disease, 3 patients with acute meningitis, 3 patients with motor neuron disease, 2 patients with multisystem atrophy, 2 patients with extrapyramidal syndromes, 2 patients with acute encephalitis, 2 patients with intracerebral hemorrhage, 1 patient with acute cerebral infarct, 1 patient with Wilson disease, 1 patient with type C Niemann-Pick disease, 1 patient with focal epilepsy, and 1 patient with Friedrich ataxia.

Plasma samples were also obtained from 31 healthy individuals and 28 patients with ONDs of the CNS (Table 1). The latter comprised 11 patients with acute cerebral infarct, 3 patients with intracerebral hemorrhage, 2 patients with degenerative dementia, 2 patients with cryptocogenic generalized epilepsy, 2 patients with Behçet disease, 2 patients with brain vasculitis, 2 patients with acute encephalitis, 1 patient with acute meningitis, 1 patient with Wilson disease, 1 patient with type C Niemann-Pick disease, and 1 patient with Sydenham chorea. None of the participants were being treated with immunosuppressive or immunomodulatory drugs during CSF or blood sampling.

ANTIGENS

Human MOG, a recombinant MOG that represents the immunoglobulinlike domain of the MOG molecule, was prepared as described previously. The specific peptide sequence is GQFRVFPRPHIRALGYDEVELPCRISPQKNATMEVGWYRPFPFVRYHLYNKGQDQADQPEYRGTRELLKDAGEGKYTLRINRVSDEGGFTCFDRDHYSQEEAEMLVKEDPFYYWS.

Human MBP was purified from neurologically healthy human brain according to Dunkley and Carnegie.

ANTIBODY DETERMINATION

Cerebrospinal fluid and plasma samples were tested for levels of anti-MOG and anti-MBP antibodies to the IgA, IgG, and IgM isotypes. The CSF levels were measured by a modified enzyme-linked immunosorbent assay with avidin-biotin amplification and the plasma levels were measured by standard enzyme-linked immunosorbent assay. Briefly, 96-well microtiter plates (Nunc-Immuno plates; Nunc A/S, Roskilde, Denmark) were coated with 100 µL of a 10-µg/mL antigen (recombinant human MOG or MBP) solution in phosphate-buffered saline solution (PBS), pH 7.4, overnight at 4°C. Unreactive sites were saturated with 10% filtered fetal calf serum (150 µL) in carbonate buffer, pH 9.6, for 2 hours at 37°C. A 75-µL volume of CSF (diluted 1:5 in PBS with 1% bovine serum albumin [BSA] and 0.05% Tween-20) or plasma (diluted 1:200 in the same solution) was added to each well, and the plates were kept at room temperature for 1 hour. After washing, goat biotinylated antihuman IgG (75 µL), diluted 1:4000 in PBS and 3% BSA or antihuman IgA, IgG and IgM conjugated to peroxidase (75 µL), diluted 1:2000 in PBS and 1% BSA and 0.05% Tween-20 were added to each well of the tested CSF and plasma samples, respectively, and the plates were incubated for an additional 2 hours at room temperature. Bovine serum albumin was included to reduce nonspecific binding. The CSF plates were washed again, then peroxidase (75 µL) that was conjugated to streptavidin and diluted 1:2000 in PBS and 1% BSA was added to each well, and the plates were incubated for 30 minutes at 37°C. Afterward, all plates were washed and a 2,2′-Azinobis(3-ethylbenzthiazoline-sulfonic acid) (ABTS; Sigma-Aldrich Corp, St Louis, Mo) substrate was added. The developing color was read as an optical density at 405 nm (OD405). Each CSF and plasma specimen was tested in duplicate (2 samples), so the results for every specimen express the average value of the OD readings. The within-assay variation was consistently less than 15%. Specimens were considered positive if their average OD reading was higher than the mean OD95 level +2 SDs of controls. Results of preliminary tests done twice with 30 patients with MS, 20 patients with ONDs, and 10 controls show a complete matching of positive specimens for anti-MOG antibodies.

CSF OLIGOCONAL BANDS

Cerebrospinal fluid oligoclonal bands were detected by electrophoresis of unconcentrated CSF on agarose gel (Pana-gel; Princeton Separations Inc, Freehold, NJ) slides, followed by fixation using picric acid, drying, and staining with amido black.

CSF IgG CONCENTRATIONS

The CSF IgG concentrations of patients with MS, patients with ONDs, and controls were measured in immunodiffusion plates (Behringwerke AG, Marburg, Germany). Measurement was done to check whether the anti-MOG and anti-MBP levels obtained were specific for the antigen tested or were attributable to the total IgG concentrations.

STATISTICAL ANALYSIS

Comparisons were made among patients with MS, patients with ONDs, and controls for anti-MOG and anti-MBP antibody levels and for CSF IgG concentrations using the Wilcoxon rank sum test. Comparisons of the frequency of positive patients were made with Yates corrected χ² test. P<.05 was considered significant.
model of experimental autoimmune encephalomyelitis induced by MOG, exacerbations correlated with an enhanced production of anti-MOG antibody. 25

Patients with MS had higher titers of CSF anti-MOG antibody, and more anti-MOG IgG antibody-secreting cells, in CSF and blood than did controls. 26,27

To evaluate whether the presence of anti-MOG antibody is specific to MS, we compared the levels of CSF and plasma anti-MOG antibody in patients with MS and the frequencies of patients with MS positive for this antibody with those of patients with other neurologic diseases (ONDs) of the CNS and healthy control subjects. We also compared the frequencies of anti-MOG antibody with those of anti-MBP antibody in the same groups.

Levels of anti-MOG and anti-MBP antibodies were determined in CSF and plasma samples from patients with MS, patients with ONDs, and controls. We compared overall values of the antibodies and frequency of positive patients. The cutoff value above which patients were considered positive for CSF antibodies was set as the mean value for controls + 2 SDs: 0.781 OD 405 for the anti-MOG antibody and 1.070 OD 405 for the anti-MBP antibody. The cutoff value above which patients were considered positive for plasma antibodies was set as the mean value for controls + 2 SDs: 0.451 OD 405 for the anti-MOG antibody and 0.554 OD 405 for the anti-MBP antibody.

As shown in Table 2, CSF levels of anti-MOG and anti-MBP antibodies of patients with MS and patients with ONDs were significantly higher than those of controls. However, only the frequency of patients who were positive for the anti-MOG antibody (but not for the anti-MBP antibody) was higher in the MS and ONDs groups than in controls. There were no significant differences in anti-MOG and anti-MBP antibody levels between the MS and ONDs groups.

Plasma levels of anti-MOG and anti-MBP antibodies of patients with MS were significantly higher than those of patients with ONDs and controls (Table 3). There were no significant differences in percentage of patients who were positive for anti-MOG or anti-MBP antibodies among patients with MS, patients with ONDs, and controls.

RESULTS

Table 1. Clinical and Laboratory Data for Patients With MS, Patients With ONDs, and Controls*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients With MS</th>
<th>Patients With ONDs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of plasma donors, range (mean), y</td>
<td>18-57 (41.5)</td>
<td>14-82 (50.1)</td>
<td>22-54 (35.5)</td>
</tr>
<tr>
<td>Positive CSF OCBs</td>
<td>19/10</td>
<td>19/14</td>
<td></td>
</tr>
<tr>
<td>RR/SP disease course</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF donors</td>
<td>21/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma donors</td>
<td>19/14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Anti-MOG and Anti-MBP Antibody Levels in the CSF of Patients With MS, Patients With ONDs of the CNS, and Controls*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients, No.</th>
<th>OD 405, Mean ± SD</th>
<th>Positive Patients, No. (%)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-MOG Antibody (Cutoff Value, 0.781 OD 405)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>31</td>
<td>0.793 ± 0.522 .005</td>
<td>10 (32)</td>
<td>.01§</td>
</tr>
<tr>
<td>ONDs</td>
<td>31</td>
<td>0.794 ± 0.459 .005</td>
<td>12 (39)</td>
<td>.03</td>
</tr>
<tr>
<td>Healthy</td>
<td>28</td>
<td>0.470 ± 0.156 .005</td>
<td>1 (4)</td>
<td>.00</td>
</tr>
<tr>
<td>Anti-MBP Antibody (Cutoff Value, 1.070 OD 405)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>30</td>
<td>0.925 ± 0.201 .005</td>
<td>3 (10)</td>
<td>.65</td>
</tr>
<tr>
<td>ONDs</td>
<td>31</td>
<td>0.896 ± 0.174 .005</td>
<td>4 (13)</td>
<td>.41</td>
</tr>
<tr>
<td>Healthy</td>
<td>28</td>
<td>0.804 ± 0.133 .005</td>
<td>1 (4)</td>
<td>.00</td>
</tr>
</tbody>
</table>

*MS indicates multiple sclerosis; ONDs, other neurologic diseases; CSF, cerebrospinal fluid; OCBs, oligoclonal bands; RR/SP, relapsing-remitting/secondary progressive; EDSS, Expanded Disability Status Scale according to Kurtzke; and ellipses, data not applicable.

Table 3. Anti-MOG and Anti-MBP Antibody Levels in the Plasma of Patients With MS, Patients With ONDs of the CNs, and Controls*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients, No.</th>
<th>OD 405, Mean ± SD</th>
<th>Positive Patients, No. (%)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-MOG Antibody (Cutoff Value, 0.451 OD 405)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>33</td>
<td>0.295 ± 0.080 .005</td>
<td>2 (6)</td>
<td>.50</td>
</tr>
<tr>
<td>ONDs</td>
<td>28</td>
<td>0.226 ± 0.064 .005</td>
<td>0 (0)</td>
<td>.99</td>
</tr>
<tr>
<td>Healthy</td>
<td>31</td>
<td>0.261 ± 0.090 .005</td>
<td>1 (3)</td>
<td>.00</td>
</tr>
<tr>
<td>Anti-MBP Antibody (Cutoff Value, 1.554 OD 405)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>33</td>
<td>0.414 ± 0.104 .005</td>
<td>4 (12)</td>
<td>.14</td>
</tr>
<tr>
<td>ONDs</td>
<td>28</td>
<td>0.300 ± 0.127 .005</td>
<td>2 (7)</td>
<td>.43</td>
</tr>
<tr>
<td>Healthy</td>
<td>31</td>
<td>0.351 ± 0.101 .005</td>
<td>1 (3)</td>
<td>.00</td>
</tr>
</tbody>
</table>

*MS indicates myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; MS, multiple sclerosis; ONDs, other neurologic diseases; CNS, central nervous system; OD 405, optical density at 405 nm. No significant statistical differences were found between patients with MS and patients with ONDs for the levels and frequency of positive patients of anti-MOG and anti-MBP antibodies; and ellipses, data not applicable.

†Levels of antibodies were compared using the Wilcoxon rank sum test.
‡Positive frequencies were compared according to the Yates corrected χ² test.
§MS vs healthy.
ONDs vs healthy.
To check whether the differences in levels of CSF antibodies can be attributed to levels of CSF immunoglobulins, we measured CSF IgG concentrations. Range of CSF IgG concentrations in patients with MS was 0.002 to 0.15 g/L (mean, 0.05 g/L). Mean CSF IgG concentration of the 10 anti-MOG antibody–positive patients with MS was 0.06 g/L, whereas mean CSF IgG concentrations of the 21 anti-MOG antibody–negative patients with MS was 0.06 g/L. There was no significant difference between these 2 subgroups of patients with MS.

Range of CSF IgG concentrations in patients with ONDs was 0.002 to 0.12 g/L (mean, 0.03 g/L). Mean CSF IgG concentrations of the 12 anti-MOG antibody–positive patients with ONDs was 0.04 g/L, whereas mean CSF IgG concentrations of the 19 anti-MOG antibody–negative patients with ONDs was 0.04 g/L. There was no significant difference in IgG concentration between these 2 subgroups of patients with ONDs.

Similarly, no significant difference in anti-MOG and anti-MBP antibodies was found between 19 patients with MS with CSF oligoclonal bands and 12 patients with MS without CSF oligoclonal bands.

Because the relative frequency of immunoglobulin-producing B cells and plasma cells is higher in the later stages of MS, we analyzed the levels and frequencies of anti-MOG and anti-MBP in patients with MS according to disease course (relapsing-remitting vs secondary progressive), degree of disability (evaluated by the Expanded Disability Status Scale of Kurtzke), and disease duration. No significant differences in the levels and frequencies of CSF anti-MOG and anti-MBP antibodies were noted on the basis of the above-mentioned clinical features of MS. However, 50% (6/12) of the patients with MS with a disease duration of 5 years or more were positive for the anti-MOG antibody compared with only 21% (4/19) of the patients with MS with a disease duration of less than 5 years ($P = .20$).

Similar to the CSF, no significant differences in the levels and frequencies of the plasma anti-MOG and anti-MBP antibodies were found by subdividing the patients with MS according to disease course, degree of disability, or disease duration.

In light of the assumption that the anti-MOG antibody participates in the pathogenesis of demyelination in MS, and may serve as a diagnostic tool for this disease, we compared the occurrence of this antibody in patients with MS, patients with ONDs, and controls. The levels of anti-MOG antibody and the frequencies of patients positive to this antibody were also compared with those of anti-MBP antibody, which is known to be elevated nonspecifically in patients with MS. Our main finding is that patients with MS had higher CSF anti-MOG and anti-MBP antibody levels than controls. However, only the percentage of anti-MOG antibody–positive patients was significantly higher in patients with MS compared with controls. Furthermore, this difference was not specific to MS because patients with ONDs showed a similar pattern of anti-MOG antibody levels and frequencies of positive patients.

There was no correlation between CSF anti-MOG antibody levels and CSF IgG concentrations. This result indicated that the detected antibodies were specific for the antigens we tested.

Although the plasma levels of the anti-MOG and anti-MBP antibodies of patients with MS were significantly higher than the antibody levels of patients with ONDs and controls, these levels were usually not higher than the cutoff value for controls.

Anti-MOG antibody has been suggested to be involved in the formation of demyelinating MS plaques. However, we found that this antibody was also present in ONDs with no demyelinating lesions. Therefore, our study shows that measuring anti-MOG antibody cannot serve as a diagnostic tool for MS and does not support the notion that anti-MOG antibody is primarily responsible for demyelination in MS.

Our findings support the possibility that increased levels of anti-MOG antibody in MS may represent a secondary phenomenon related to myelin damage in this disease. Taking into account its demyelinating property, this antibody may be pathogenic in the setting of blood-brain barrier breach, complement, and inflammatory infiltrates that occur in acute MS lesions. However, it may serve as a defense line against immunemediated damage after myelin breakdown by opsonization of myelin debris to allow phagocytosis by macrophages and microglial cells. Therefore, as with other MS nonspecific autoantibodies, the meaning of elevated anti-MOG antibody levels in CSF is not straightforward.

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REFERENCES


