Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study

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Summary
In experimental animal models of multiple sclerosis demyelinating antibody responses are directed against the myelin oligodendrocyte glycoprotein (MOG). We have investigated whether a similar antibody response is also present in multiple sclerosis patients. Using the recombinant human extracellular immunoglobulin domain of MOG (MOG-Ig) we have screened the sera and CSFs of 130 multiple sclerosis patients, 32 patients with other inflammatory neurological diseases (OIND), 30 patients with other non-inflammatory neurological diseases (ONND) and 10 patients with rheumatoid arthritis. We report that 38% of multiple sclerosis patients are seropositive for IgG antibodies to MOG-Ig compared with 28% seropositive for anti-myelin basic protein (MBP). In contrast, OIND are characterized by similar frequencies of serum IgG antibody responses to MOG-Ig (53%) and MBP (47%), whereas serum IgG responses to MOG-Ig are rare in ONND (3%) and rheumatoid arthritis (10%). Anti-MBP IgG antibodies, however, are a frequent finding in ONND (23%) and rheumatoid arthritis (60%). Our results provide clear evidence that anti-MOG-Ig antibodies are common in CNS inflammation. However, in OIND these antibody responses are transient, whereas they persist in multiple sclerosis. We demonstrate that the serum anti-MOG-Ig response is already established in early multiple sclerosis (multiple sclerosis-R0; 36%). In later multiple sclerosis stages frequencies and titres are comparable with early multiple sclerosis. In contrast, the frequency of anti-MBP antibodies is low in multiple sclerosis-R0 (12%) and increases during disease progression in relapsing–remitting (32%) and chronic progressive multiple sclerosis (40%), thus suggesting that anti-MBP responses accumulate over time. Finally we provide evidence for intrathecal synthesis of IgG antibodies to MOG-Ig in multiple sclerosis.

Keywords: multiple sclerosis; autoantibodies; myelin oligodendrocyte glycoprotein; myelin basic protein; brain inflammation

Abbreviations: ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; MOG-Ig = extracellular immunoglobulin domain of MOG; multiple sclerosis-CP = multiple sclerosis, chronic progressive disease course; multiple sclerosis-R0 = multiple sclerosis, first attack; multiple sclerosis-RR = multiple sclerosis, relapsing–remitting disease course; OIND = other inflammatory neurological diseases; ONND = other non-inflammatory neurological diseases; Q-Alb = albumin quotient

Introduction
The molecular and cellular mechanisms responsible for the selective destruction of CNS myelin in multiple sclerosis are unknown. Multiple sclerosis is believed to be an immune mediated disease, in which a cascade of autoimmune events leads to the formation of persistently demyelinated gliotic scar tissue. The immunopathology of multiple sclerosis lesions is heterogeneous suggesting that lesion formation is mediated by different effector mechanisms in discrete subsets of patients (Ozawa et al., 1994; Lucchinetti et al., 1996). In particular there is increasing evidence that antibody-
dependent effector mechanisms can play an important role in the immunopathogenesis of demyelination.

Immunoglobulin (Ig) deposition within white matter lesions (Prineas, 1985), Ig-associated receptor mediated phagocytosis of myelin debris by macrophages (Prineas and Graham, 1981; Prineas, 1985) and complement activation within the CNS (Gay and Esiri, 1991; Storch et al., 1998a) all provide indirect evidence for the involvement of antibody in disease pathogenesis. This concept is further supported by the colocalization of IgG and C9neo, a marker for the final lytic stage of the complement cascade at the active leading edge of demyelinating lesions (Storch et al., 1998a), and the presence of myelin debris coated with complement membrane attack complex in the CSF (Scolding et al., 1989). However, the antigen(s) recognized by this putative pathogenic autoantibody response remain to be identified.

A promising target for this demyelinating antibody response is the myelin oligodendrocyte glycoprotein (MOG), which was initially identified as the dominant target for the demyelinating autoantibody response in experimental autoimmune encephalomyelitis induced by immunization with CNS tissue homogenates (Lebar et al., 1986; Linnington and Lassmann, 1987). MOG is a minor component of CNS myelin that is exclusively expressed at the outer surface of the myelin sheath and oligodendrocyte plasma membrane (Brunner et al., 1989). A single Ig V-like domain (Gardinier et al., 1992) exposed at the membrane surface (Kroepfl et al., 1996) provides the target for the demyelinating antibody response (Adelmann et al., 1995). This Ig domain is unique in that it contains encephalitogenic T-cell epitopes which synergize with this demyelinating antibody response to induce a demyelinating variant of experimental autoimmune encephalomyelitis in rats (Adelmann et al., 1995), mice (Amor et al., 1994) and marmosets (Genain et al., 1995). The pathology of these animal models closely resembles that seen in a subset of multiple sclerosis patients (Ozawa et al., 1994; Lucchinetti et al., 1996; Storch et al., 1998a, b).

Although enhanced T- and B-cell responses to MOG have been reported in multiple sclerosis (Sun et al., 1991; Xiao et al., 1991; Kerlero de Rosbo et al., 1993; Wallström et al., 1998) the role of these responses in disease pathogenesis is uncertain. In particular, no information is available with respect to either the frequency or titre of antibody responses directed against the extracellular MOG Ig domain (MOG-Ig), which is the target of the pathogenic autoantibody response to this antigen.

In this retrospective study we used recombinant human MOG-Ig to determine the frequency and titres of MOG specific autoantibodies in multiple sclerosis patients. Moreover, we have also determined the frequency of autoantibodies against another myelin antigen, myelin basic protein (MBP). We analysed paired serum and CSF samples from 130 multiple sclerosis patients, 32 patients with other inflammatory neurological diseases (OIND), 30 patients with other non-inflammatory neurological diseases (ONND) and 10 patients with rheumatoid arthritis. We demonstrate that multiple sclerosis is associated with a persistent antibody response to MOG-Ig, whereas this response is only transient in OIND. Our results further suggest that antibodies to MOG-Ig arise early in the course of multiple sclerosis, whereas antibodies to MBP accumulate over time.

**Methods**

**CSF and serum samples**

Paired CSF and serum samples were obtained between 1993 and 1998 during diagnostic or therapeutic lumbar puncture from patients with given informed consent, and were stored at –20°C until use. All CSF samples were routinely analysed for mononuclear cell count, albumin-quotients (Q-Alb), CSF oligoclonal bands and IgG-indices (Link and Tibbling, 1977; McLean et al., 1990). The clinical and CSF data of all patients analysed in this study are shown in Table 1. Multiple sclerosis patients were diagnosed according to the criteria of Poser and colleagues (Poser et al., 1983) and the degree of disability was scored using the expanded disability status scale (EDSS; Kurtzke, 1983). The multiple sclerosis cohort (130 patients, age range 13–72 years) included 50 patients with relapsing–remitting disease course (multiple sclerosis-RR, age range 14–70 years) and 38 patients with chronic progressive disease course (multiple sclerosis-CP, age range 16–72 years). We have also included 42 patients with their first attack of multiple sclerosis (laboratory-supported definite multiple sclerosis; multiple sclerosis-R0, age range 13–59 years), all of whom have later developed clinically definite multiple sclerosis. None of the multiple sclerosis patients analysed in this study had received either corticosteroids and/or other immunosuppressive treatment in the 6 months prior to CSF and serum sampling.

Thirty-two patients with OIND (age range 12–89 years) and 30 patients with ONND (age range 12–80 years) served as controls. Patients with OIND included 16 patients with viral neurological diseases (viral meningitis, 10 patients; meningoencephalitis, 4 patients; and encephalitis, 2 patients); and 16 patients with bacterial neurological diseases (bacterial meningitis, 7 patients; and meningoencephalitis, 9 patients). OIND patients included patients with headaches (13 patients); back pain (4 patients); cerebrovascular disease (3 patients); amyotrophic lateral sclerosis (2 patients); dementia (2 patients); psychosis (2 patients); cerebral neoplasm (1 patient); non-inflammatory neuropathy (1 patient); head trauma (1 patient) and Parkinson’s disease (1 patient). The clinical and CSF data of all controls analysed in this study are also shown in Table 1.

Ten patients with rheumatoid arthritis served as a control for a chronic systemic inflammatory disease without CNS involvement (Table 1).

Ten multiple sclerosis patients seropositive for anti-MOG-Ig antibodies (3 multiple sclerosis-R0, 4 multiple sclerosis-RR and 3 multiple sclerosis-CP patients) have been reinvestigated between 9 and 43 months (mean 21.2 ± 10.6 months) after...
Antibodies to MOG-Ig and MBP in multiple sclerosis

first analysis. At the time of this second investigation 7 patients had multiple sclerosis-RR and 3 patients had multiple sclerosis-CP disease courses. Six anti-MOG-Ig seropositive OIND patients (2 patients with viral and 4 patients with bacterial inflammation) have also been reinvestigated between 0.5 and 23 months (mean 8.6 ± 9.2 months) after first analysis.

Antigens and antibodies

A 102-amino acid long N-terminal MOG peptide (amino acids 28–130) including the MOG immunoglobulin superfamily domain (without the leader peptide sequence) was expressed in the QuiaExpress Type III expression system (Qiagen, Hilden, Germany). The recombinant protein contains six histidine residues fused to the C-terminus of MOG-Ig in order to allow affinity purification on a Ni-NTA agarose column (Qiagen). Briefly, the coding cDNA sequence (GenBank Accession X74511) was amplified from a human brain cDNA by the polymerase chain reaction using primers introducing artificial PstI and BglII restriction sites (5'-GGGCTGCAGGGCAGTTCAGAGTG- I and BglII/PstI digested PQE50 and BglII/BglII digested pQE17 plasmid vector arms, thus creating a MOG-Ig expression plasmid. Ligated plasmids were then used for transformation of Escherichia coli M15 host-cells. The identity of MOG-Ig expression plasmids was verified by sequence analysis. Expression and affinity purification of the recombinant protein was performed as recommended by the manufacturer Briefly, recombinant MOG-Ig was affinity purified on Ni-NTA agarose columns (Qiagen) under denaturing conditions in urea-buffer (8 M urea, 100 mM NaH2PO4, 10 mM Tris–HCl), pH 8.0. Bound fusion proteins were eluted by a shift in pH from 8 to 4.5. Fractions containing recombinant MOG-Ig were pooled and applied at a Mono-SP-cationic exchange column (Pharmacia, Uppsala, Sweden) in urea-buffer, pH 4.5. After washing with 100 mM NaCl in urea-buffer, pH 4.5, bound MOG-Ig fusion proteins were eluted with a linear NaCl-gradient from 200 to 500 mM in urea-buffer, pH 4.5. Fractions containing MOG-Ig were pooled, dialysed against 4 M urea, 100 mM NaH2PO4, 10 mM Tris–HCl, pH 4.5, and stored at –20°C.

As a control we have used recombinant mouse dihydro folate reductase encoded by the control plasmid pQE-16 (Qiagen). Dihydrofolate reductase was produced and purified according to the manufacturer’s instructions.

MBP was purified from normal human brain according to the procedure of Eylar and colleagues (Eylar et al., 1979). Purity of the MOG-Ig and MBP preparations was confirmed by SDS (sodium dodecyl sulphate)–PAGE (polyacrylamide gel electrophoresis) and Western blot analysis using the following monoclonal antibodies: anti-MBP 130–137 (Boehringer–Mannheim, Mannheim, Germany) and anti-MOG 8.18-C5 (Linington et al., 1984).

Western blot analysis and ELISA

Western blot analysis was performed using standard methods. Briefly, either 1 µg recombinant MOG-Ig or 2 µg MBP was loaded per lane and separated in 14% SDS-polyacrylamide gels (Novex, San Diego, Calif., USA). Separated proteins were electrotransferred to nitrocellulose membranes (Pharmacia). Efficiency of transfer was monitored by use of the precasted low range SDS–PAGE standard (Bio-Rad, Hercules, Calif., USA) and by staining of the filters with Ponceau S (Sigma, St Louis, Mo., USA) after transfer. Blots were blocked with 2% milk powder in PBS (phosphate-buffered saline) containing 0.05% Tween-20 (PBS-T). Blots were then dried, cut into 2 mm nitrocellulose strips using a membrane cutter (Novex) and probed with

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Females</th>
<th>Age (years)*</th>
<th>Disease duration (years)*</th>
<th>EDSS*</th>
<th>CSF oligoclonal bands</th>
<th>Q-Alb*</th>
<th>IgG-index*</th>
<th>CSF cell count*/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-R0</td>
<td>42</td>
<td>34 (81%)</td>
<td>33 (12)</td>
<td>0.1 (0.2)</td>
<td>2 (1)</td>
<td>42 (100%)</td>
<td>5 (3)</td>
<td>1.1 (0.5)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>MS-RR</td>
<td>50</td>
<td>33 (66%)</td>
<td>33 (10)</td>
<td>4.9 (3.6)</td>
<td>2 (1)</td>
<td>50 (100%)</td>
<td>6 (2)</td>
<td>1.1 (0.7)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>MS-CP</td>
<td>38</td>
<td>25 (66%)</td>
<td>48 (12)</td>
<td>12.0 (9.7)</td>
<td>4 (2)</td>
<td>38 (100%)</td>
<td>8 (5)</td>
<td>1.2 (0.8)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>All multiple sclerosis</td>
<td>130</td>
<td>92 (71%)</td>
<td>37 (13)</td>
<td>5.4 (7.3)</td>
<td>3 (2)</td>
<td>130 (100%)</td>
<td>6 (3)</td>
<td>1.1 (0.7)</td>
<td>7 (11)</td>
</tr>
<tr>
<td>bIND</td>
<td>16</td>
<td>6 (38%)</td>
<td>47 (26)</td>
<td>&lt;1</td>
<td>–</td>
<td>2/3 (66%)</td>
<td>47 (45)</td>
<td>0.7 (0.1)</td>
<td>2528 (6192)</td>
</tr>
<tr>
<td>vIND</td>
<td>16</td>
<td>6 (38%)</td>
<td>40 (19)</td>
<td>&lt;1</td>
<td>–</td>
<td>4/5 (80%)</td>
<td>11 (6)</td>
<td>0.8 (0.9)</td>
<td>131 (107)</td>
</tr>
<tr>
<td>All OIND</td>
<td>32</td>
<td>12 (38%)</td>
<td>44 (23)</td>
<td>&lt;1</td>
<td>–</td>
<td>6/8 (75%)</td>
<td>29 (37)</td>
<td>0.7 (0.6)</td>
<td>1330 (4477)</td>
</tr>
<tr>
<td>ONND</td>
<td>30</td>
<td>14 (47%)</td>
<td>40 (14)</td>
<td>&lt;1</td>
<td>–</td>
<td>0/16 (0%)</td>
<td>5 (2)</td>
<td>0.5 (0.1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>10</td>
<td>9 (90%)</td>
<td>63 (11)</td>
<td>&gt;1</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

n = number of patients; EDSS = expanded disability status scale; Q-Alb = albumin-quotient; MS-R0 = multiple sclerosis, first attack; MS-RR = multiple sclerosis, relapsing–remitting disease course; MS-CP = multiple sclerosis, chronic progressive disease course; bIND = bacterial inflammatory neurological diseases; vIND = viral inflammatory neurological diseases; OIND = other inflammatory neurological diseases; ONND = other non-inflammatory neurological diseases; NA = CSF not available. *Mean values with standard deviations given in parenthesis.

Table 1 Clinical and CSF data of analysed patients
diluted human sera (1:1000 in 2% milk powder in PBS-T) or CSFs (1:10 in 2% milk powder in PBS-T, or if necessary normalized for CSF IgG) overnight at 4°C. Approximately 10 µg of serum IgG and 5 µg of CSF IgG were used for Western blot analysis. Nitrocellulose strips were then washed three times with PBS-T and incubated with alkaline phosphatase conjugated anti-human IgG (JGH055003; Axell, Westbury, NY, USA) or anti-mouse IgG (JGM055003; Axell) for 1 h at room temperature. All antibodies were diluted 1:5000 in 2% milk powder in PBS-T. After washing, bound antibodies were detected by p-nitro-blue tetrazolium chloride (Boehringer–Mannheim) and 5-bromo-4-chloro-3-indolyl phosphate (Boehringer–Mannheim) in 100 mM Tris–HCl, 50 mM MgCl₂, 100 mM NaCl pH 9.5.

ELISA was performed using 96-well Nunc-Immuno Maxisorp microtiter plates (Nunc, Roskilde, Denmark) coated with 100 µl of a 10 µg/ml recombinant MOG-Ig solution in PBS overnight at 4°C (1 µg/well). Plates were then washed six times with PBS-T and blocked with 3% BSA (bovine serum albumin) (Sigma) in PBS for 2 h at room temperature. After washing, 100 µl of diluted CSF (1:5) or serum (1:500 for IgG), both diluted in 0.5% BSA in PBS-T, were added and incubated for 1 h at room temperature. After washing, 100 µl of diluted alkaline phosphatase conjugated anti-human IgG antibodies (see above), diluted in 0.5% BSA in PBS-T, were added and incubated for 1 h at room temperature. Reaction products were visualized with p-nitrophenylphosphate (Sigma) in 1 M diethanolamine, 5 mM MgCl₂, pH 9.8, and the optical density determined at 405 nm. Control wells were coated with BSA and all data were corrected by subtraction of these background values. End-point titres of Western blot positive sera and CSFs were measured by ELISA using serial dilutions of sera (1:500 to 1:64 000 for IgG) and CSFs (1:5 to 1:640).

**Statistical evaluation**

Statistical analysis (means, standard deviations, medians) and significance of group differences (χ² and Mann–Whitney U tests) were evaluated using the SPSS statistical analysis program (SPSS Inc., Chicago, Ill., USA). P values < 0.05 were considered as statistically significant.

Anti-MOG-Ig specific IgG-indices were calculated in a similar way to the total IgG-index (Link and Tibbling, 1977) using the following formula: (CSF titre/serum titre)/(CSF albumin/serum albumin).

**Results**

**Differential serum antibody responses to MOG-Ig and MBP in multiple sclerosis patients and other neurological diseases**

We screened the sera of 130 multiple sclerosis and 72 control patients for IgG autoantibodies to recombinant human MOG-Ig by Western blot analysis. Specificity of our assay was confirmed by control experiments using the pre-absorption of sera with a similarly prepared recombinant control protein. Since antibody responses to MBP also occur in multiple sclerosis patients (Paterson et al., 1981; Newcombe et al., 1985; Olsson et al., 1990; Warren and Catz, 1994; Sellebjerg et al., 1995) we used human myelin derived MBP for comparative studies. Antibodies bound to MOG-Ig or MBP were visualized with alkaline phosphatase-conjugated anti-human or anti-mouse IgG and colorimetric substrates for detection (see Methods). Molecular weights standards (kilodaltons) are shown at the left.

As shown in Table 2, the frequency of anti-MOG-Ig antibody responses in multiple sclerosis patients was comparable with that seen in OIND. However, the frequency of patients seropositive for an anti-MBP antibody response was significantly lower in multiple sclerosis patients than in OIND. In contrast, almost all ONND were negative for anti-MOG-Ig serum antibodies, but 23% of these patients exhibited an anti-MBP antibody response. Therefore, in multiple sclerosis the antibody response to these two myelin antigens is dominated by the anti-MOG-Ig response, in OIND the frequency of anti-MOG-Ig and anti-MBP responses is approximately equal, whereas in ONND the immune response is dominated by MBP.

Finally we were interested in whether these antibody responses observed in CNS inflammation are also present in chronic systemic inflammatory diseases without CNS involvement. Therefore we analysed serum samples from patients with rheumatoid arthritis. Interestingly only one out of 10 of this control group was seropositive for anti-MOG-Ig antibodies, whereas six patients were seropositive for anti-
Antibodies to MOG-Ig and MBP in multiple sclerosis

Table 2  **Frequencies of serum IgG antibodies to MOG-Ig and MBP**

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Anti-MOG-Ig</th>
<th>Anti-MBP</th>
<th>Anti-(MOG-Ig + MBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>130</td>
<td>49 (38%)</td>
<td>36 28%</td>
<td>14 (11%)</td>
</tr>
<tr>
<td>OIND</td>
<td>32</td>
<td>17 (53%)</td>
<td>15 (47%)</td>
<td>9 (28%)</td>
</tr>
<tr>
<td>ONND</td>
<td>30</td>
<td>1 (3%)</td>
<td>7 (23%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>10</td>
<td>1 (10%)</td>
<td>6 (60%)</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>

The number and percentage of positive sera reacting with recombinant human MOG-Ig and MBP purified from neurologically healthy human brain was determined by Western blot analysis (see Methods). Group differences were calculated using the $\chi^2$ test; $P$ values < 0.05 were considered as statistically significant. Values which are significantly different from each other are linked by a square bracket. $n =$ number of patients analysed.

Table 3  **Serum IgG antibodies to MOG-Ig and MBP—longitudinal studies**

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Months*</th>
<th>Anti-MOG-Ig</th>
<th>Anti-MBP</th>
<th>Anti-(MOG-Ig + MBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis, 1st analysis</td>
<td>10</td>
<td>0</td>
<td>10 (100%)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Multiple sclerosis, 2nd analysis</td>
<td>10</td>
<td>21 (11)</td>
<td>10 (100%)</td>
<td>6 (60%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>OIND, 1st analysis</td>
<td>6</td>
<td>0</td>
<td>6 (100%)</td>
<td>3 (50%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>OIND, 2nd analysis</td>
<td>6</td>
<td>9 (9)</td>
<td>0 (0%)</td>
<td>1 (17%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

The number and percentage of positive sera reacting with recombinant human MOG-Ig and MBP purified from neurologically healthy human brain were determined by Western blot analysis (see Methods). $n =$ number of patients analysed; months = time of second analysis in months. *Mean values with standard deviations given in parenthesis.

MBP antibodies (Table 2) indicating that in non-neurological inflammation anti-MBP antibodies are more common than antibodies to MOG-Ig.

**The antibody response to MOG-Ig persists in multiple sclerosis, but is transient in OIND**

Antibody responses to MOG-Ig and MBP were analysed in follow-up sera from anti-MOG-Ig seropositive patients with multiple sclerosis and OIND by Western blot analysis. As shown in Table 3 the anti-MOG-Ig response persisted in the sera of all 10 multiple sclerosis patients available for this study, and an additional 30% of the patients became seropositive for MBP. In contrast, the anti-MOG-Ig antibody response was no longer detectable in OIND sera after a mean interval of 9 months.

**Frequencies of serum antibodies to MOG-Ig and MBP in different multiple sclerosis subgroups**

The antibody response to MOG-Ig was subsequently analysed in patients stratified for the defined subsets of multiple sclerosis (Table 4). Whereas the frequency of anti-MOG-Ig antibody positive patients is similar in multiple sclerosis-R0, multiple sclerosis-RR and multiple sclerosis-CP, there are clear differences between these multiple sclerosis subgroups regarding the anti-MBP antibody response. Anti-MBP antibody responses are low in early multiple sclerosis and increase significantly with disease duration and conversion to either multiple sclerosis-RR or multiple sclerosis-CP. Antibody responses in multiple sclerosis-R0 are therefore dominated by the anti-MOG-Ig response, whereas the incidence of anti-MBP antibodies increases with time.

We have then used a human MOG-Ig specific ELISA to determine end-point titres of anti-MOG-Ig Western blot positive sera. Both assays showed similar sensitivities (1 ng of anti-MOG-Ig specific antibodies). Serum anti-MOG-Ig antibody titres ranged from 1 : 1000 to 1 : 32000 (Fig. 2). However, anti-MOG-Ig titres are comparable in all groups analysed indicating that titres do not increase with multiple sclerosis disease severity.

**Intrathecal synthesis of anti-MOG-Ig autoantibodies**

In view of the reported compartmentalization of anti-myelin antibody responses in the CNS of multiple sclerosis patients (Moller et al., 1989; Olsson et al., 1990; Baig et al., 1991b; Sun et al., 1991; Xiao et al., 1991; Warren and Catz, 1994; Sellebjerg et al., 1995) we analysed the CSFs obtained from patients with multiple sclerosis, OIND and ONND for the presence of MOG-Ig specific IgG antibodies by Western blotting (Table 5). Titres of anti-MOG-Ig antibody positive patients were subsequently determined by ELISA (Fig. 2).

In general, the frequency of the anti-MOG-Ig response in multiple sclerosis and OIND was similar in CSF and serum suggesting that the CSF antibody response to MOG-Ig is related to that seen in the patients’ sera. The highest frequency of CSF anti-MOG-Ig antibodies was seen in OIND, whereas ONND CSF was uniformly negative for anti-MOG-Ig antibodies. In order to determine whether in multiple sclerosis
Table 4 Serum IgG responses to MOG-Ig and MBP in multiple sclerosis subgroups

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Anti-MOG-Ig</th>
<th>Anti-MBP</th>
<th>Anti-(MOG-Ig + MBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-R0</td>
<td>42</td>
<td>15 (36%)</td>
<td>5 (12%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>MS-RR</td>
<td>50</td>
<td>18 (36%)</td>
<td>16 (32%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>MS-CP</td>
<td>38</td>
<td>16 (42%)</td>
<td>15 (40%)</td>
<td>8 (21%)</td>
</tr>
</tbody>
</table>

The number and percentage of positive sera reacting with recombinant human MOG-Ig and MBP purified from neurologically healthy human brain was determined by Western blot analysis (see Methods). Group differences were calculated using the χ² test; P values < 0.05 were considered as statistically significant. Values which are significantly different from each other are linked by a square bracket. n = number of patients analysed.

Table 5 Frequencies of CSF IgG antibodies to MOG-Ig

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Anti-MOG-Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-R0</td>
<td>42</td>
<td>14 (33%)</td>
</tr>
<tr>
<td>MS-RR</td>
<td>50</td>
<td>16 (32%)</td>
</tr>
<tr>
<td>MS-CP</td>
<td>38</td>
<td>13 (34%)</td>
</tr>
<tr>
<td>All multiple sclerosis</td>
<td>130</td>
<td>43 (33%)</td>
</tr>
<tr>
<td>OIND</td>
<td>32</td>
<td>17 (53%)</td>
</tr>
<tr>
<td>ONND</td>
<td>30</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

The number and percentage of positive CSFs reacting with recombinant human MOG-Ig was determined by Western blot analysis (see Methods). Group differences were calculated using the χ² test; P values < 0.05 were considered as statistically significant. Values which are significantly different from each other are linked by a square bracket. n = number of patients analysed.

Fig. 2 Serum (A) and CSF (B) IgG antibody titres to human recombinant MOG-Ig in multiple sclerosis patients with first attack (MS-R0; n = 15), relapsing–remitting disease course (MS-RR; n = 18), chronic progressive disease course (MS-CP; n = 16) and patients with other inflammatory neurological diseases (OIND; n = 17). Anti-MOG-Ig end-point titres of Western blot positive sera were determined by ELISA (see Methods). Median titres are displayed by bars, individual titres are indicated by open circles.

The synthesis of anti-MOG-Ig antibodies is to any extent compartmentalized within the CNS, we compared MOG-Ig specific IgG-indices, total IgG-indices, CSF cell counts and Q-Albs for CSF anti-MOG-Ig IgG positive and negative patients (Table 6). MOG-Ig specific and total IgG-indices were significantly higher in CSF anti-MOG-Ig positive multiple sclerosis patients than in their CSF anti-MOG-Ig positive OIND counterparts, whereas Q-Albs and CSF cell counts were significantly higher in OIND. CSF anti-MOG-Ig IgG antibodies in multiple sclerosis are therefore strongly associated with high anti-MOG-Ig specific and total IgG-indices indicating intrathecal anti-MOG-Ig antibody synthesis, whereas in OIND CSF anti-MOG-Ig antibodies are associated with pathological CSF mononuclear cell numbers and Q-Albs indicating extensive blood–brain barrier damage and leakage from the peripheral blood. In contrast, the CSF anti-MOG-Ig positive multiple sclerosis patients had significantly higher IgG-indices and CSF cell counts than CSF anti-MOG-Ig negative multiple sclerosis patients, whereas the Q-Alb values were comparable.

Discussion

Neuropathological findings suggest that antibodies play an important role in lesion formation in a subset of multiple sclerosis patients (Prineas and Graham, 1981; Prineas, 1985; Gay and Esiri, 1991; Ozawa et al., 1994; Lucchinetti et al., 1996; Storch et al., 1998a), but their target specificity is unknown. Autoantibodies recognizing several myelin proteins including MBP (Paterson et al., 1981; Newcombe et al., 1985; Olsson et al., 1990; Warren and Catz, 1994; Sellebjerg et al., 1995), proteolipid protein (Warren and Catz, 1994; Sellebjerg et al., 1995), myelin-associated glycoprotein (Moller et al., 1989; Baig et al., 1991b) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (Walsh and Murray, 1998) are present in multiple sclerosis patients, but their role in disease pathogenesis is enigmatic and controversial. Animal models and in vitro studies failed to demonstrate a pathogenic
role for autoantibody responses against any of these myelin proteins. Antisera raised against MBP (Mithen et al., 1982a, b), proteolipid protein (Mithen et al., 1980; Seil and Agrawal, 1980) or myelin-associated glycoprotein (Seil et al., 1981) are unable to mediate demyelination either in vivo or in myelinating organotypic CNS cultures in vitro. In contrast MOG specific autoantibodies mediate demyelination in vitro (Kerlero de Rosbo et al., 1990) and in vivo (Schluesener et al., 1987; Linington et al., 1988). In rodent and primate models of experimental autoimmune encephalomyelitis, MOG is the immunodominant target for this demyelinating autoantibody response, which is directed against the extracellular Ig domain of the protein (Adelmann et al., 1995; Genain et al., 1995). However, although the pathogenic role of the anti-MOG antibody response is well established in animal models, there is as yet no evidence that this is also the case in multiple sclerosis.

MOG specific autoantibody and B-cell responses have been reported in multiple sclerosis, but their epitope specificity, in particular whether or not they recognized the pathogenic Ig domain, was not described (Sun et al., 1991; Xiao et al., 1991). However, a recent study has demonstrated the association of MOG specific autoantibodies with myelin debris in multiple sclerosis lesions, indicating that they may well be involved in lesion formation (Genain et al., 1999). In the present study we demonstrate that a substantial subset of multiple sclerosis patients mount a sustained autoantibody response to the extracellular Ig domain of MOG that may be directly involved in disease pathogenesis.

This MOG-Ig specific antibody response is, however, not a specific feature of multiple sclerosis, as similar frequencies and titres are present in the sera and CSFs of patients with acute CNS inflammation. Acute viral or bacterial CNS diseases are monophasic, which correlates with the observed transient autoantibody response to both MOG-Ig and MBP. In contrast, multiple sclerosis was characterized by a persistent anti-MOG-Ig antibody response suggesting that a chronic inflammatory response is necessary to sustain this autoantibody response.

Intriguingly, the characteristics of the antibody response to MOG-Ig differed dramatically from the response to MBP. Unlike the anti-MOG-Ig response that was associated with multiple sclerosis and OIND, anti-MBP antibodies were also found in patients with non-inflammatory CNS diseases. Moreover, the anti-MOG-Ig antibody response appears to be established early in the course of multiple sclerosis and does not then differ significantly between the different patient subsets. In contrast, the anti-MBP response appears to accumulate with time. Our finding of enhanced anti-MBP antibody responses in advanced multiple sclerosis, OIND, ONND and rheumatoid arthritis is comparable with previous reports (Frick and Stickl, 1980; Paterson et al., 1981; Newcombe et al., 1985; Baig et al., 1991a; Solders et al., 1992; Wang et al., 1992; Olsson et al., 1993) indicating that an anti-MBP antibody response is a relatively common sequel to any CNS injury. Moreover, even in our small number of patients with chronic systemic inflammatory disease we could demonstrate a common antibody response to MBP but not MOG-Ig. However, although our data indicate that anti-MOG-Ig antibodies tend to be more associated with CNS inflammation than anti-MBP antibodies, further studies are needed to confirm this particular observation.

The differential humoral responses to MOG-Ig and MBP observed in this study may reflect differences in antigen concentration, sequestration by the blood–brain barrier and immunogenicity. MBP accounts for approximately 30% of the CNS myelin membrane protein, whereas MOG is a minor component of the myelin sheath (0.01–0.05% of the total membrane protein; Brunner et al., 1989). In addition, MBP is not a CNS myelin specific protein; it is also expressed in the peripheral nervous system and cells of the immune system (Pribyl et al., 1996). In contrast, MOG is exclusively expressed in oligodendrocytes (Gardinier et al., 1992) and is therefore sequestered from the immune system by the endothelial blood–brain barrier and lack of direct lymphoid drainage from the CNS compartment. However, it is apparent that immune sequestration does not render MOG uniformly immunogenic in all patients, as only 40–50% of multiple sclerosis and OIND samples were anti-MOG-Ig antibody positive.

The association of a sustained anti-MOG-Ig antibody response with multiple sclerosis is intriguing, but the demyelinating potential of this response has still to be proven. Moreover, the pathogenic potential of anti-MOG antibodies

Table 6 Evidence for intrathecal synthesis of IgG antibodies to MOG-Ig in multiple sclerosis

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>CSF cell count/µl*</th>
<th>Q-Alb*</th>
<th>Total IgG-index*</th>
<th>Anti-MOG-Ig IgG-index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis, negative</td>
<td>87 (67%)</td>
<td>6 (12)</td>
<td>6.0 (3.6)</td>
<td>1.0 (0.6)</td>
<td>–</td>
</tr>
<tr>
<td>Multiple sclerosis, positive</td>
<td>43 (33%)</td>
<td>8 (9)</td>
<td>6.3 (3.2)</td>
<td>1.4 (0.8)</td>
<td>1.6 (1.0)</td>
</tr>
<tr>
<td>OIND, positive</td>
<td>17 (53%)</td>
<td>2211 (6035)</td>
<td>44.9 (44.7)</td>
<td>0.8 (0.8)</td>
<td>0.6 (0.5)</td>
</tr>
<tr>
<td>OIND, negative</td>
<td>15 (47%)</td>
<td>331 (856)</td>
<td>10.6 (5.8)</td>
<td>0.6 (0.1)</td>
<td>–</td>
</tr>
</tbody>
</table>

The numbers and percentage of positive CSFs reacting with recombinant human MOG-Ig were determined by Western blot analysis, and total IgG-indices and specific anti-MOG-Ig IgG-indices were calculated (see Methods). Group differences were calculated using the Mann–Whitney U test; P values < 0.05 were considered as statistically significant. Values which are significantly different from each other are linked by a square bracket. n = number of patients analysed. *Mean values with standard deviations given in parenthesis.

Antibodies to MOG-Ig and MBP in multiple sclerosis
may be critically dependent on synergy with a T-cell mediated inflammatory response in the CNS. Circulating pathogenic anti-MOG antibodies are unable to induce demyelination per se. Only when blood–brain barrier permeability is enhanced can antibody enter the CNS, bind to the myelin surface and initiate demyelination (Litzenburger et al., 1998). In animal models, an encephalitogenic T-cell response provides the ‘key’ that opens the blood–brain barrier to both serum proteins and activated macrophages. The presence of both T-cell (Kerlero de Rosbo et al., 1993; Wallström et al., 1998) and antibody responses to MOG in multiple sclerosis patients suggests that a similar pathomechanism may be involved in demyelination in the human disease. However, it should be pointed out that any encephalitogenic T-cell response, irrespective of its antigen specificity, will modulate blood–brain barrier function. Furthermore, it is unlikely that MOG is the only autoantigen expressed on the myelin surface that can be targeted by a pathogenic autoantibody response in multiple sclerosis. Additional studies are required to identify the full potential range of autoantigen targets in multiple sclerosis and the effector mechanisms involved in lesion formation.

In summary, in this study we demonstrate an enhanced and persistent anti-MOG-Ig antibody response in a subgroup of multiple sclerosis patients that may play an important role in lesion formation in these multiple sclerosis patients. These findings are consistent with recent reports demonstrating antibody and complement precipitation in an immunopathologically defined subset of multiple sclerosis patients (Prineas and Graham, 1981; Prineas, 1985; Gay and Esiri, 1991; Ozawa et al., 1994; Lucchinetti et al., 1996; Storch et al., 1998a). Further studies are required to determine whether or not the presence of anti-MOG-Ig antibodies in early multiple sclerosis may provide a useful paraclinical marker identifying patients for whom therapies targeting the B-cell arm of the immune response may be beneficial.

Acknowledgements
We wish to thank all our patients who contributed most to this study, Dr Johann Gruber, Department of Internal Medicine, University of Innsbruck, for the gift of rheumatoid arthritis sera and Ingrid Gstrein for excellent technical assistance. This study was supported by the European Union (Biomed 2), the DFG, SFB 217, Teilprojekt C14. C.L. holds a Hermann–Lilly Stiftungs professorship.

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Received March 17, 1999. Revised May 5, 1999.
Accepted May 26, 1999