PAPER

Antimyelin antibodies and the risk of relapse in patients with a primary demyelinating event

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Aim: To investigate whether the presence of serum antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) in patients with a clinically isolated syndrome (CIS) predicts the interval to develop more frequently and earlier a first relapse (clinically definite multiple sclerosis: CDMS) than seronegative patients.

Methods: Sera from 45 patients with a CIS and positive intrathecal IgG-synthesis were retrospectively tested for the presence of IgM antibodies against both MOG and MBP. Antibodies were detected by immunoblot using recombinant MOG (1–125) and human MBP antigen preparations. Clinical follow ups were performed retrospectively by telephone interviews and documented neurological examination.

Results: Using the Cox proportional hazards model there was no significant increased risk for developing CDMS in anti-MOG and anti-MBP positive patients compared with negative. However regarding the median of the time span between CIS and CDMS over the whole follow up, antibody positive patients (MOG/MBP +/+) developed significantly earlier relapses (median 5.5 months (range 3–20)) than the antibody negative ones (median 25.0 months (range 7–43); $p < 0.006$). On testing sera from 56 apparently healthy students, quite high frequencies of anti-MOG and anti-MBP antibodies (21% and 28% respectively) were detected. This limited specificity of anti-MOG and anti-MBP antibodies has been seen earlier and restricts their diagnostic relevance in MS despite their role as a predictor of relapses after a CIS.

Conclusions: This study confirms previous data only in a subanalysis indicating that patients with positive anti-MOG/MBP antibodies develop earlier relapses than patients who are antibody negative. However, the authors could not verify that the presence of these antibodies anticipates the overall risk of developing CDMS—according to study criteria—after a first demyelinating event within the study period of 21–106 months (mean 60 (SD 25)).

Multiple sclerosis (MS) is a common inflammatory neurological disease, predominantly affecting young adults.¹ It usually starts with a clinically isolated syndrome (CIS), caused by an inflammatory demyelinating lesion of the central nervous system. About 30% of patients with a CIS exhibit a second demyelinating event with dissemination within 12 months, leading to the diagnosis of clinically definite multiple sclerosis (CDMS).^{2–4}

The pathogenesis of MS is not completely understood. Apart from the evidence that myelin-specific T cell responses are crucial to disease pathogenesis, it is suggested that B cells and autoantibodies may also play a major role in the process of demyelination.^{5–8}

According to a recent study by Berger *et al.*,⁹ seropositivity for myelin oligodendrocyte glycoprotein (MOG) and/or myelin basic protein (MBP) in patients with a CIS was associated with a markedly increased risk of a first relapse during the study follow up (mean 50.9, range 12–96). In the present study, the incidence of anti-MOG and anti-MBP IgM antibodies was investigated retrospectively in a cohort of patients with a CIS. The main issue of this study was to investigate whether MOG and MBP positive CIS patients develop more frequently and earlier a first relapse than seronegative patients. In addition, to further examine the diagnostic specificity of these antibodies, incidences of MOG and MBP antibodies were analysed in a group of healthy individuals not suffering from any inflammatory disease.

MATERIAL AND METHODS

Patients

Patients aged 16–55 years were screened retrospectively in 2004 on the basis of our cerebrospinal fluid (CSF) data bank.

CSF, serum, and clinical data were collected in the time between November 1995 and December 2002. Inclusion criteria for this retrospective analysis were as follows:

- Clinical evidence of a first demyelinating event. Patients with any previous neurological symptoms in their case history that might suspect an earlier demyelinating event were excluded, as well as those patients with primary progressive disease course. The period between the beginning of clinical symptoms and lumbar puncture must not have exceeded four months.
- Intrathecal IgG synthesis (oligoclonal bands (OCB) or elevated IgG index (>0.8) according to the Reiber formula¹⁰), but without CSF evidence of infection (CSF cell count $<50/\mu\text{l}$, CSF protein $<950\text{ mg/l}$).
- Typical demyelinating lesions or normal MRI.¹¹
- No disease modifying treatment given before the second demyelinating event (CDMS).
- There must be no other explanation for the neurological symptoms than a demyelinating disease.

Data collection

Patients who gave informed consent were interviewed by telephone with respect to their clinical follow up using a standardised questionnaire. The main focus of the interview was the assessment of relapses and the time period between

Abbreviations: CDMS, clinically definite multiple sclerosis; CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; OCB, oligoclonal bands.

CIS and the second demyelinating event. A relapse was defined by new neurological symptoms occurring at least 30 days after the CIS and lasting not less than 24 hours. If relapse and CIS revealed equal neurological symptoms, paraclinical evidence (for example, visual evoked potentials) of a dissemination in space according to the McDonald criteria¹² was required for defining CDMS. The relapse had to be confirmed by a documented neurological examination.

Controls

Sera from 56 students (F/M 38/18, age range 21–25 years) without overt neurological disease were taken as controls. They were not age and sex matched with the patient group.

Sera

Sera from patients and controls were stored at -80°C prior to investigation.

Immunological methods

Antigens

Recombinant MOG_(1–125) antigen was expressed and purified as earlier described.¹³ Human MBP antigen (Biodesign, Kennebunk, USA) was purified according to G Diebler.¹⁴

SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed by a modified method of Schägger and von Jagow¹⁵ on a Mini-PROTEAN II cell (Bio Rad, Hercules, USA) using a 12% separating gel and a 4% stacking gel. The amounts of antigens were as follows: Human MBP 100 $\mu\text{g/gel}$. Recombinant MOG 15 $\mu\text{g/gel}$. The antigens were blotted to a nitrocellulose membrane (PROTRAN, Schleicher und Schuell, Dassel, Germany).

Western blot was performed by standard methods as described by Towbin *et al.*^{15–16} Sera were diluted in phosphate buffered saline containing 2% skimmed milk (PBS-M) 1:100 for the detection of IgM antibodies for both antigens. Sera were incubated overnight at 4°C on a shaker. Immunodetection was done with peroxidase conjugated rabbit anti-human IgM (Dianova, diluted 1:500 (MBP) and 1:2000 (MOG) respectively in 10% (wt/vol) skimmed milk in PBS). Diaminobenzidine (Sigma, Munich, Germany) was

used as substrate. A monoclonal mouse antibody (anti-MOG 8.18-C5) was used to verify reactivity to recombinant MOG antigen (dilution 1:2000). A polyclonal rabbit-hyperimmune serum (dilution: 1:500) (Myelin Basic Protein Ab-1, NeoMarkers) was used to identify the MBP antigen in immunoblot. The immunoreactivity of the serum samples was judged by three independent investigators, who were blinded for the clinical data. A serum sample was considered to be positive if the immunoreactivity was equal to or greater than that of a control sample. As controls a monoclonal anti MOG antibody (8.18-C5)¹⁷ and a polyclonal rabbit anti human MBP (Myelin Basic Protein Ab-1, NeoMarkers, Fremont, USA) antibody and negative and positive human serum samples were used.

Statistical analysis

The following one way analyses were used to compare patients and controls according to antibody status: χ^2 testing or Kruskal-Wallis testing. The cumulative risk for CDMS was demonstrated by the Kaplan-Meier method. The cox proportional hazards model was used to assess the predictive value of anti-MOG and anti-MBP antibodies in a multivariable analysis, with adjustment for potential confounding variables. The following variables were included in the initial model: age, sex, the disease duration, anti-MOG and MBP antibody status, the logarithms of total CSF cell count, total protein, and the IgG index (calculated according to the formula [cerebrospinal fluid IgG/serum IgG]/[cerebrospinal fluid albumin/serum albumin], with a usual upper limit of 0.65).

RESULTS

Forty five patients fulfilled the inclusion criteria. Mean follow up was 60.4 months (SD 25.1) (range 21–106). Demographic and clinical data are given in table 1.

Twenty eight (62%) patients developed CDMS during the observation period of 21 to 106 months. In 25 patients the relapse was confirmed by a neurologist; in three patients the neurological examination at the time of relapse was performed by a general practitioner. However, unambiguous neurological symptoms of a relapse were documented in all cases on records.

Table 1 Demographic data, clinical symptoms, and relapses of all patients and the groups according to antibody status

Antibody status MOG/MBP	Total	group A (–/–)	group B (+/+)	group C (+/–)	group D (–/+)	p Value
Percent	n = 45 (100%)	n = 14 (31%)	n = 15 (33%)	n = 8 (18%)	n = 8 (18%)	
Female*	31 (69%)	8 (57%)	11 (73%)	5 (62%)	7 (87%)	NS
Age (years)†	31 (7) (16–51)	30 (6)	30 (6)	33 (10)	32 (7)	NS
Time to lumbar puncture (days)†	29 (26) (3–90)	38 (34)	28 (18)	19 (16)	26 (17)	NS
CSF cell count (per μl)†	9 (9) (1–35)	12 (12)	8 (9)	11 (7)	5 (2)	NS
CSF total protein (mg/l)†	409 (150) (196–925)	427 (163)	415 (173)	396 (127)	380 (125)	NS
CSF IgG index‡	1.0 (0.5) (0.5–2.2)	1.0 (0.4)	0.8 (0.3)	1.4 (0.6)	0.8 (0.3)	NS
Symptoms (first event)*						
Sensory	36 (80%)	9 (64%)	12 (80%)	8 (100%)	7 (87%)	NS
Motor	24 (53%)	8 (57%)	9 (60%)	3 (37%)	4 (50%)	NS
Optic neuritis	7 (15%)	4 (28%)	2 (13%)	1 (12%)	0	NS
Cerebellar	6 (13%)	3 (21%)	2 (13%)	0	1 (12%)	NS
Steroid treatment before lumbar puncture*	3 (6%)	2 (14%)	1 (6%)	0	0	NS
Follow up (months)†	60 months (25) (21–106)	63 (29)	59 (23)	64 (23)	54 (26)	NS
Patients with relapse in follow up period	28 (62%)	9 (64%)	8 (53%)	6 (75%)	5 (62%)	NS
Time to relapse (months)§	12.5 (7.5) (3–62)	25.0 (12) (7–43)	5.5 (2.5) (3–20)	19.0 (6) (12–62)	11.0 (7) (3–56)	¶

Group A: anti-MOG negative and anti-MBP negative = (–/–); group B: anti-MOG positive and anti-MBP positive (+/+); group C: anti-MOG positive and anti-MBP negative (+/–); group D: anti-MOG negative and anti-MBP positive (–/+).

*Absolute/frequency.

†Mean (standard deviation) (minimum–maximum)

‡IgG index calculated from (CSF-IgG: serum-IgG): (CSF-albumin: serum-albumin).

§Median, (standard deviation of median) (minimum–maximum).

¶p Values (Kruskal-Wallis test): A/B $p < 0.006$; A/C $p < 0.906$; A/D $p < 0.162$; B/C $p < 0.010$; B/D $p < 0.555$; C/D $p < 0.082$.

Thirty two patients revealed typical demyelinating brain lesions in MRI; seven of those patients showed additional inflammatory lesions in the spinal cord; 10 patients had spinal cord inflammatory lesions only (no brain MRI was available in two of those patients). In two patients brain MRI was normal; one of those patients suffered from clinical symptoms suggesting myelitis, but MRI of the cervical spinal cord was also normal. The other patient suffered from bilateral optic neuritis and paresthesia in the left leg. In another patient with symptoms suspicious for myelitis the MRI of the spinal cord was normal and brain MRI was not performed initially; this patient revealed typical relapses three and 12 months after CIS. The first MRI of the brain was performed 50 months after CIS and revealed typical MS lesions. MRI data revealed no correlation to the anti-myelin antibody status (data not shown).

Positive anti-MOG IgM immunoblots were detected in 23 (51%) of 45 patients and 12 (21%) of 56 controls ($p < 0.05$). Positive anti-MBP IgM immunoblots were seen in 23 (51%) of 45 patients and 15 (27%) of 56 controls ($p < 0.05$). Fifteen (33%) sera of patients and three (5%) of controls were positive for both antibody specificities.

For further investigation patients were divided into four groups, according to their antibody status (group A: anti-MOG negative and anti-MBP negative = $-/-$); group B: anti-MOG positive and anti-MBP positive ($+/+$); group C: anti-MOG positive and anti-MBP negative ($+/-$); group D: anti-MOG negative and anti-MBP positive ($-/+$) (table 1 and fig 1). The demographic and CSF data and the types of symptoms seen at the first demyelinating event were similar among the groups delineated (table 1). Also, the mean observation period and the frequency of conversion to CDMS during the observation period did not differ significantly (table 1).

In a univariate log rank test only a total protein level of more than 500 mg/l was associated with an increased risk of relapse during the study period ($p = 0.0351$). All the other variables tested were negative. Thus, the final cox proportional hazards model included as potential confounding variable only the logarithm of total protein. Using the Cox model there was no significant increased risk for developing a

relapse (CDMS) in anti-MOG and anti-MBP positive patients compared with negative ones ($p > 0.05$). The same was true when anti-MOG and anti-MBP positive patients were analysed separately.

Considering the time span between CIS and CDMS, group B patients developed significantly earlier a relapse than patients in group A ($p < 0.006$) (table 1). Due to low numbers, patients of group C and group D were not included in further analyses.

Twenty sera were analysed independently in the laboratories of Innsbruck and Freiburg to compare reproducibility of the immunoblot; concordance rate for MOG and MBP was 17/20 (85%) for both tests.

DISCUSSION

The objective of the present study was to investigate whether the risk of developing a relapse (CDMS) in patients with suspected MS due to a CIS and positive intrathecal IgG synthesis is associated with seropositivity to anti-MOG and anti-MBP antibodies. According to Cox regression analysis the antibody status did not predict the risk for CDMS in the present cohort during the retrospective study period of 21–106 months (mean 60 (SD 25)) (fig 1). This is in line with a recent paper by Lim *et al*¹⁸ although the study cohorts and designs of both trials differ markedly. The latter authors analysed 47 CIS patients (46 with optic neuritis, no lumbar puncture) prospectively for 12 months and found that anti-myelin antibodies did not predict the development of MS or CDMS within one year after a first demyelinating event. In addition, in our study there was no significant correlation between the antibody status and the risk of CDMS if anti-MOG and anti-MBP antibodies were evaluated separately (data not shown).

Both papers cited above contradict a former study by two of us,⁹ which found that patients with anti-MOG and anti-MBP antibodies had more frequent and earlier relapses than patients without these antibodies. The latter authors detected a striking risk of developing a relapse after a CIS in antibody positive patients (adjusted hazard ratio 76.5 (95% CI 20.6 to 284.6); $p < 0.001$), compared with the antibody negative group. Considering the demographic and clinical data of both investigations—which are roughly comparable—the apparent discrepancy may be partly related to methodical factors: the most substantial difference between both studies comprises the retrospective study design of the present study, compared with the prospective follow up by Berger *et al*. Another difference is related to inclusion criteria: Berger *et al* only included patients who revealed demyelinating lesions in brain MRI whereas in our study 10 patients with exclusively spinal cord lesions and positive OCB in CSF were also recruited; nevertheless, such patients may of course represent an early stage of MS. Removing these patients with isolated myelitis from our study did not lead to an increased association between MOG/MBP seropositivity and relapse rate (data not shown). However, within a follow up of 12 months there was a trend in our study ($p < 0.068$): group B patients (MOG/MBP $+/+$) more frequently developed CDMS than group A patients (MOG/MBP $-/-$) (fig 1). Taken together group A and B contain only 29 patients. Thus, it seems possible that a higher number of patients and a prospective study design may confirm this trend or would even produce significant figures but, according to our present data, it seems not to be expected that these data would reach a striking correlation as reported previously.⁹

In addition we estimated the time span from CIS to CDMS with respect to the antibody status. Anti-MOG/MBP positive patients (group B) developed significantly earlier CDMS (median 5.5 months) than antibody negative patients (group A; median 25.0 months; $p < 0.006$). This result can also be

Figure 1 Kaplan-Meier estimates of the risk of clinically definite multiple sclerosis, according to antibody status. There was no statistical significant difference between the groups ($p > 0.05$). Plus signs denote seropositive and minus signs seronegative. The curves are related to the antibody status groups of table 1 as follows: group A: anti-MOG negative and anti-MBP negative = $-/-$; group B: anti-MOG positive and anti-MBP positive ($+/+$); group C: anti-MOG positive and anti-MBP negative ($+/-$); group D: anti-MOG negative and anti-MBP positive ($-/+$).

drawn as a tendency from the Kaplan-Meier curve considering the time span 0–20 months (fig 1). These data reveal a minor correlation with those of Berger *et al*:⁹ the antibody positive patients developed a relapse after a mean of 7.5 (SD 4.4) months whereas the antibody negative cases revealed the first relapse after a mean of 45.1 (SD 13.7) months ($p < 0.001$). However, the estimation of time spans in our study—though statistically significant—has to be interpreted with caution; due to the small study groups these results may be influenced markedly if the latencies of only a few patients changed.

In order to test the specificity of the antimyelin antibody response, we investigated 56 sera from students without overt neurological or inflammatory disease. Anti-MOG IgM antibodies were found in 12/56 (21%) sera and anti-MBP IgM antibodies were observed in 15/56 (27%) sera. This reveals a diagnostic specificity of 79% for anti-MOG and 73% for anti-MBP antibodies respectively. It has been shown and also stressed earlier that antimyelin antibodies are not specific for multiple sclerosis,^{13–19–21} but limited specificity diminishes further the possible use of these antibodies as surrogate markers in MS.

In conclusion, the anti-MOG/MBP antibody status did not predict the risk for a relapse in patients with a CIS in our study. But in a subanalysis we could verify previous results in that patients with positive anti-MOG/MBP antibodies develop a relapse (CDMS) earlier than patients who are antibody negative. Together with the trial by Lim *et al*¹⁸ this is the second investigation which could not or only partly confirm the findings by Berger *et al*. This raises the question of whether the high correlation between the antimyelin antibody status and the risk of developing CDMS after a CIS was overestimated in the latter trial. Nevertheless, these findings indicate that the establishment of a surrogate marker for prediction of the prognosis in MS requires comparable patient groups and similar study designs. Due to the discrepancies ruled out we suggest that further studies are desirable to evaluate the definite value of antimyelin antibody testing for estimation of clinical prognosis and consecutive decisions on the initiation of early immunomodulatory therapy in patients with suspected MS.

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REFERENCES

- 1 **Noseworthy JH**, Lucchinetti C, Rodriguez M, *et al*. Multiple sclerosis. *N Engl J Med* 2000;**343**:938–52.
- 2 **O'Riordan JI**, Thompson AJ, Kingsley DP, *et al*. The prognostic value of brain MRI in clinically isolated syndromes of the CNS. A 10-year follow-up. *Brain* 1998;**121**:495–503.
- 3 **Jacobs LD**, Kaba SE, Miller CM, *et al*. Correlation of clinical, magnetic resonance imaging, and cerebrospinal fluid findings in optic neuritis. *Ann Neurol* 1997;**41**:392–8.
- 4 **Brex PA**, Ciccarelli O, O'Riordan JI, *et al*. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. *N Engl J Med* 2002;**346**:158–64.
- 5 **Archelos JJ**, Storch MK, Hartung HP. The role of B cells and autoantibodies in multiple sclerosis. *Ann Neurol* 2000;**47**:694–706.
- 6 **Storch MK**, Piddlesden S, Haltia M, *et al*. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. *Ann Neurol* 1998;**43**:465–71.
- 7 **Lucchinetti C**, Bruck W, Parisi J, *et al*. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;**47**:707–17.
- 8 **Lassmann H**, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. *Trends Mol Med* 2001;**7**:115–21.
- 9 **Berger T**, Rubner P, Schautzer F, *et al*. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N Engl J Med* 2003;**349**:139–45.
- 10 **Reiber H**, Felgenhauer K. Protein transfer at the blood cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. *Clin Chim Acta* 1987;**163**:319–28.
- 11 **Fazekas F**, Offenbacher H, Fuchs S, *et al*. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. *Neurology* 1988;**38**:1822–5.
- 12 **McDonald WI**, Compston A, Edan G, *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;**50**:121–7.
- 13 **Reindl M**, Linnington C, Brehm U, *et al*. Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study. *Brain* 1999;**122**:2047–56.
- 14 **Diebler G**, Martenson R, Kies R. Large scale preparation of myelin basic protein from central nervous tissues of several mammalian species. *Prep Biochem* 1972;**2**:39–165.
- 15 **Schägger H**, von Jagow G. Tricine-sodium dodecyl-sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;**166**:368–79.
- 16 **Towbin HH**, Staehelin H, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;**76**:4350–4.
- 17 **Linnington C**, Webb M, Woodhams PL. A novel myelin-associated glycoprotein defined by a mouse monoclonal antibody. *J Neuroimmunol* 1984;**6**:387–96.
- 18 **Lim ET**, Berger T, Reindl M, *et al*. Anti-myelin antibodies do not allow earlier diagnosis of multiple sclerosis. *Mult Scler* 2005;**11**:492–4.
- 19 **Karni A**, Bakimer-Kleiner R, Abramsky O, *et al*. Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis. *Arch Neurol* 1999;**56**:311–5.
- 20 **Lampasona V**, Franciotta D, Furlan R, *et al*. Similar low frequency of anti-MOG IgG and IgM in MS patients and healthy subjects. *Neurology* 2004;**62**:2092–4.
- 21 **Lutterotti A**, Reindl M, Gassner C, *et al*. Antibody response to myelin oligodendrocyte glycoprotein and myelin basic protein depend on familial background and are partially associated with human leukocyte antigen alleles in multiplex families and sporadic multiple sclerosis. *J Neuroimmunol* 2002;**131**:201–7.