Similar low frequency of anti-MOG IgG and IgM in MS patients and healthy subjects

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Abstract—The authors used a liquid-phase radiobinding assay to measure serum anti-myelin oligodendrocyte protein (MOG) immunoglobulin (Ig) G in 87 patients with multiple sclerosis (MS), in 12 patients with encephalomyelitis, and in 47 healthy subjects. Anti-MOG IgM was determined in samples obtained at onset from 40 of 87 patients with MS and in control subjects. The frequency of positive samples with low titers of anti-MOG IgG (≤5.7%) and IgM (≤8.3%) was similar in all the groups and subgroups. Binding competition experiments showed that these antibodies had low affinity. Anti-MOG antibodies are not disease specific.

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Anti-myelin oligodendrocyte glycoprotein (MOG) antibodies induce extensive demyelination in T-cell-mediated experimental autoimmune encephalomyelitis (EAE)[1] and bind to disintegrating myelin in acute multiple sclerosis (MS) and in the marmoset model of EAE.[2] These observations motivate the search for anti-MOG antibodies in MS, in which increased MOG-specific T- and B-cell responses are reported.[3] The reported data have had a negligible impact on patient care, but Berger et al.[4] showed that serum anti-MOG immunoglobulin (Ig) M strongly predicted the early conversion of first demyelinating events to clinically definite MS.

We devised a radiobinding assay (RBA) to measure anti-MOG IgG and IgM in patients with MS, in patients with encephalomyelitis, and in healthy subjects.

Patients and methods. *Patients.* The table shows the demographic characteristics of the 87 patients with definite MS included in the study.[5] All the patients had been drug free for at least 6 months before sample collection, which was performed at onset in 40 and during clinically active disease in another 22 patients. Twenty-nine patients were monosymptomatic at onset. Patients were considered in an active phase of the disease when experiencing an episode of symptoms, documented on neurologic examination, indicative of an abnormality attributable to acute demyelination in one or more regions of the CNS. CSF IgG oligoclonal band frequency was 98%. Sera were also obtained from 12 patients with demyelinating/necrotizing encephalomyelitis (D/NE; acute demyelinating encephalomyelitis, 4; herpetic encephalomyelitis, 4; subacute sclerosing panencephalitis, 2; and viral encephalomyelitis of unknown agent, 2) and 47 healthy subjects.

Immunized animals. Serum samples were collected from 1) 11 C57BL/6 mice with $MOG_{40–55}$-induced EAE before immunization, and at varying intervals, with a 6-week postimmunization cutoff;[6] and 2) rabbits immunized either with the rat $MOG_{35–55}$ peptide or a purified recombinant human $MOG_{1–125}$ peptide. These sera were included in all assays as reference.

Recombinant MOG. The complete human MOG coding sequence was amplified by reverse transcription-PCR from brain cDNA and cloned into the pSPUTK vector (Stratagene, La Jolla, CA). Plasmid DNA was prepared with QuantumPrep spin column (Bio-Rad, Hercules, CA) and in vitro transcribed and translated in the presence of ^{35}S-methionine (Amersham Biosciences, Piscataway, NJ) with the TnT SP6 Quick-coupled rabbit reticulocyte lysate system (Promega, Madison, WI). Unincorporated ^{35}S-methionine was removed with gel chromatography on an NAP5 column (Amersham Biosciences). Each step was performed according to manufacturers' instructions. The correct MOG molecular size was verified with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

Anti-MOG radiobinding assay and Western blot analysis. We modified an RBA developed for anti-glutamic acid decarboxylase (GAD) and anti-IA-2 determination.[7] Briefly, 2 μL of serum was added to 25 μL of 1% Tween 20 in phosphate-buffered saline (PBST) containing 25,000 cpm of ^{35}S-MOG and incubated overnight on ice. Immune complexes were recovered by the addition of 1 mg either of protein G-Sepharose (for mouse sera) or of protein A-Sepharose (Amersham Biosciences; for human and rabbit sera), preswollen and resuspended in 50 μL of PBST; incubated for 1 hour at 4 °C with shaking; washed five times with 750 μL of PBST; centrifuged to pellet beads between washing; transferred in 100 μL of PBST to a 96-well Optiplate (Applied Biosystems, Foster City, CA); added with 150 μL of Microscint 40 (Applied Biosystems); and counted in a TopCount scintillation counter (Applied Biosystems). For IgM, immune complex recovery was performed with streptavidine-sepharose resin beads coated with biotinylated antihuman IgM (PharMingen, San Diego, CA).

Selected sera were also tested using Western blot analysis

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Table *Demographic characteristics of patients with relapsing-remitting or primary progressive multiple sclerosis, patients with demyelinating/necrotizing encephalomyelitis, and healthy controls*

Characteristics	MS	D/NE	HC
Subjects, n	87	12	47
Male/female	33/54	8/4	20/27
Age, y, mean ± SD (range)	33 ± 10 (17–60)	34 ± 11 (22–58)	36 ± 10 (20–47)
RR MS/PP MS	83/4	NA	NA
EDSS, mean ± SD (range)	2.6 ± 1.8 (0.0–7.0)	NA	NA
Disease duration, y, mean ± SD (range)	5.4 ± 6.2 (0.0–29.0)	NA	NA

MS = multiple sclerosis; D/NE = demyelinating/necrotizing encephalomyelitis; HC = healthy controls; RR = relapsing-remitting; PP = primary progressive; NA = not applicable; EDSS = expanded disability status scale.

according to Berger et al.,[4] except that bound antibodies were detected with chemiluminescence (Pierce, Rockford, IL).

Binding competition measurement. For the measurement of antibody binding to ^{35}S-MOG, 2 μL of serum was preincubated with 1 μg of either bacterially expressed recombinant MOG or $MOG_{40–55}$ peptide for 2 hours at 4 °C. ^{35}S-MOG was then added, and RBA was performed as described previously.

Results. Figure 1 shows the data points for each animal and human antibody determination. In sera of mice with MOG-induced EAE, anti-MOG antibody titers peaked at 5 weeks postimmunization (mean cpm value ± SEM: preimmunization, 66.7 ± 4.8; postimmunization, 872.7 ± 164.3; Wilcoxon test, p = 0.0005). The highest levels of anti-MOG antibodies were detected in sera of rabbits immunized with the $MOG_{35–55/1–125}$ peptides. In humans, mean + 2 SD of the responses of healthy subjects was chosen as a cutoff level for positivity for anti-MOG IgG/IgM. Percentages of anti-MOG seropositivity were 5.7% in the whole MS group, 0% in the D/NE group, and 4.1% in healthy subjects for IgG and 7.5% in MS patients with samples at onset, 8.3% in the D/NE group, and 6.2% in healthy subjects for IgM. Whether in the entire MS group or in patients with samples at onset, anti-MOG antibodies were unrelated to exacerbations, time of sampling, or clinical forms.

Figure 2 shows the correlations between the quantitative (RBA) vs semiquantitative (Western blot analysis) anti-MOG antibody determinations. RBA was positive and Western blot analysis was negative in one of the two patients with MS. Immunoprecipitation of ^{35}S-MOG indicates the presence of antibodies in all samples at different levels, in agreement with the liquid-phase RBA.

In the competition assay, the excess of unlabeled cold protein inhibited the binding to ^{35}S-MOG in sera of immunized animals more efficiently than in sera of subjects with the highest anti-MOG antibody titers (mean percentage of inhibition ± SEM: MS patients, 19.0 ± 5.8; healthy subjects, 24.2 ± 9.4; immunized animals, 74.8 ± 4.8; $p < 0.05$, Kruskal–Wallis test vs the two human groups). Competition with $MOG_{40–55}$ peptide resulted in complete inhibition in mice sera but not in the serum of rabbits immunized with $MOG_{1–125}$.

Discussion. We found that anti-MOG IgG and IgM were detected at the same frequency whether in patients with MS or encephalomyelitis or in healthy control subjects. Our data would not confirm either the reported high prevalence of serum anti-MOG IgG in MS (38%) and viral neurologic diseases (53%)[8] or a role for anti-MOG IgM as predictors for definite MS in early cases.[4]

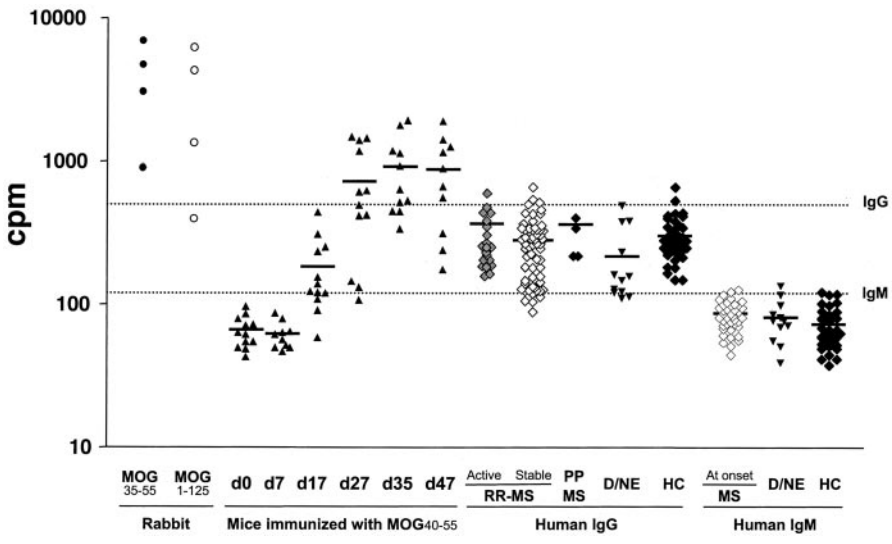

Figure 1. The cpm values for anti-myelin oligodendrocyte glycoprotein (MOG) immunoglobulin (Ig) G and IgM in animal and human sera are shown. Sera of immunized rabbits were 10-fold diluted (up to 1:1000) for assay and then corrected on plot. Serial serum samples of mice with MOG-induced experimental autoimmune encephalomyelitis show the kinetics of anti-MOG IgG response. Horizontal dotted lines indicate the mean + 2 SD of anti-MOG IgG and IgM levels in healthy control subjects (HC). Bars represent mean values. RR-MS = relapsing-remitting multiple sclerosis; active = clinically active; stable = clinically stable; PP-MS = primary progressive MS; D/NE = demyelinating/necrotizing encephalomyelitis; and at onset MS = MS at onset.

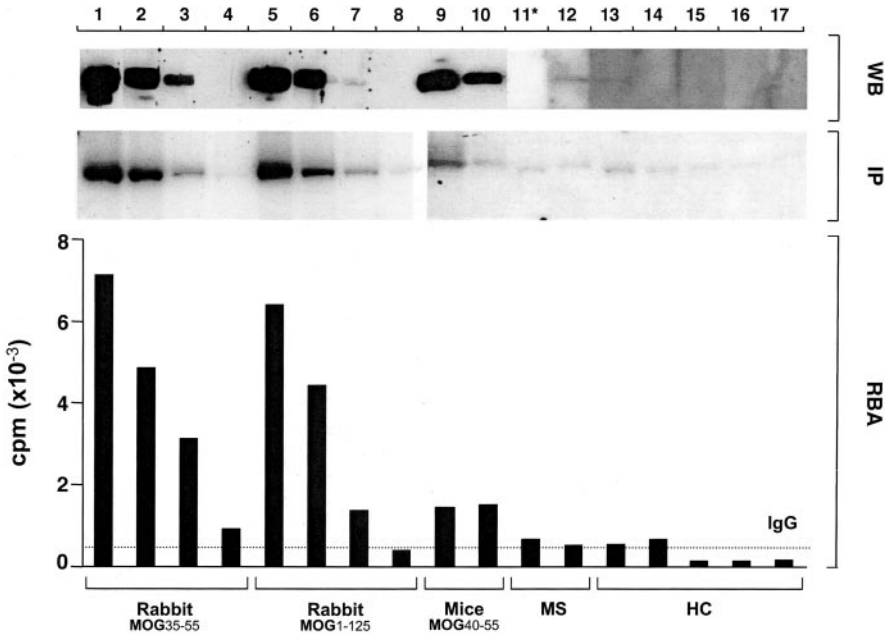

Figure 2. A parallel between three methods for anti-myelin oligodendrocyte glycoprotein (MOG) antibody determination. Native recombinant MOG was used as antigen in Western blot (WB) analysis and immunoprecipitation (IP), which was followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Lanes 1 to 4, serial 10-fold dilutions (up to 1:1000) of a serum sample from a rabbit immunized with the rat $MOG_{35–55}$ peptide; Lanes 5 to 8, the same as previous except for the human $MOG_{1–125}$ peptide; Lanes 9 and 10, sera of mice with $MOG_{40–55}$-induced experimental allergic encephalomyelitis; Lanes 11 and 12, sera of patients with multiple sclerosis (MS); and Lanes 13 to 17, sera of healthy controls (HCs). RBA = radiobinding assay. In RBA, the horizontal dotted line indicates the mean + 2 SD of anti-MOG immunoglobulin (Ig) G levels in HCs. The asterisk indicates the RBA-positive and WB-negative patient.

Evidence from type 1 diabetes, in which specific autoantibodies correlate with risk for disease onset, active disease, and disease progression,[9] indicates that an accurate prediction depends on the assay's analytical sensitivity and specificity. Our RBA, which derives from an RBA for anti-GAD determination in diabetes,[7] uses nondenatured, radiolabeled in vitro translated and transcribed full-length MOG that is immunoprecipitated in liquid phase with the patient's serum. In contrast, most assays for anti-MOG antibody determination use bacterially expressed, denatured, recombinant proteins immobilized on solid phase. Sensitivity and specificity in solid-phase assays are generally lower than in liquid-phase assays.[10] Our RBA accurately measured anti-MOG antibodies in sera of mice with MOG-induced EAE and of rabbits immunized with $MOG_{35–55/1–125}$ peptides. Moreover, our assay recognized more than one epitope in MOG amino acids 1 to 125. Except for one case, in which RBA was more sensitive, quantitative results obtained with RBA correlated with the findings obtained with a Western blot method similar to that used by Berger et al.[4,8] Antibodies to conformational or glycosylated MOG epitopes may be the only antibodies able to cause demyelination, thus explaining our negative findings.[3] However, increased frequency and titer of anti-MOG antibodies were determined in MS using Western blot analysis[4,8] when the antigen is nonglycosylated and in a completely denatured form.

We found that anti-MOG antibodies had low affinity when detected at low titer. Similar results were previously reported for anti-myelin basic protein antibodies in MS.[10] In contrast, the anti-MOG antibodies that we found in animals had high affinity because the animals were actively immunized with specific MOG peptides. Such affinity was similar to that of anti-GAD antibodies in diabetes.[7]

Discrepant results in autoantibody determinations are not new. Technical differences in antigen preparation and antibody determination, together with differences in patient populations, could explain the discrepancies between our findings and published data.[3,4,8]

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