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Mannose-Binding Lectin Contributes to the Severity of Guillain-Barré Syndrome[1]

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In Guillain-Barré syndrome (GBS), complement activation plays a crucial role in the induction and extent of the postinfectious immune-mediated peripheral nerve damage. Mannose-binding lectin (MBL) activates the complement system via the lectin pathway after recognition of repetitive sugar groups on pathogens. We investigated whether the *MBL2* genotype, serum MBL level, and MBL complex activity are associated with the development and severity of GBS. Single nucleotide polymorphisms in the promoter region (−550 H/L and −221 X/Y) and exon 1 (A/O) of the *MBL2* gene were determined in 271 GBS patients and 212 healthy controls. The frequencies of the H allele, HY promoter haplotype, and HYA haplotype, which are related to high MBL activity, were all increased in GBS patients compared with healthy controls ($p \leq 0.03$), particularly in severely affected GBS patients (MRC-sum score <40) ($p \leq 0.02$). Severe weakness was also associated with high MBL concentrations and MBL complex activity in sera from GBS patients ($p < 0.01$). The *MBL2* B allele was associated with functional deficiency and relatively mild weakness. These results support the hypothesis that complement activation mediated by MBL contributes to the extent of nerve damage in GBS, which is codetermined by the *MBL2* haplotype. ***The Journal of Immunology,*** **2006, 177: 4211–4217.**

Guillain-Barré syndrome (GBS)[3] is a postinfectious immune-mediated polyneuropathy characterized by subacute monophasic paresis and sensory deficits. GBS is considered to be a true case of a molecular mimicry mediated disease in which antecedent infections in susceptible hosts induce cross-reactive Abs to nerve gangliosides, resulting in Ab-mediated complement activation and subsequent peripheral nerve damage (1). GBS is highly variable with respect to clinical severity. Patients may have mild sensory and motor symptoms not requiring therapy, but may also develop complete paralysis of arms, legs, and respiratory muscles requiring ventilation for months and rehabilitation for years. The factors which determine the extent of the peripheral nerve damage are unknown, but polymorphisms in host immune response genes are one of the candidates.

Complement activation is a crucial factor in the pathogenesis of GBS, as has been demonstrated in studies both in patients and animal models for GBS. 1) Complement activation products are elevated in serum and cerebrospinal fluid of GBS patients (2–4). 2) Deposits of activated complement factors, C3d and C5b-9, are present on the outer membrane of Schwann cells in acute inflammatory demyelinating polyneuropathy (5), and on the axolemma of motor fibers in acute motor axonal neuropathy (6). 3) In the mouse diaphragm-phrenic nerve model, complement activation is crucial for the induction of the Ab-mediated pathophysiological effects and for axonal cytoskeletal breakdown and perisynaptic Schwann cell damage (7–9). The complement dependency of the pathogenic effect of anti-ganglioside Abs was confirmed in an in vitro assay using neuronal cells expressing gangliosides (10). 4) The complement inhibitor APT070 completely prevents the pathogenic effects of these Abs (11). 5) The therapeutic effect of i.v. Ig (IVIg) in GBS patients is also partly due to the prevention of complement activation (12).

The complement system can be activated by mannose-binding lectin (MBL), a C-type lectin that recognizes repetitive sugar groups such as mannose and *N*-acetyl-glucosamine on the surface of bacteria and viruses. The activation of the complement system is predominantly mediated through a serine protease MBL-associated serine protease-2, which cleaves C4 and C2 to generate a C3 convertase (13, 14). MBL also recognizes structures on apoptotic cells and facilitates the clearance of these cells by macrophages and dendritic cells (15–17).

Serum levels and functional activity of MBL are highly variable in the human population. This variation is mainly determined by single nucleotide polymorphisms (SNPs) located in the promoter region and in exon 1 of the *MBL2* gene (18–20). SNPs in exon 1 affect the polymeric structure of the MBL molecule, resulting in low serum levels of functional MBL and an impaired ability to activate the complement system (21). Two SNPs in the promoter region affect the levels of MBL by regulating the transcriptional activity of the *MBL2* gene: the −550 H/L polymorphism and −221 X/Y polymorphism (22, 23).

Given the role of MBL in pathogen recognition, complement activation, and clearance of apoptotic cells, we hypothesized that the functional activity of the lectin pathway partly determines the susceptibility to develop GBS and/or the extent of nerve damage. To study this hypothesis, we determined the *MBL2* genotype in GBS patients and healthy controls, and the serum MBL level and functional activity in GBS patients to assess the involvement in GBS susceptibility and severity.

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[3] Abbreviations used in this paper: GBS, Guillain-Barré syndrome; IVIg, i.v. Ig; MBL, mannose-binding lectin; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

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Materials and Methods

Study population

In this study, peripheral blood samples were obtained from 271 Dutch Caucasian GBS patients (median age at onset of disease, 46 years; range, 7–82 years; male-female ratio, 1.06), who participated in one of the Dutch trials or national survey studies (24–27). All patients fulfilled the diagnostic criteria for GBS (28). The protocol of this study was reviewed and approved by the Medical Ethical Committee of the Erasmus Medical Center, and all patients gave their written informed consent. Of these patients, 153 participated in one of the Dutch GBS trials and are therefore documented in detail (25, 26). At randomization, these patients were clinically examined and pretreatment blood samples were obtained for screening for the most common antecedent infections in GBS (*Campylobacter jejuni*, CMV, EBV, and *Mycoplasma pneumoniae*) and the presence of the most frequent Abs in GBS to the gangliosides GM1 and GD1a (29, 30). Sera were all tested in 1/100 dilutions in ELISA and were considered to be positive according to criteria defined previously (30). During a follow-up of 6 mo, the patients were neurologically examined on 13–16 time points according to the protocol. At these time points, several clinical parameters were scored: 1) the Medical Research Council (MRC)-sum score, representing the muscle strength of six bilateral muscle groups of arms and legs and ranging from 60 (normal muscle strength) to 0 (tetraparalysis) (31); 2) the GBS disability score (32); and 3) the involvement of sensory and cranial nerves. Severe GBS was defined as an MRC-sum score lower than 40 at nadir and mild GBS as an MRC-sum score of 40 or higher (33, 34).

Genomic DNA of 212 Dutch Caucasian healthy subjects (median age: 35 years, range: 19–60 years, male-female-ratio: 0.65) was provided by the Laboratory for Histocompatibility and Immunogenetics (Sanquin Bloodbank South West Region, Rotterdam, The Netherlands). All healthy subjects had given a written informed consent.

Isolation of genomic DNA

Isolation of genomic DNA from EDTA anticoagulated blood samples was performed using the Invisorb MaxiBlood kit (Invitek) according to the manufacturer's instructions. DNA samples were dissolved in 0.1× TE (1 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA) buffer and stored at −80°C.

Detection of polymorphisms in the promoter region and exon 1 of the MBL2 *gene*

In this study, we detected the (−550) H/L and (−221) X/Y polymorphism in the promoter region of the human *MBL2* gene using a single LightCycler (Roche Diagnostics) assay with two sets of hybridization probes (TibMolBiol). The forward primer 5′-GCCAGAAAGTAGAGAGGTATTTA GC-3′ and the reverse primer 5′ TGTGACATGCGTGACTAGTAC-3′ were used to amplify a fragment spanning the two SNPs in the promoter region. For genotyping the H/L polymorphism at position −550, we used the fluorescein-labeled detection probe 5′-TTTTAGACAGGCTTGCCT GGGT-3′FLU which is complementary to the L allele and the 5′-LC Red 705- AGCATTTTCTCTGGAAATTTCTTACTACGTTGG-3′-phosphorylated anchor probe. The 5′-CTATAAACATGCTTTCGGTGGCAGT-3′FLU detection probe and the 5′-LC Red 640-AACAAATGGGACCGT GCATTGCCA-3′-p anchor probe were used for genotyping the X/Y polymorphism.

In another assay, the three SNPs in codons 52, 54, and 57 of exon 1 were detected using one set of hybridization probes. The area of the *MBL2* gene spanning exon 1 was amplified by using the forward primer 5′-TGAG TATGGTCAGCGTCTTA-3′ and the reverse primer 5′-TGGGCTG GCAAGACAACTATTAG-3′. The 5′-LC-Red 640-TTCTTCCTTGGT GCCATCACACCCA-3′-p detection probe and the fluorescein-labeled anchor probe 5′-CAGCCCAACACGTACCTGGTTCCCCCT-3′FLU were used to detect the three SNPs in exon 1 (TibMolBiol). The "A" allele represents the wild type, while the "O" allele represents the variant alleles B, C, and D together.

The PCR and the melting curve were performed in LightCycler capillaries (Roche Diagnostics) with a final volume of 20 μl, containing 10 ng of genomic DNA, 0.5 μM of each primer, 0.15 μM of each hybridization probe, 1× LightCycler DNA Master Hybridization Probes (Roche Molecular Biochemicals), and 2 mM $MgCl_2$. The PCR amplification profile of both experiments consisted of 10 min at 95°C, followed by 45 cycles of 95°C for 3 s, 60°C (63°C in case of detection of the *MBL* exon 1 polymorphism) for 15 s, and 72°C for 10 s. Next, the melting curve profile was determined, which consisted of 1 cycle of 95°C for 3 min, 55°C for 1 min, 45°C for 30 s, and 40°C for 3 min after which the temperature was slowly increased (0.1°C/sec) to 80°C under continuous detection of the emitted light. Data were analyzed using the melting curve program. In each experiment, we used sequence-verified control donors for each genotype.

Detection of MBL concentration in serum

MBL concentration levels were measured in all 82 trial patients from which detailed clinical information was available, and sufficient amounts of serum were obtained pretreatment and within 2 wk of onset of weakness. These patients did not significantly differ from the group of 71 excluded trial patients with respect to demographic features, MBL genotype, disease severity, and outcome. Fifty-one of these patients were mildly affected and 31 were severely affected based on the MRC-sum score at nadir. Serum IgM and/or IgG Abs against GM1 and/or GD1a were absent in 59 patients and present in 23 patients. Additionally, in eight randomly selected GBS patients, MBL concentrations were measured in follow-up serum samples obtained at 2 wk, 4 wk, 3 mo, and 6 mo after randomization. An IVIg solution preparation (Gammagard SD; Baxter Healthcare, Hyland Division) was tested for the presence of MBL. MBL concentrations were determined using a human MBL oligomer ELISA kit (Antibodyshop), which specifically detects oligomerized forms of MBL. The ELISA and the calculation of the concentrations were performed according to the manufacturer's instruction.

Detection of MBL complex activity

To assess the MBL complex activity, we determined the specific C4b-depositing capacity of the MBL pathway in serum from 78 of the group of 82 GBS patients (serum from 2 mildly and 2 severely affected patients was not available for testing MBL complex activity), using an ELISA-based method as described before (35) with slight modifications. In short, Maxisorb microtiter wells (Nunc) were coated with 10 μg of mannan (Sigma-Aldrich) in 100 μl of coating buffer (100 mM $NaHCO_3$, 100 mM Na_2CO_3 (pH 9.6)) and incubated overnight at room temperature. After this coating step, plates were washed three times with PBS/0.05% Tween 20. To avoid nonspecific binding, plates were blocked with PBS-1% BSA during 1 h at 37°C. All samples were diluted in cold GVB^{++} buffer (Veronal-buffered saline, 0.08% gelatin, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.05% Tween 20) containing 1 M NaCl, overnight at 4°C. Plates were washed with PBS/0.05% Tween 20 containing 5 mM $CaCl_2$, followed by incubation with purified C4 (1 μg/ml), diluted in GVB^{++}, for 1 h at 37°C. Activation of C4 was assessed as described (21).

Statistical analyses

Pearson's χ^2 test was used to verify whether the SNPs were in Hardy-Weinberg equilibrium and to compare the genotype, allele, and haplotype frequencies between patients and healthy subjects. Conditional logistic regression was used to determine the independent contribution of each SNP. The Mann-Whitney *U* test was used to compare MBL concentrations and complex activities in mildly vs severely affected GBS patients. The Spearman rank correlation coefficient was used for correlation analyses. The Friedman test was used to compare the MBL levels in follow-up serum samples from individual patients. The Wilcoxon sign rank test was used to compare MBL concentrations in follow-up serum samples at two different time points. Values of $p \leq 0.05$ were considered to be statistically significant.

Results

MBL2 *genotype, allele, and haplotype frequencies in GBS patients and controls*

The polymorphisms in the promoter region and exon 1 of the *MBL2* gene could be determined in 269 of 271 GBS patients and in 210 of 212 healthy subjects. Because all SNPs were in Hardy-Weinberg equilibrium ($p > 0.3$) and in linkage disequilibrium ($p < 0.001$), we were able to compare the genotype, allele, and haplotype frequencies between GBS patients and healthy controls (Table I). More frequent in GBS patients compared with controls were the HH genotype (18.1% vs 9.1%; odds ratio (OR) (95% confidence interval (CI)) = 2.6 (1.4–4.7)) and HL genotype (46.9 vs 42.9%; OR (95% CI) = 1.5 (1.0–2.2)) at position −550 of the promoter region ($p = 0.004$). GBS patients also had significantly higher frequencies of the H allele, HY promoter haplotype, and the HYA haplotype (all *p* values ≤0.03). The differences in haplotype distributions were mainly due to the presence of an H allele, which was independent of the SNPs in exon 1 ($p = 0.001$). The relation between the number of H alleles or HYA haplotypes and disease susceptibility was best described by a multiplicative model: the

Table I. MBL2 *haplotype frequencies in GBS patients and healthy controls*[a]

Haplotype	GBS Patients (n = 269) n (%)	Controls (n = 210) n (%)	OR (95% CI)	p[b]	Severely Affected Patients (n = 71)[c] n (%)	Mildly Affected Patients (n = 82)[c] n (%)	OR (95% CI)	p[b]
HYA homozygotes	33 (12.3)	15 (7.2)	2.1 (1.1–4.1)	0.01/0.03	11 (15.5)	9 (11.0)	2.0 (0.7–5.4)	0.04/0.1
HYA/HYA	33 (12.3)	15 (7.2)			11 (15.5)	9 (11.0)		
HYA heterozygotes	110 (40.9)	73 (34.8)	1.5 (1.0–2.2)		31 (43.7)	26 (31.7)	1.9 (0.9–3.9)	
HYA/LYA	44 (16.4)	28 (13.3)			17 (23.9)	8 (9.7)		
HYA/LXA	28 (10.4)	24 (11.4)			5 (7.0)	7 (8.5)		
HYA/HYO	13 (4.8)	2 (1.0)			6 (8.5)	2 (2.5)		
HYA/LYO	25 (9.3)	19 (9.0)			3 (4.2)	9 (11.0)		
Non-HYA	126 (46.8)	122 (58.0)	Reference		29 (40.8)	47 (57.3)	Reference	
LYA/LYA	14 (5.2)	18 (8.6)			3 (4.2)	7 (8.5)		
LXA/LXA	12 (4.5)	9 (4.3)			2 (2.8)	2 (2.5)		
LYA/LXA	29 (10.8)	30 (14.2)			5 (7.0)	11 (13.4)		
LYA/LYO	18 (6.7)	19 (9.0)			4 (5.7)	9 (11.0)		
LYA/HYO	12 (4.4)	11 (5.3)			4 (5.7)	2 (2.5)		
LXA/LYO	16 (5.9)	18 (8.6)			2 (2.8)	8 (9.7)		
LXA/HYO	9 (3.4)	4 (1.9)			4 (5.7)	1 (1.2)		
O/O	16 (5.9)	13 (6.2)			5 (7.0)	7 (8.5)		

[a] The OR and 95% CI were calculated in comparison with the reference (most frequent group of haplotypes). The haplotype analysis compares 269 GBS patients to 210 healthy controls since missing values for the *MBL2* haplotype were found in two GBS patients and two healthy controls.

[b] The first p value represents the comparison between HYA homozygotes/heterozygotes versus non-HYA and the second one the comparison between HYA homozygotes, HYA heterozygotes, and non-HYA. The "A" represents the wild-type alleles for the exon 1 SNPs and the "O" the variant alleles (either B, C, or D).

[c] Severely affected patients were defined as an MRC-sum score < 40 and mildly affected patients as an MRC-sum score ≥ 40. The MRC-sum score ranges from 0 (tetraparalysis) to 60 (normal).

presence of a single H allele or HYA haplotype was associated with an increased risk for GBS, but the presence of two H alleles or HYA haplotypes increased this risk even more ($p \leq 0.03$). These data indicate that GBS is associated with *MBL2* genotypes that promote high production of MBL.

The distribution of exon 1 variant alleles did not significantly differ between patients and controls. In the control group, the frequencies of the four different exon 1 alleles were: 75.7% (wild-type A allele), 15.6% (B allele), 1.9% (C allele), and 6.8% (D allele). In GBS patients, these frequencies were 76.6% (A allele), 13.2% (B allele), 2.0% (C allele), and 8.2% (D allele). The X/Y promoter SNP at position −221 also did not significantly differ in genotype distribution between GBS patients and controls. The Y allele was present in 77.1% of the controls and in 80.4% of the GBS patients.

High-producing MBL2 *genotypes, alleles, and haplotypes are associated with severe GBS*

The frequency distribution of the *MBL2* SNPs was compared in GBS patients with severe weakness (MRC-sum score <40) (n = 71) vs patients with mild weakness (MRC-sum score ≥40) (n = 82). The HH genotype (23.9 vs 14.6%, OR (95% CI) = 3.1(1.2–8.0)) and HL genotype (50.7 vs 37.8%, OR (95% CI) = 2.5 (1.2–5.3)) were more frequent in severe GBS compared with mild GBS (p = 0.02). Moreover, the frequencies of the H allele and HY promoter haplotype were significantly increased in severely affected GBS patients (p values ≤0.02; data not shown).

Mildly affected GBS patients and healthy controls did not differ with respect to the frequencies of these MBL genotypes and haplotypes. However, the severely affected GBS patients and healthy controls were significantly different regarding the genotype distribution ($p < 0.001$) and the haplotype distributions ($p \leq 0.02$). The frequencies of the H allele, HH genotype, HY promoter haplotype, and HYA haplotype were all significantly increased in severely affected GBS patients (all p values ≤0.02). The observed differences in haplotype distribution were mainly due to the presence of an H allele, the effect of which was independent of the SNPs in exon 1 ($p < 0.001$). A multiplicative model was the best-fitted model to describe the relation between the number of H alleles and HYA alleles and the severity of GBS ($p < 0.02$). These data indicate that the *MBL2* haplotypes that promote high production of MBL are a susceptibility factor for developing a severe form of GBS.

In further subgroup analyses, the O allele was more frequently found in the group of patients with a positive serology for either *C. jejuni*, CMV, EBV, or *M. pneumoniae* (30.4 vs 19.2%, p = 0.02), but not with a positive serology for one antecedent infection in particular. The SNPs in the *MBL2* promoter and exon 1 were not associated with sex, age, sensory, and cranial nerve deficits, GBS functional disability score, ventilation, and the presence of Abs to GM1 and/or GD1a.

Serum MBL levels and complex activity in relation to MBL2 *genotypes*

The effect of the *MBL2* genotype on serum MBL concentration and complex activity was determined in a subgroup of 78 GBS patients. The serum MBL concentration was highly correlated with the MBL complex activity (Fig. 1*A*). The serum MBL concentrations were significantly different when comparing the genotypes of exon 1. Highest concentrations were found in A/A homozygotes (median level 2950 ng/ml), intermediate concentrations in A/O heterozygotes (median level 620 ng/ml), and lowest concentrations in O/O homozygotes (median level 30 ng/ml; $p < 0.0001$). Similar differences were observed for the median level of serum MBL complex activity: highest in A/A homozygotes (386 U/ml), intermediate in A/O heterozygotes (66 U/ml), and undetectable in O/O homozygotes ($p < 0.0001$) (Fig. 1*B*). As previously described (22), also the H/L polymorphism at position −550 in the promoter region affected the MBL concentration. The median concentration of MBL was higher in H/H homozygotes (3450 ng/ml) compared with L/L homozygotes (1160 ng/ml; p = 0.02). A similar difference was found with respect to MBL function: median complex

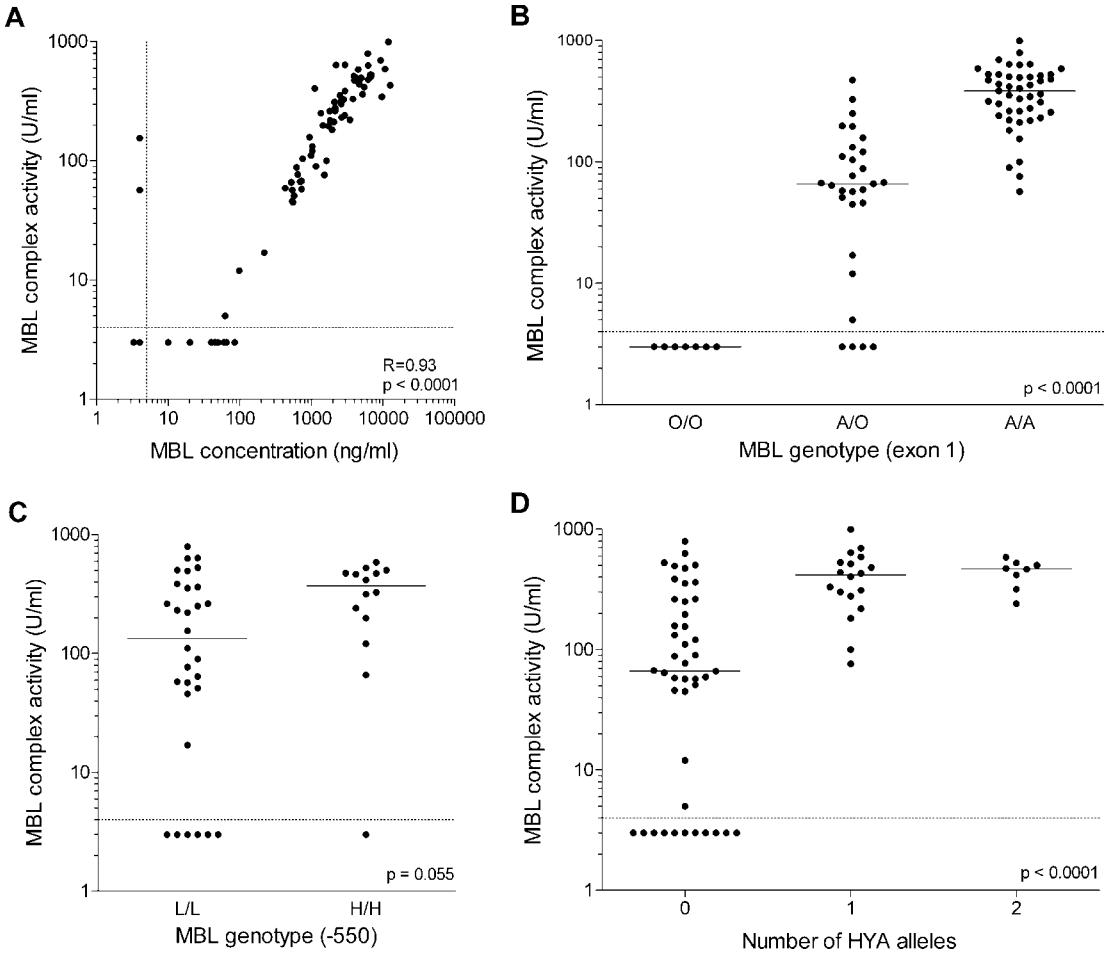

FIGURE 1. *MBL2* genotype in relation to serum MBL concentration and complex activity in GBS patients. Serum MBL concentration in relation to MBL complex activity in GBS patients ($n = 78$) (*A*). The two outliers with no detectable MBL concentrations despite the presence of MBL complex activity represent two patients with LXA/LXA haplotypes. *MBL2* exon 1 genotypes in relation to MBL complex activity (*B*). *MBL2* promoter genotype at position −550 in relation to MBL complex activity (*C*). *MBL2* haplotypes in relation to MBL-complex activity (*D*). The continuous line represents the median MBL serum level and complex activity. The dotted lines represent the detection limits of the assays.

activity of 372 U/ml in H/H homozygotes vs 133 U/ml in L/L homozygotes ($p = 0.055$) (Fig. 1*C*). The MBL concentration and MBL complex activity were also significantly different when comparing MBL2 haplotypes. The median concentration of MBL was highest in HYA homozygotes (4550 ng/ml), high in HYA heterozygotes (3375 ng/ml), and low in non-HYA genotypes (630 ng/ml; $p < 0.001$). A similar difference was found with respect to MBL function: median complex activity of 469 U/ml in HYA homozygotes, 417 U/ml in HYA heterozygotes, and 67 U/ml in non-HYA genotypes ($p < 0.0001$) (Fig. 1*D*).

High serum MBL levels and complex activity are associated with severe GBS

To confirm the functionality of the association found between *MBL2* haplotypes and disease severity, we examined the serum MBL concentration and complex activity in relation to clinical

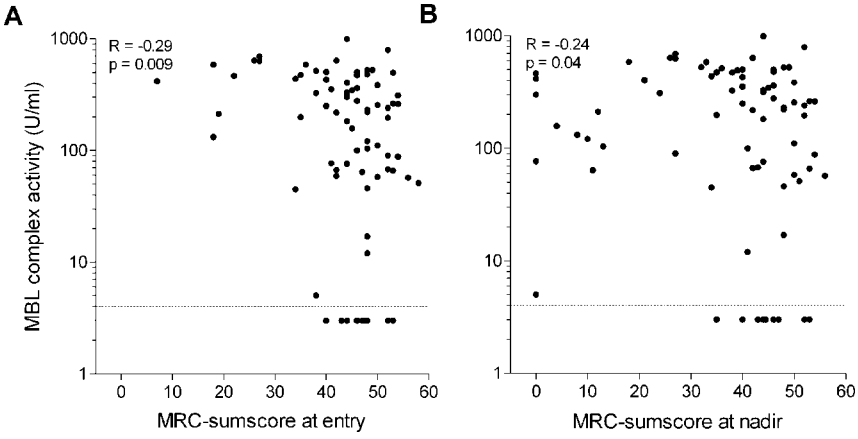

FIGURE 2. Serum MBL complex activity in relation to severity of weakness in GBS patients. MBL complex activity in relation to the extent of muscle weakness (indicated by the MRC-sum score) at entry (*A*) and nadir (*B*) in GBS patients ($n = 78$). The MRC-sum score ranges from 0 (tetraparalysis) to 60 (normal). The dotted lines represent the detection limits of the assays.

Table II. *Serum MBL concentration and complex activity in subgroups of GBS patients*[a]

	Severity of Disease			Anti-GM1 and/or GD1a Abs		
	Severe (n = 29)[b]	Mild (n = 49)	p	Present (n = 20)[b]	Absent (n = 58)	p
MBL concentration (ng/ml)	3325 (±523)	2506 (±418)	0.08	4088 (±792)	2309 (±322)	0.04
MBL complex activity (U/ml)	337 (± 41)	223 (± 32)	0.01	373 (± 54)	214 (± 28)	0.02

[a] MBL concentration (ng/ml) and MBL complex activity (U/ml) are represented by mean value and the SEM in parentheses. Severe weakness were defined as an MRC-sum score < 40 and mild weakness as an MRC-sum score ≥ 40.

[b] MBL-complex activity was determined in 78 GBS patients: 29 severely and 49 mildly affected GBS patients and 20 GBS patients with and 58 patients without Abs to the gangliosides GM1 and/or GD1a.

subgroups. High MBL concentrations were correlated with low MRC-sum scores at entry ($R = -0.27$; $p = 0.01$, data not shown) and nadir ($R = -0.20$; $p = 0.07$, data not shown). High MBL complex activity was also associated with low MRC-sum scores at entry ($p = 0.009$; Fig. 2*A*) and nadir ($p = 0.04$; Fig. 2*B*). The MBL-complex activity was significantly higher in the severely affected patients compared with the mildly affected GBS patients (Table II). The MBL concentration and MBL complex activity were also significantly higher in patients with Abs to GM1 and/or GD1a compared with patients without these Abs (Table II).

MBL *allele associated with functional MBL deficiency confers protection against severe GBS*

Data presented in Fig. 2 indicate that GBS patients with low MBL complex activity have less severe disease. Ten of 11 patients with an MBL complex activity below the detection limit had mild GBS (MRC-sum scores at nadir ≥40). As presented in Fig. 1*B*, patients with undetectable MBL complex activity carry one ($n = 4$) or two O alleles ($n = 7$). To further investigate the effect of MBL exon 1 O alleles on disease severity, MRC-sum scores were compared between GBS patients with identical MBL genotypes except for the presence of one B allele (Fig. 3). Heterozygous carriers of B alleles are known to have a more severe MBL deficiency than heterozygous carriers of D alleles (21). Carriers of the LYB/HYA genotype compared with those with the LYA/HYA had a milder form of GBS as indicated by the significantly higher MRC-sum scores at entry ($p = 0.04$; data not shown) and at nadir (Fig. 3*A*; $p = 0.03$). The MBL complex activity showed a profound difference between both groups (Fig. 3*B*; $p = 0.0003$). Also MBL concentration was much lower in the LYB/HYA group than in the LYA/HYA group ($p = 0.04$, data not shown). Together, these data indicate that the B allele is associated with functional MBL deficiency and with protection from severe GBS.

MBL serum levels during the disease course

The kinetics of serum MBL concentration during a follow-up of 6 mo in relation to severity of weakness was studied in eight randomly selected GBS patients. Most severe weakness, defined by the lowest MRC-sum score, was reached at entry in six patients and within 14 days after entry in two patients. The median MRC-sum score at nadir was 44.5, ranging from 13 to 50. The median MRC-sum score after 6 mo of follow-up was 57, ranging from 45 to 60. The MBL levels changed significantly during follow-up ($p < 0.001$; Fig. 4). Compared with pretreatment samples, MBL levels were significantly higher in serum obtained at 2 wk (1.2-fold increase, $p = 0.02$) and significantly lower in serum obtained at 6 mo (1.8-fold decrease, $p = 0.03$). IVIg preparations, used for patient treatment, contained no detectable levels of MBL (data not shown).

Discussion

Antecedent infections in combination with genetic host factors most likely precipitate the cross-reactive immune response and complement activation at peripheral nerves in GBS. MBL plays a crucial role in both pathogen recognition and activation of the complement system and may be one of the host factors in the susceptibility to develop GBS. In the present study, we showed that the frequency of *MBL2* haplotypes associated with high levels of MBL is increased in GBS patients, in particular in those patients with severe paresis. *MBL2* haplotypes associated with MBL deficiency, in contrast, were associated with relatively mild weakness in GBS patients. Functional studies confirmed that the MBL concentration and complex activity in serum from GBS patients are related to disease severity. The level of MBL activity may therefore contribute to the extent of peripheral nerve damage in GBS.

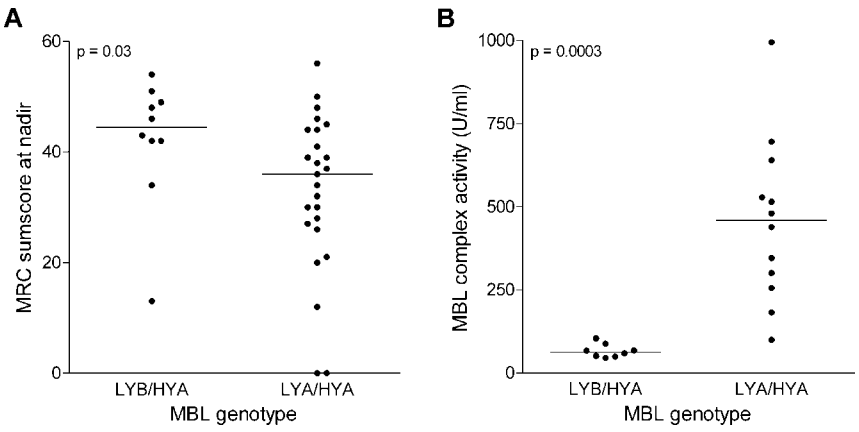

FIGURE 3. Effect of *MBL2* B allele on severity of weakness and serum MBL complex activity in GBS patients. Effect of the *MBL2* B allele on MRC-sum score at nadir (*A*) and serum MBL-complex activity (*B*) by comparing GBS patients with LYB/HYA vs LYA/HYA genotype. The MRC-sum score ranges from 0 (tetraparalysis) to 60 (normal). The lines represent the median MRC-sum scores and MBL complex activity.

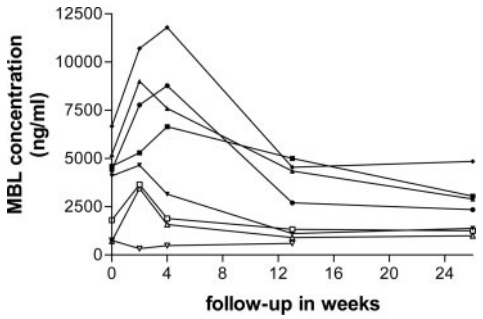

FIGURE 4. Serum MBL concentration during a follow-up of 6 mo in eight GBS patients.

MBL serum concentration and functional activity are mainly determined by *MBL2* gene polymorphisms. In this study, we investigated in a large group of Dutch Caucasoid GBS patients the SNPs in the *MBL2* gene which are known to influence the serum MBL levels. We did not determine the +4 P/Q SNP at the 5′ untranslated region of exon 1, because the LYPA haplotype is almost absent in Caucasoid individuals and the Q variant is only present in combination with the LY promoter haplotype (22). A recent study reported other SNPs in a haplotype block at the 3′ end of the *MBL2* gene of which the functional effects on MBL have not been established (36). The observed genotype frequencies in our healthy control group and the high associations between genotype/haplotype and serum MBL levels in GBS patients are consistent with the findings of others in healthy controls, indicating that our data are accurate and representative (21, 22, 37, 38).

The high frequency of the HH genotype and HY and HYA haplotypes in GBS patients, particular in severely affected GBS patients, was largely determined by the presence of an H allele at position −550 of the promoter region. H alleles have a direct effect on *MBL2* gene expression by regulating the transcriptional activity. Accordingly, we demonstrated an association between high serum MBL levels and the presence of H alleles and HYA genotypes (Fig. 1, *C* and *D*), and between high serum MBL levels and severe weakness (Fig. 2). However, no association was found between severe weakness and other *MBL2* gene polymorphisms related to high MBL levels. The effect of H alleles on disease severity in GBS is therefore partly explained by a high MBL production, but the involvement of other genes in linkage disequilibrium with H alleles cannot be excluded.

Our study indicates that GBS patients with MBL deficiency (no detectable MBL complex activity) do not develop severe weakness (Fig. 2). MBL deficiency in this population of GBS patients was related to the presence of a B allele in exon 1 of the *MBL2* gene. Heterozygous carriers of the B allele had low serum MBL levels and relatively mild weakness (Fig. 3). This finding may indicate that an MBL activity above a certain threshold level is required to develop a severe form of GBS. Studies in follow-up serum samples demonstrated that MBL levels initially rise in the acute phase of GBS and than decline to baseline (Fig. 4). This transient rise in MBL most likely is related to an acute phase response after infection, but could not explain the relation between high MBL levels and GBS or the extent of peripheral nerve damage. During acute phase reactions, MBL levels may rise up to 3-fold. The >1000-fold differences in MBL levels found in the pretreatment serum samples from the GBS patients could only be explained by genetic variation (14).

MBL levels may determine the extent of peripheral nerve damage in GBS patients by affecting both the afferent and efferent part of the immune response. MBL binds to repetitive sugar residues on pathogens, thereby possibly enhancing the immune response to antecedent bacterial and viral infections associated with GBS. Activation of the complement system facilitates phagocytosis by opsonization and enhances Ag presentation and Ab production (14, 39). Accordingly, MBL could be involved in protection of the host against such infections, as suggested by the increased frequency of antecedent infections in carriers of MBL exon 1-O alleles in our study. In contrast, complement activation upon such infections may increase the susceptibility to develop a cross-reactive immune response to peripheral nerves. Accordingly, an association between high MBL levels and the presence of Abs to GM1 and GD1a was found, which are known to be frequently cross-reactive with infectious agents and are associated with severe GBS (29).

MBL may also be involved in the efferent part of the immune response by enhancing tissue damage. MBL activates the lectin pathway upon recognition of Abs of the IgA subclass (40) and of nongalactosylated IgG Abs (41). Alternatively, MBL might activate this pathway upon recognition of carbohydrate clusters formed via cross-linking of gangliosides by anti-ganglioside Abs. MBL can also bind directly to apoptotic and necrotic cells (16). Binding of MBL to damaged endothelium is involved in complement activation and tissue damage following ischemia/reperfusion injury (42). Recently, a role for MBL in tissue injury has been suggested for several inflammatory diseases such as rheumatoid arthritis (43), ulcerative colitis (44), and diabetic vasculopathy (45). Moreover, a recent report demonstrated an association between high levels of MBL and chronic rheumatic heart disease in patients previously diagnosed with rheumatic fever (46). In GBS and rheumatic fever antecedent infections, molecular mimicry and cross-reactive Abs play a similar role in pathogenesis. Therefore, the association between MBL and the severity of GBS shown in the present study might be explained by binding of MBL to initially damaged nerve tissue, followed by complement activation, attraction of inflammatory cells, and aggravation of tissue injury.

The overlap in MBL levels between mildly and severely affected GBS patients illustrates the complexity and heterogeneity of GBS. If complement activation indeed plays such an important role in pathogenesis, the extent of peripheral nerve damage and motor deficits will also depend on local regulatory components, such as complement-inhibitory molecules like the decay-accelerating factor (CD55). Moreover, other host factors like higher age at onset and environmental factors such as *C. jejuni* infection have already been shown to be independent prognostic factors for the development of a severe GBS (29, 33).

In conclusion, our study demonstrated an association between *MBL2* gene polymorphisms and severity of weakness in GBS. The higher levels of MBL protein and complex activity in patients with severe GBS associated with these polymorphisms further suggests that complement activation mediated by MBL contributes to the extent of nerve damage in GBS. Because the lectin pathway can be involved in several phases of the immune response, further studies are warranted to elucidate the underlying mechanism.

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Disclosures

The authors have no financial conflict of interest.

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