Multifocal motor neuropathy in the Netherlands: immunology, genetics and treatment

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Cover design Mere Vos, www.merevos.nl
Layout Renate Siebes, Proefschrift.nu
Printed by Ipskamp Drukkers B.V.
ISBN 978-90-393-5679-1

Multifocal motor neuropathy in the Netherlands: immunology, genetics and treatment

Multifocale motorische neuropathie in Nederland: immunologie, genetica en behandeling

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 20 december 2011 des ochtends te 10.30 uur

door

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geboren op 31 oktober 1979
te Utrecht

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Dit proefschrift werd (mede) mogelijk gemaakt met financiële steun van het Prinses Beatrix Fonds, Abbott, Baxter B.V., Biogen Idec International B.V., de J.E. Jurriaanse Stichting, Novartis Pharma B.V., Tergooiziekenhuizen, Sanquin, UCB Pharma B.V.

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Introduction and outline

MULTIFOCAL MOTOR NEUROPATHY

Multifocal motor neuropathy (MMN) is a rare chronic neuropathy characterized by slowly progressive asymmetric weakness of muscles in the distal arm and leg and the electrophysiological finding of conduction block (CB).[1-4] Asymmetric weakness of distal arm or leg muscles without sensory deficits is a highly uncommon presentation for a peripheral neuropathy. Until its first detailed description in 1988,[1, 2] MMN was often diagnosed as amyotrophic lateral sclerosis (ALS) with a surprisingly benign disease course. Unlike ALS, which has a poor prognosis with a median survival of approximately three years,[5] patients with MMN have a normal life expectancy. Diagnostic consensus criteria (summarized in Table 1.1) may be a helpful tool to distinguish patients with MMN from patients with motor neuron disorders. Outcome of MMN and determinants of disability have not been studied in detail, probably due to the rarity of the disorder.

PATHOGENESIS

The favorable response to intravenous immunoglobulin (IVIg) and the finding of antibodies against the ganglioside GM1 suggest that MMN is an immune-mediated disorder. The presence of serum IgM antibodies against the ganglioside GM1 was acknowledged in the first descriptions of MMN and has been since confirmed by many subsequent studies, although the estimation of their prevalence has been hampered by differences in methodology used.[6-8] Gangliosides are glycolipids which consist of a ceramide anchor coupled to a varying number of oligosaccharide residues and sialic acids.[9] Gangliosides are abundantly expressed in nervous tissues, but are not randomly distributed. GM1 is expressed at relatively high densities on the surface of Schwann cells, paranodal myelin, and probably axonal membranes of motor nerves, but also in dorsal root ganglia.[9]

The biological functions of gangliosides have not been completely clarified, but it is clear that gangliosides are important antigens in several immune-mediated neuropathies. The Guillain-Barré syndrome (GBS) is the prototype polyneuropathy associated with ganglioside-specific antibodies. The specificity of ganglioside-specific antibodies and antibodies against complexes of gangliosides is clearly associated with clinical features in patients with GBS.[9-11] Experimental exposure to gangliosides or bacterial constituents exposing ganglioside-like structures induces inflammatory neuropathy in rabbits and humans.[9] Anti-ganglioside IgG antibodies were shown to induce inflammation and to disrupt nerve function by activation of complement and leukocytes after binding to their targets.[12, 13] The pathogenic role of anti-ganglioside IgM antibodies in MMN is not fully clarified. Anti-GM1 IgM antibodies in sera from MMN patients have been shown to activate complement.[14] The range of specificities of ganglioside-specific antibodies in patients with MMN and the association with clinical features has not been studied in detail. Associations similar to those in GBS would support

Table 1.1 Diagnostic criteria for MMN

Clinical criteria

1. Slow or stepwise progressive limb weakness
2. Asymmetrical limb weakness
3. Fewer than 7 affected limb regions (upper/lower arm, upper/lower leg on both sides, maximum 8)
4. Tendon reflexes in affected limbs are decreased or absent
5. Signs and symptoms are more pronounced in arms than in legs
6. Age 20–65 years at onset of disease
7. No objective sensory abnormalities except for vibration sense
8. No bulbar signs or symptoms
9. No upper motor neuron features
10. No other neuropathies
11. No myopathy

Laboratory criteria

1. CSF protein <1 g/L
2. Elevated serum IgM anti-GM1 antibody titer
3. High signal intensity on T2-weighted MRI of the brachial plexus

Electrodiagnostic criteria

1. Definite motor conduction block: CMAP area reduction on proximal versus distal stimulation of at least 50% over a long segment (between Erb (shoulder) and axilla, upper arm, lower arm, lower leg), or a CMAP amplitude reduction on proximal versus distal stimulation of at least 30% over a short distance (2.5 cm) detected by inching. CMAP amplitude on stimulation of the distal part of the segment with motor conduction block of at least 1 mV.
2. Probable motor conduction block: CMAP amplitude reduction on proximal versus distal stimulation of at least 30% over a long segment of an arm nerve. CMAP amplitude on stimulation of the distal part of the segment with motor conduction block of at least 1 mV.
3. Slowing of conduction compatible with demyelination: MCV 75% of the lower limit of normal; DML or shortest F wave latency 130% of the upper limit of normal or absence of F waves all after 16–20 stimuli. CMAP amplitude on distal stimulation of at least 0.5 mV.
4. Normal sensory nerve conduction in arm segments with motor conduction block. Normal SNAP amplitudes on distal stimulation.

Definite MMN

1–11 on clinical criteria, 1 on laboratory criteria,1 and 4 on electrodiagnostic criteria

Probable MMN

1–3 and 6–11 on clinical criteria, 1 on laboratory criteria, 2 and 4 on electrodiagnostic criteria

Possible MMN

1 and 7–11 on clinical criteria, 2 or 3 on laboratory criteria, 3 and 4 on electrodiagnostic criteria

CSF indicates cerebrospinal fluid; MRI, magnetic resonance imaging; CMAP, compound muscle action potential; MCV, motor conduction velocity; DML, distal motor latency; SNAP, sensory nerve action potential. Adapted from: Van Asseldonk JT, Franssen H, Van den Berg-Vos RM, Wokke JH, Van den Berg LH (2005) Multifocal motor neuropathy. Lancet Neurol 4:309-319.

the assumption that anti-ganglioside IgM antibodies also play a role in MMN pathogenesis. Possible pathological mechanisms in MMN are depicted in Figure 1.1.

In contrast to GBS, it is not clear why anti-GM1 IgM antibodies are often found in serum from patients with MMN. Assuming a pathogenic role of anti-GM1 IgM antibodies, MMN could be a post-infectious complication (similar to GBS), an autoimmune disease, or an immune-mediated neuropathy secondary to monoclonal proliferation of B-cells.[15, 16]

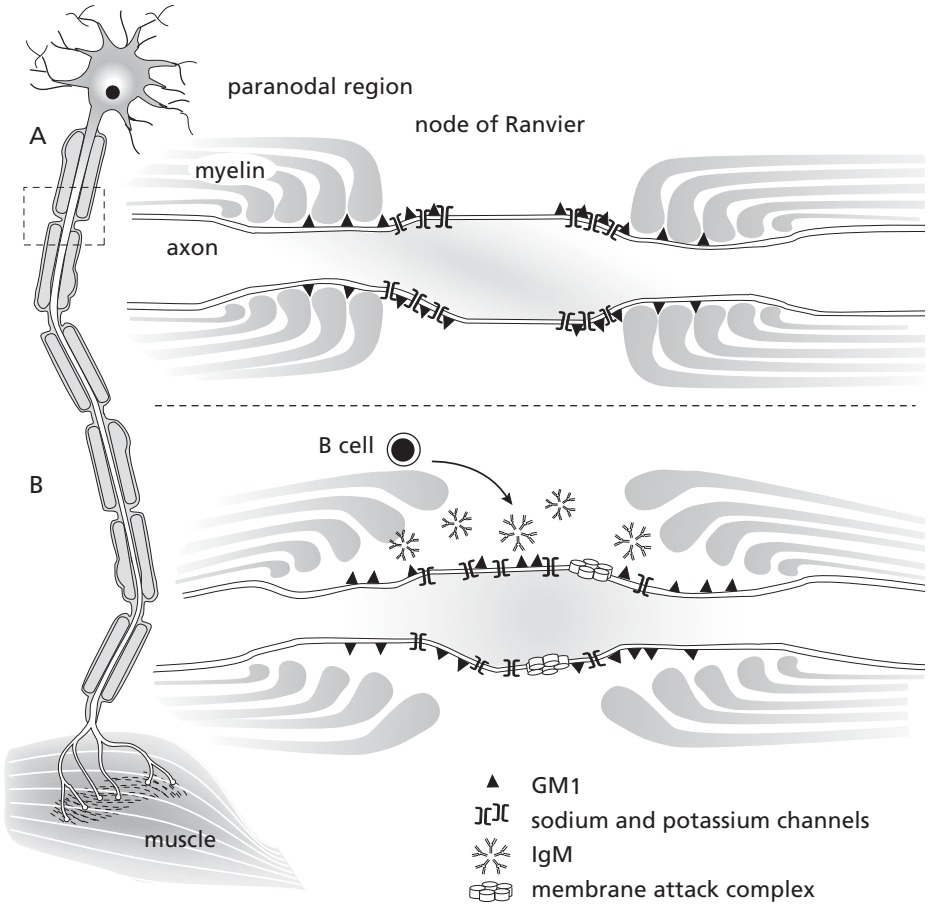

Figure 1.1 Possible pathological mechanisms in MMN.
A The nodes of Ranvier ensure saltatory conduction. GM1 is important for maintaining tight junctions at the paranodal regions, anchoring potassium channels and clustering of sodium channels.
B GM1-specific IgM antibodies may bind to GM1 in paranodal myelin or at nodes of Ranvier, and activate complement. This could cause disruption of Schwann cell-axolemma junctions, displacement and disruption of ion-channel clusters. Deposition of complement factors, including membrane attack complex (MAC), could compromise membrane integrity and cause axonal damage.

Susceptibility to MMN may be partially genetically determined. Frequencies of single nucleotide polymorphisms (SNPs) in a few candidate genes have been studied in small scale studies.[17] Candidate genes include the highly polymorphic human leukocyte antigen (HLA) system, since many autoimmune diseases are associated with specific HLA alleles.[18] There is also a growing body of evidence that SNPs in other genes may increase susceptibility to several autoimmune diseases.[19] This suggests that some pathogenic pathways are shared by many autoimmune diseases. Genetic association studies might therefore help to unravel the pathogenesis of MMN.

TREATMENT

Treatment with IVIg has been shown to be beneficial in randomized controlled trials.[17, 20-23] Disadvantages of IVIg treatment are costs and the fact that it is time-consuming. Cyclophosphamide has been reported effective but its toxicity limits usefulness for patient with MMN.[24, 25] Plasma exchange and prednisone are well-established therapies in other immune-mediated neuropathies,[26-28] but are not effective in patients with MMN and may even aggravate symptoms.[29, 30] Cyclophosphamide is an efficient B-cell inhibiting drug. IVIg exerts multiple effects on the humoral part of the immune system including anti-idiotype effects and modulation of B-cell receptors and the complement system, which may all contribute to its beneficial effect.[31, 32]

OUTLINE OF THE THESIS

The aims of this thesis were to identify correlates of outcome in patients with MMN, to further dissect the immune pathogenesis, to study genetic variants that predispose to MMN and to evaluate efficacy and convenience of IVIg treatment in MMN. This thesis is divided in four parts:

PART I - CLINICAL FEATURES OF MMN

In 2007 we initiated a cross-sectional descriptive study in The Netherlands. We identified and re-examined all patients with MMN in the Netherlands to document the variety of clinical phenotypes and response to IVIg treatment. We also investigated the correlates of outcome .

PART II- IMMUNE PATHOGENESIS OF MMN

We focused on the prevalence and specificity of antibodies against single gangliosides and ganglioside complexes in serum from 88 MMN patients and their association with clinical features and the clonality of anti-GM1 IgM antibodies in MMN patients. We evaluated if

associated autoimmune diseases are more common in MMN patients and their first-degree relatives than in population-based controls. Because of the importance of the complement system in the pathogenesis of experimental models for antibody-mediated neuropathy, we studied variation in the activity of the classical and lectin pathway of the complement system in MMN patients and controls.

PART III- GENETICS OF MMN

We studied frequencies of HLA class I and II alleles in patients with MMN and controls, and studied the possible association of HLA alleles with clinical characteristics of MMN. Frequencies of SNPs in other candidate genes, i.e. *PTPN22*, *BANK1*, *Blk*, *FCGR2B*, *CD1A/E*, *TAG-1* and *MBL2*, were also studied in MMN patients and controls.

PART IV- TREATMENT IN MMN

We assessed safety, efficacy and convenience of a 10% ready-to-use liquid IVIg in comparison with a freeze-dried 5% IVIg preparation. We also studied perceived advantages and disadvantages of home-based IVIg treatment and hospital-based IVIg treatment. To evaluate which immunemodulatory effects might explain the beneficial effects in MMN, we studied the effects of IVIg on the titers and function of anti-GM1 IgM antibodies and the complement system in patients with MMN.

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Clinical features of MMN

2

Correlates of outcome and response to IVIg in 88 patients with multifocal motor neuropathy

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Neurology 2010;75(9):818-825.

ABSTRACT

Objective: Identification and examination of all patients with multifocal motor neuropathy (MMN) in The Netherlands to document the clinical spectrum, response to IVIg and to determine correlates of outcome.

Methods: A national cross-sectional descriptive study was performed. Ninety-seven patients were identified; 88 participated. Logistic regression analysis was used to study determinants of outcome.

Results: Age at onset was younger in men than in women (38 versus 45 years, $p = 0.05$). Onset of weakness was in distal arm (61%) or distal leg (34%), and occasionally in the upper arm (5%). Initial diagnosis was motor neuron disease in one-third of patients. Brisk, but not pathological, reflexes in weakened muscles were found in 8%. Conduction blocks were most frequently detected in the ulnar (80%) and median (77%) nerves, but occasionally only between Erb and axilla (6%), or in the musculocutaneous nerve (1%). Ninety-four percent responded to IVIg therapy: non-responders had longer disease duration before the first treatment ($p = 0.03$). Seventy-six percent received IVIg maintenance treatment at time of this study (median duration 6 years; range 0–17): the median dose increased over the years from 12 to 17 grams per week ($p < 0.01$). Independent determinants of more severe weakness and disability were axon loss ($p < 0.001$; $p < 0.0001$) and longer disease duration without IVIg ($p = 0.03$; $p = 0.07$).

Conclusion: The results of this study may aid to recognize the clinical picture of MMN. Early IVIg treatment may help to postpone axonal degeneration and permanent deficits.

Classification of evidence: This study provides Class IV evidence that IVIg improves muscle strength of patients with MMN and disability (defined as an increase of ≥1 MRC grade in at least two muscle groups without decrease in other muscle groups) in 94% (95% confidence interval, 86.8 to 97.4%) of patients.

INTRODUCTION

Multifocal motor neuropathy (MMN) was first described more than 20 years ago, as a pure motor neuropathy with conduction block (CB) characterized by slowly progressive, asymmetrical weakness of limbs.[1, 2] It is important to differentiate MMN from motor neuron disease (MND) because it is amenable to treatment and has a more favorable prognosis. Various open trials and four placebo-controlled trials have shown that treatment with IVIg leads to muscle strength improvement in MMN.[3-7] Of the immunosuppressants, cyclophosphamide has been reported effective but has severe side effects.[8] Plasmapheresis and corticosteroids are not effective and may even aggravate symptoms.[9, 10]

Several small and medium-sized cohort studies and a few elaborate reviews have highlighted clinical features of MMN,[11-16] but the relative rarity has precluded detailed studies of the phenotype and correlates of outcome in larger numbers of patients. We, therefore, tried to identify and re-examine all patients with MMN in the Netherlands (population 16.4 million)

to document the variety of clinical phenotypes and response to IVIg treatment. The relatively large number of patients enabled us to investigate the correlates of outcome in a multivariate analysis.

METHODS

Patients

The study had a cross-sectional descriptive study design and was conducted in The Netherlands from January until December 2007. To identify MMN patients, all Dutch neurologists (~900) were asked by letter to enroll MMN patients, a national neuromuscular database (CRAMP)[17] was screened and patients were informed by the Dutch Neuromuscular Patient Association.

Inclusion criteria were a diagnosis of definite, probable or possible MMN, according to previously published criteria.[18] In summary, all patients had slow or stepwise progressive limb weakness, no objective sensory abnormalities except for vibration sense at onset, no bulbar signs, no upper motor neuron signs, and no other cause of neuropathy or myopathy. Patients with "definite MMN" had at least one definite motor CB, patients with "probable MMN" had at least one probable motor CB and patients with "possible MMN" had conduction slowing compatible with demyelination in addition to elevated serum IgM anti-GM1 antibody titers or abnormal brachial plexus magnetic resonance imaging (MRI).[19] Patients had normal sensory nerve conduction in segments with motor CB and normal distal sensory nerve action potential amplitudes at the first nerve conduction study. IVIg responsiveness was not part of the diagnostic criteria.

Patients were requested to fill out a questionnaire and to attend the University Medical Center (UMC) Utrecht for a full neurological re-examination.

Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the institutional committee of the UMC Utrecht. All patients gave written informed consent prior to the study.

Questionnaires and physical examination

All patients completed a questionnaire designed to clarify their medical history, site of onset, initial diagnosis and treatment. Muscle strength was assessed bilaterally using a modified 10-grade scale of the Medical Research Council (MRC) ranging from 0 (= no movement, no contraction) to 5 (= normal). Eleven muscle groups of arms and seven muscle groups of legs were tested. Weakness was defined as an MRC score lower than 5. The MRC sum score was

calculated (maximum 180). Tendon reflexes of biceps, triceps, knee and ankle were scored as absent, normal or brisk. Sensory function was assessed bilaterally in arms and legs using a Rydel-Seiffer tuning fork for vibration sense.[20] Vibration sense was graded from normal (grade 0) to disturbed at the acromioclavicular joint or anterior superior iliac spine (grade 4).[21]

Laboratory studies

Serum samples were taken from all patients and tested for IgM anti-GM1 antibodies, using standardized enzyme-linked immunosorbent assay (ELISA).[22]

Nerve conduction studies

Nerve conduction studies were performed and studied by one investigator (H.F.). Prior to investigation, the extremities were warmed in water at 37°C for 30 minutes. Motor nerve conduction was investigated up to Erb's point in the median, ulnar, radial and musculocutaneous nerves, and up to the popliteal fossa in the peroneal and tibial nerves.[23] To assess CB, the CMAP on stimulation of the distal part of the segment with CB had to be at least 1mV; and 0.5mV to assess demyelinative slowing. Conduction abnormalities were defined as: definite CB (CMAP area reduction of at least 50%); probable CB (CMAP area reduction of 30–50% in an arm nerve[24]); demyelinative slowing (motor conduction velocity (MCV) <75% of the lower limit of normal, distal motor latency (DML) or shortest F-wave latency 130% of the upper limit of normal).

Presence of a decreased distal CMAP (distal CMAP amplitude below the lower limit of normal[25]) was scored for the median, ulnar, radial, musculocutaneous, peroneal and tibial nerves on both sides, and was considered to reflect axon loss.[25, 26]

Functional impairment and fatigue

Functional impairment was assessed using the Overall Disability Sum Score (ODSS) ranging from 0 (normal) to 5 for the arms and to 7 for the legs.[27]

Fatigue was scored using the Fatigue Severity Scale (FSS).[28] The patient was asked to read nine statements and circle a number from 1 (not very appropriate) to 7 (agreement). Final scores were calculated by determining the mean of responses. Severe fatigue was defined as a score exceeding the FSS 95th percentile (= 5) in healthy controls.

Statistical analysis and determinants of outcome

Differences were tested with the Mann-Whitney U test and the χ^2 test. To identify determinants of outcome, logistic regression analysis was performed. More severe weakness was defined as lower MRC sum score than the median, and more severe disability as a lower than median

ODSS score for arms and legs. Determinants for weakness and disability were first analyzed with univariate analysis. Next, multivariate analysis was performed to determine the independent contribution of each potential determinant. The determinants gender (male/female), symptom onset in a leg (yes/no), IgM anti-GM1 antibodies (positive/negative), severe fatigue (FSS 5 or more/FSS less than 5) were analyzed as dichotomous variables; age at symptom onset, years untreated (disease duration without IVIg), duration of IVIg treatment and axon loss (number of nerves with decreased distal CMAP) were analyzed as continuous variables.

Classification of evidence

This study provides Class IV evidence that IVIg improves muscle strength (defined as an increase of ≥1 MRC grade in at least two muscle groups without decrease in other muscle groups) in patients with MMN and disability.

RESULTS

Patients

Ninety-seven patients with MMN were identified. Eighty-eight patients (91%) agreed to participate. Fifty-five patients were recruited from the UMC Utrecht; 29 patients were enrolled by notified neurologists, and screening the neuromuscular database resulted in identification of an additional four patients.

Patient characteristics are shown in Table 2.1. The male to female ratio was 2.7:1. Age at onset was younger in men (38 years) than in women (45 years) ($p = 0.05$).

Onset of muscle weakness was most frequently in the distal arm (61%) or distal leg (34%). Muscle weakness occasionally started in the upper arm (5%), but never in the upper leg. Symptom onset was more often in the dominant hand ($p = 0.04$).

The initial diagnosis was different from MMN in the majority of patients. The median delay from first symptoms to the diagnosis of MMN has decreased ($p < 0.0001$) since the first descriptions[1,2]; from 1988 to 1995 median time to diagnosis was five years (range one to 15), from 1996 to 2000 three years (range one to 10) and from 2001 to 2006 two years (range one to five).

Weakness

The median MRC sum score was 166 (range 108–179), with a pattern of predominant distal weakness, more pronounced in arms than legs. Table 2.2 shows the distribution and severity of muscle weakness. Finger flexors were relatively spared compared to other distal muscle groups in arms. Weakness of the upper leg was found in patients with disease duration of 20 years or longer.

Table 2.1 Clinical features of 88 patients with MMN

Gender (male)	64 (73)
Age at symptom onset (yrs) (median, range)	40 (22-66)
Age at inclusion (yrs) (median, range)	52 (27-78)
Disease duration at inclusion (yrs) (median, range)	11 (2-43)
First symptom	
Reduced grip strength hand	22 (25)
Reduced dexterity hand	18 (21)
Extension weakness of fingers	14 (16)
Weakness adduction upper arm	4 (5)
Foot drop	28 (32)
Reduced mobility toes	2 (2)
Number of affected limb regions at inclusion	
one	12 (14)
two	15 (17)
three	13 (15)
four	19 (22)
five	12 (14)
six	11 (13)
seven	2 (2)
eight	4 (5)
Initial diagnosis	
MMN	31 (35)
Motor neuron disease	28 (32)
Mononeuropathy	11 (13)
Polyneuropathy	13 (15)
Radiculopathy	2 (2)
Chronic inflammatory demyelinating neuropathy	1 (1)
Hereditary neuropathy	1 (1)
Minor stroke	1 (1)
Time from disease onset to first IVIg treatment (yrs) (median, range)	5 (0-36)
Maintenance treatment IVIg	67 (76)
Titer serum IgM anti-GM1 antibodies	
1:400	14 (16)
1:800	9 (10)
1:1600	5 (6)
1:3200	0
1:6400	4 (5)
1:12800	1 (1)
1:25600	2 (2)
1:51200	3 (3)

Data are presented as numbers (%), unless otherwise specified; limb regions = upper arm, lower arm, upper leg and lower leg on both sides (maximally eight); yrs = years.

Table 2.2 Severity of muscle weakness of 88 patients with MMN

	MRC 5	MRC 4	MRC 0–3	Mean MRC grade
Upper arm				
Elbow extension	61 (69%)	25 (28%)	2 (2%)	4.7
Elbow flexion	58 (66%)	27 (31%)	3 (3%)	4.6
Shoulder abduction	50 (57%)	35 (40%)	3 (3%)	4.5
Lower arm				
Flexion fingers	70 (80%)	17 (19%)	1 (1%)	4.8
Wrist flexion	15 (17%)	65 (74%)	8 (9%)	4.6
Wrist extension	13 (15%)	55 (63%)	20 (23%)	3.8
Extension fingers	12 (14%)	40 (45%)	36 (41%)	3.3
Hand				
Adduction thumb	34 (39%)	44 (50%)	10 (11%)	4.2
Opposition thumb	26 (30%)	46 (52%)	16 (18%)	3.9
Spreading fingers	7 (8%)	44 (50%)	40 (42%)	3.3
Abduction thumb	6 (7%)	38 (43%)	44 (50%)	2.9
Upper leg				
Hip flexion	79 (90%)	9 (10%)	0	4.9
Knee flexion	79 (90%)	9 (10%)	0	4.9
Knee extension	81 (92%)	7 (8%)	0	4.9
Lower leg/ Foot				
Foot plantar flexion	67 (76%)	13 (15%)	8 (9%)	4.6
Flexion toes	52 (59%)	22 (25%)	14 (16%)	4.2
Foot dorsal flexion	28 (32%)	37 (42%)	23 (26%)	3.6
Extension toes	22 (25%)	38 (43%)	28 (32%)	3.6

Data are presented as numbers (%). MRC = MRC grade of weakest side; mean MRC grade = mean MRC grade of all patients.

Tendon reflexes

Seventy-three patients (83%) had one or more absent reflexes. In seven patients (8%) all reflexes were normal. In eight patients (9%) some or all reflexes were brisk (all reflexes three times; biceps and triceps reflexes three times; knee reflex two times); seven of these eight patients (8% of total) had weakness in the same segment where the tendon reflex was brisk. Brisk reflexes were easily elicitable reflexes, but without spread or clonus.

Sensory function

Abnormal vibration sense in distal leg was found in 19 patients (22%); grade 1 (disturbed at the interphalangeal joint of the hallux) in 16 patients and grade 2 (disturbed at the medial malleolus) in three patients. The median disease duration was longer (median 18 years, range 5–43, $p < 0.02$) compared to patients without sensory symptoms.

Laboratory studies

Sera from 38 patients (43%) contained IgM anti-GM1 antibodies with titers 1:400 or higher (Table 2.1).

Nerve conduction studies

Seventy-one patients (81%) had at least one definite CB, and 16 patients (18%) had no definite but at least one probable CB. Six patients (7%) had only one definite CB (median, ulnar, radial or musculocutaneous nerve), another six patients (7%) had only one probable CB (ulnar or median nerve). One patient (1%) had MCV and DML values compatible with demyelination but no CB. This patient had elevated IgM anti-GM1 antibody titers and abnormal brachial plexus MRI, and fulfilled the criteria of "possible MMN".

CB was most often detected in the ulnar (80%) and median nerves (77%) (Table 2.3). In five patients (6%), CB was exclusively detected in the proximal (Erb-axilla) segment (three times ulnar, two times radial nerves), and in one patient (1%) exclusively in the musculocutaneous nerve.

A decreased distal CMAP was most frequently found in the peroneal (66%) or median (60%) nerves followed by the tibial (47%), ulnar (37%), radial (21%) and musculocutaneous (10%) nerves. The number of nerves with a decreased distal CMAP in individual patients ranged from zero to 10.

Functional impairment and fatigue

Median ODSS of arms and legs combined was 4 (range 0–9). Sixteen patients (18%) reported minimal or no disability of the arms (ODSS 0 and 1). Fifty-four patients (61%) had moderate impairment of the arms (ODSS 2), whereas 18 patients (21%) reported severe disability (ODSS 3). Twenty-five patients (28%) had normal function of the legs (ODSS 0). Walking was mildly affected in 15 patients (17%) (ODSS 1). Forty-two patients (48%) walked independently but had abnormal gait (ODSS 2) and six patients (7%) needed unilateral support or a wheelchair (ODSS 3 to 6).

The mean response to the fatigue severity scale was 4.7 (range 2.7–7.0). Severe fatigue (FSS > 5) was present in 45 patients (51%).

Treatment

Eighty-four patients (95%) had received a first IVIg course at a cumulative dose of 2 g/kg. Four patients had never received IVIg, because they experienced no problems in their daily life or had limited weakness. Seventy-nine of 84 patients (94%) responded to therapy, defined as an increase of ≥1 MRC grade in at least two muscle groups without a decrease in other muscle

Table 2.3 Frequency and distribution of conduction block

	Definite CB				Probable CB				Definite or probable CB
Nerve	N (%) 88 (100)	Proximal (Erb-axilla)*	Upper arm*	Lower arm/ leg*	N (%) 88 (100)	Proximal (Erb-axilla)*	Upper arm*	Lower arm/ leg*	N (%) 88 (100)
Median	43 (50)	17	15	27	47 (55)	19	30	34	66 (77)
Ulnar	34 (40)	15	12	13	51 (59)	22	17	30	69 (80)
MusCut	2 (2)	2	0	0	7 (8)	9	0	0	8 (9)
Radial	17 (20)	7	11	0	27 (31)	18	14	0	35 (41)
Peroneal	6 (7)	NA	NA	6	0	NA	NA	0	6 (7)
Tibial	12 (14)	NA	NA	14	0	NA	NA	0	12 (14)
Total CB			139				193		

CB = conduction block; N = number (%) of patients with CB; * = individual patients may have more than one CB in the same nerve; Proximal (Erb-axilla) = number of CBs in the segment between Erb's point and the axilla; Upper arm = number of CBs in the segment of the upper arm; Lower arm/ leg = number of CBs in the segment of the lower arm or leg; MusCut = musculocutaneous nerve; NA = not applicable.

groups (95% confidence interval, 86.8–97.4%). Five patients (6%) did not respond to IVIg. Lack of response was associated with more axon loss ($p < 0.01$), and a longer disease duration before the first IVIg treatment ($p = 0.03$). Sixty-seven patients (76%) received IVIg maintenance treatment at time of this study. Median duration of maintenance treatment was six years (range 0–17) and median dose, converted to grams per week, gradually increased over the years from 12 to 17g/week ($p < 0.01$).

Seventeen patients (19%) did not use maintenance treatment for the following reasons: no beneficial effect (five patients), stable disease course without treatment (eight patients), a concomitant disease (bipolar disorder and Waldenström's macroglobulinemia) or adverse effects (severe erythema and thrombo-embolic complication).

Thirty-five (40%) patients had unsuccessfully used other immune modulating therapy; interferon beta (20%)[29], mycophenolate mofetil (15%)[30], cyclophosphamide (3%) or prednisone (2%).

Correlates of outcome

Univariate analysis suggested that more severe disability was associated with more axon loss, years untreated, symptom onset in a leg and presence of IgM anti-GM1 antibodies; multivariate analysis identified only axon loss ($p < 0.0001$) as an independent determinant of more severe disability (Table 2.4). More severe weakness was associated with axon loss (univariate $p < 0.0001$; multivariate $p < 0.001$) and years untreated (univariate $p < 0.01$; multivariate $p = 0.03$).

Table 2.4 Logistic regression analysis for determinants of disability

Determinant	Univariate	*p* (2-tailed)	Multivariate	*p* (2-tailed)
Axon loss	2.3 (1.6–3.3)	< 0.0001	2.1 (1.4–3.2)	< 0.0001
Years untreated	1.1 (1.0–1.2)	< 0.01	1.1 (1.0–1.2)	0.07
Symptom onset leg	5.0 (1.8–13.5)	< 0.01	2.6 (0.8–8.9)	0.13
Serum IgM anti-GM1 antibodies	2.7 (1.1–6.4)	0.03	2.4 (0.7–8.0)	0.14
Age at symptom onset (yrs)	1.0 (0.9–1.0)	0.37		
Gender	0.7 (0.3–1.8)	0.46		
Duration of IVIg treatment (yrs)	1.0 (0.9–1.1)	0.83		
Definite CB	1.0 (0.3–2.8)	0.95		
Severe fatigue	1.1 (0.5–2.5)	0.84		

Multivariate analysis was performed with selected determinants from the univariate analysis (with $p \leq 0.1$); Axon loss = number of nerves with decreased distal compound muscle action potential (minimum 0, maximum 12); Years untreated = years without maintenance treatment with IVIg; Definite CB = presence of at least one definite conduction block; Severe fatigue = Fatigue Severity Score > 5; Data are expressed as odds ratios (95% confidence interval).

DISCUSSION

We performed a nationwide study on MMN, and identified 97 patients in The Netherlands, which corresponds to a prevalence of at least 0.6 per 100,000 inhabitants.

Our study did not only confirm that MMN is more prevalent in men than women[12, 13, 16] but also showed that symptom onset was at a younger age in males. Clinical presentation and patterns of weakness were fairly homogeneous, with onset in distal muscle groups of an arm or leg in the great majority of patients. Ulnar, median, radial and tibial nerves were most commonly affected, with striking differences in weakness of muscles innervated by a common terminal nerve. For example marked weakness of the abductor pollicis brevis, but relatively preserved function of the flexor muscles of the first three fingers (both innervated by the median nerve), and weakness of intrinsic hand muscles without weakness of the flexor muscles of fingers four and five (ulnar nerve). These findings suggest that pathogenic mechanisms are length-dependent but also patchy.[15, 16]

Our results suggest that there are few reliable clinical hallmarks which suggest the diagnosis of MMN in a patient with asymmetrical limb weakness. Brisk, but not pathological, reflexes may occur even in weakened segments. Hence the finding of brisk reflexes but without spread or clonus may not always differentiate MMN from MND. Significant sensory disturbances from onset exclude MMN, and rather suggest Lewis-Sumner syndrome[31], but recently published studies showed reduced sensory nerve action potentials years after MMN onset.[32, 33] We found minor vibration sense disturbances at neurological examination in 22%. Sensory nerve conduction studies to confirm these clinical findings were not performed for this study. The median disease duration in the subgroup with sensory deficits in this study (18 years) and other studies was relatively long.[11-13]

Nerve conduction studies are crucial to distinguish MMN from MND and other mimics. All participating patients fulfilled the criteria for MMN that were published by our own group.[18] In contrast to other criteria, this allowed inclusion of patients with "possible MMN" (conduction slowing compatible with demyelination in addition to elevated IgM anti-GM1 antibody titers or abnormal brachial plexus MRI). The use of other sets of diagnostic criteria would have resulted in the exclusion of some patients. Using the criteria proposed by the American Association of Electrodiagnostic Medicine, we would have excluded 13 patients (12 with only one CB and one patient without CB).[24] However, the findings that all 13 patients responded to IVIg, and seven had IgM anti-GM1 antibodies further support the diagnosis MMN. The use of the electrophysiological criteria of the European Federation of Neurological Societies/ Peripheral Nerve Society Guideline[34] would have resulted in exclusion of the only patient without CB, but with nerve conduction slowing, IgM anti-GM1 antibodies and abnormal brachial plexus MRI. This patient responded to IVIg. This case and the use of an extensive nerve conduction protocol illustrate that CB can occasionally only be detected in proximal nerve segments or in nerves that are not routinely investigated, and that CB may also be absent in patients with a clinical phenotype of MMN.[35, 36] These rare patients should not be denied a trial with IVIg.

MMN is considered as a benign disease, but functional impairment can be quite serious. One-fifth of our patients reported severe disability of the arms, and more than half of MMN patients reported severe fatigue. Fatigue has a comparable prevalence among patients with other immune-mediated disorders of the peripheral nervous system.[37] Future studies are needed to study the origin, and possibly treatment, of fatigue in MMN. A relation with IVIg treatment cannot be excluded.

To analyze determinants of outcome, we used multivariate analysis, which showed that axon loss and years without treatment were associated with more severe weakness and disability. A decreased distal CMAP was used as criterion for axon loss in our study.[25, 26] Theoretically, a decreased distal CMAP may also reflect distal CB, or marked slowing distal to the most distal stimulation site.[38] However, stimulation of the median nerve distal to the wrist in 20 MMN patients with a decreased thenar CMAP did not reveal CB (unpublished observation) and marked distal slowing was found only in 3% of nerves.[25] Needle EMG was not routinely performed for this study, but we previously published results of 20 MMN patients in whom needle EMG was performed of each muscle from which a CMAP was recorded[39] showing a relatively good correlation: from 26 nerves with a low distal CMAP, 23 of the respective innervated muscles showed neurogenic changes on needle EMG (88%). In the same study we showed that axon loss, but not CB or demyelinative slowing, was an independent determinant of weakness in corresponding muscles. This was confirmed in the present study in which we were able to analyze more determinants due to the larger number of patients. The lack of correlation between CB or demyelinative slowing and severity of weakness may be explained by the finding that a substantial number (approximately one-third) of electrophysiological abnormalities are found in nerves innervating non-weakened muscles.[25]

In patients who received maintenance IVIg treatment, the median dose gradually increased over the years indicating less response over time. This is consistent with a previous study showing that IVIg has a beneficial long-term effect on muscle strength and disability but could not prevent a slight decrease in muscle strength and increase of axon loss.[23] Our present finding that 'years untreated' and not 'duration of IVIg treatment', was a determinant for more severe weakness and disability indicates that progression of weakness and axon loss were more pronounced in the years without than in the years with IVIg treatment. Other studies have also shown that axon loss is more extensive in patients with long disease duration without treatment,[7, 39] and that IVIg treatment may prevent axon loss.[40] Results of this study indicate that an early start of IVIg, followed by maintenance treatment, is at present the only intervention that may prevent axonal degeneration and a more severe outcome. Elucidation of the pathogenesis of axon loss in MMN would facilitate the development of new treatment strategies.

ACKNOWLEDGMENT

The authors thank the patients and referring physicians, especially Dr I.N. van Schaik and Dr J.T.H. van Asseldonk.

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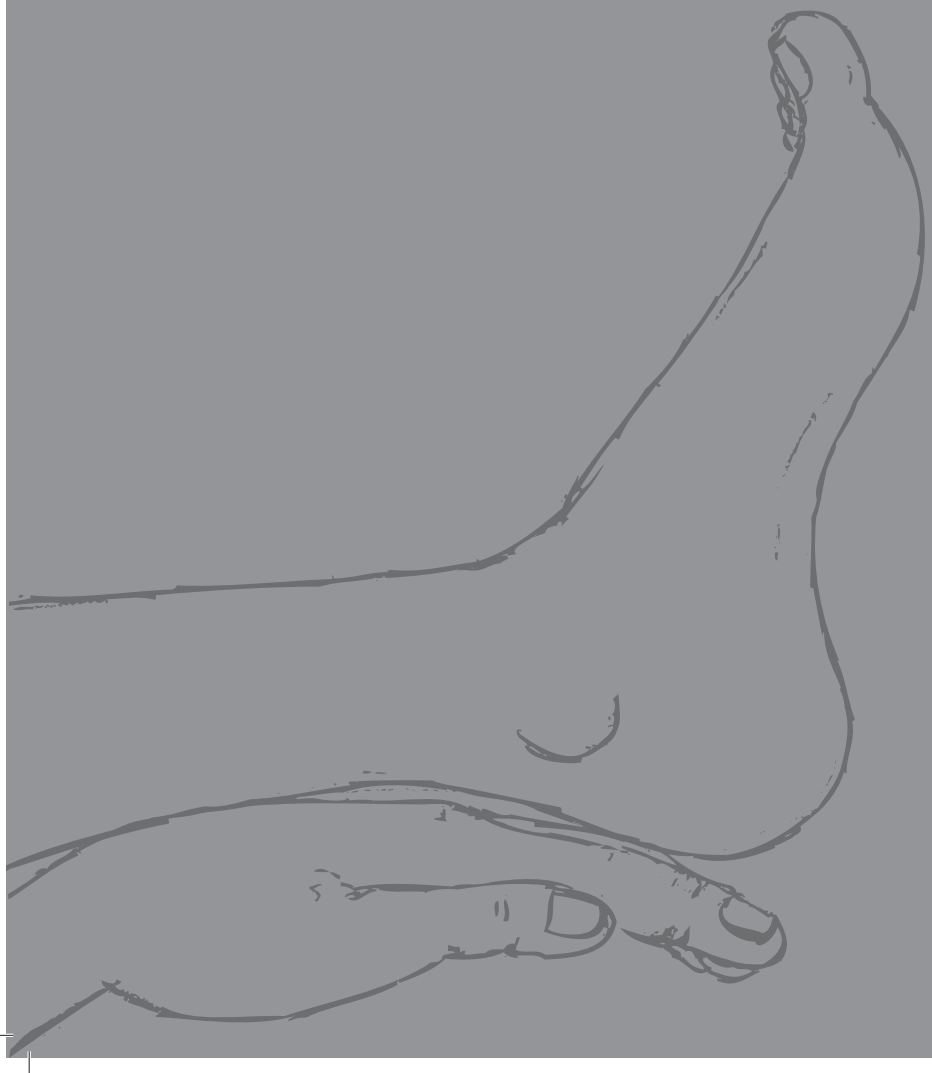
Immune pathogenesis of MMN



3

Multifocal motor neuropathy: association of anti-GM1 IgM antibodies with clinical features

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Neurology 2010;75(22):1961-1967.

ABSTRACT

Objective: To determine the prevalence and specificity of antibodies against single gangliosides and ganglioside complexes in serum from 88 patients with multifocal motor neuropathy (MMN) and to study the association with clinical features.

Methods: ELISA was used to detect IgM, IgG and IgA antibodies against GM1, GM2, GD1a, GD1b, GM1b, GT1a, GT1b, GQ1b, GalNAcGD1a and the glycolipid SGPG; absorption studies were performed to study cross-reactivity. Presence of antibodies against ganglioside complexes consisting of any of combinations of GM1, GM2, GD1a, GD1b, GT1b and GQ1b was also tested.

Results: Anti-GM1 IgM, IgG and IgA antibodies were detected in serum from 43%, 1% and 5% of MMN patients. Anti-GM2 IgM antibodies were detected in 6% and anti-GD1b IgM antibodies in 9% of patients. MMN patients with anti-GM1 IgM antibodies had more severe weakness ($p < 0.01$), more disability ($p < 0.01$) and more axon loss ($p = 0.05$) than patients without anti-GM1 IgM antibodies. Anti-GM1 IgM antibody titers correlated with MRC scores (correlation coefficient = 0.43; $p < 0.0001$). Anti-GD1b IgM antibody activity was associated with reduced vibration sense ($p < 0.01$). Absorption studies showed that anti-GD1b and anti-GM2 IgM antibodies cross-reacted with GM1. Antibodies against ganglioside complexes were not detected. Complexes containing GD1a, GD1b, GT1b or GQ1b with GM1 lowered antibody activity against GM1.

Conclusion: Anti-ganglioside IgM antibodies in MMN display limited specificity and are associated with severity and clinical characteristics. Results of this study suggest that anti-GM1 IgM antibodies may play a role in MMN pathogenesis.

INTRODUCTION

Multifocal motor neuropathy (MMN) is a chronic, immune-mediated neuropathy, characterised by asymmetric predominantly distal weakness of the limbs,[1, 2] conduction block (CB) and often by the presence of anti-GM1 IgM antibodies. Differences in laboratory techniques may explain the striking differences in reported prevalence (20-85%) of anti-GM1 IgM antibodies in serum from MMN patients.[3-6] IgM antibody activity against other gangliosides than GM1 has also been reported, but this has not yet been investigated in detail, most likely due to the rarity of the disease.[6, 7]

Gangliosides are glycolipids consisting of a ceramide moiety linked to varying numbers of externally exposed carbohydrate structures. Differences in numbers of oligosaccharides and sialylation explain their heterogeneity.[6] Ganglioside-specific antibodies are associated with inflammatory neuropathies, in particular the Guillain-Barré syndrome (GBS), and their specificity is associated with clinical features. Anti-GM1, GD1a, GalNAcGD1a, GD1b and GM1b antibodies are associated with axonal pure motor variants of GBS;[8-13] antibodies against GD3, GT1a and GQ1b are associated with ophtalmoplegia and the Miller Fisher syndrome and anti-GD1b antibodies with sensory atactic neuropathy.[6, 14, 15] In addition to antibodies against

single gangliosides, serum antibodies against complexes of GM1/GalNAc-GD1a, GD1a/GD1b, and GD1b/GT1b have also been detected.[16, 17]

Detailed association studies of ganglioside-specific antibodies with clinical characteristics of MMN have not been performed. We investigated in a national cross-sectional study in the Netherlands of 88 patients the prevalence and specificity of antibodies against single gangliosides and complexes of gangliosides and the association with clinical features.

METHODS

Patients

All 88 patients participated in a national cross-sectional study on MMN that was conducted in the Netherlands from January until December 2007. Patient characteristics are shown in Table 3.1.[18] Inclusion criteria were a diagnosis of definite (71 patients; 81%), probable (16 patients; 18%) or possible (one patient; 1%) MMN according to criteria published previously.[19] These criteria consist of a combination of clinical, laboratory and electrophysiological characteristics. Briefly, all patients had slow or stepwise progressive limb weakness, no objective sensory abnormalities except for vibration sense abnormalities at onset, no bulbar signs, no upper motor neuron signs and no other cause of neuropathy. Patients with "definite MMN" had at least one definite motor CB, patients with "probable MMN" had at least one probable motor CB and patients with "possible MMN" had slowing of conduction compatible with demyelination in addition to serum anti-GM1 IgM antibodies and abnormal brachial plexus magnetic resonance imaging.[20] Patients had normal sensory nerve conduction in segments with motor CB and normal sensory nerve action potential amplitudes on distal stimulation at the time of the first nerve conduction study.

All patients completed a questionnaire and were interviewed to document their medical history, onset of symptoms, and treatment history. At study entry, muscle strength of 18 muscle groups was assessed by one investigator (EAC) using a 10 point scale of the Medical Research Council (MRC). MRC sum scores were calculated, range from 0 (tetraplegic) to 180 (normal). Vibration sense was assessed bilaterally in arms and legs using a Rydel-Seiffer tuning fork.[21] Functional impairment was assessed using the Overall Disability Sum Score (ODSS) which ranks functional impairment in limbs from 0 (normal) to 5 for the arms and to 7 for the legs.[22]

Nerve conduction studies were performed in all patients according to a standardized protocol.[19] All nerve conduction studies were reviewed by one investigator (HF). Prior to investigation, the extremities were warmed in water at 37°C for 30 minutes. Motor nerve conduction was investigated on both sides in the median (recording from thenar and forearm muscles), ulnar, radial and musculocutaneous nerves up to Erb's point, and in the peroneal and tibial nerves up to the popliteal fossa.[19] The compound muscle action potential (CMAP) on distal stimulation of a nerve segment had to be at least 1mV to assess CB and at least 0.5mV

Table 3.1 Clinical characteristics and disability scores of 88 patients with MMN

Gender (male)	64 (73)
Age (years)	52 (27-78)
Age at symptom onset (years)	40 (22-66)
Time to diagnosis (years)	5 (1-36)
Medical Research Council sum score of 18 muscle groups	166 (108-179)
Abnormal vibration sense	19 (22)
Maintenance treatment intravenous immunoglobulin	67 (76)
IVIg grams per week	14 (5-100)
Nerve conduction study	
Definite conduction block	71 (81)
Probable conduction block	16 (18)
Features of demyelination without conduction block	1 (1)
Number of nerves with decreased distal CMAP	2 (0-10)
Overall Disability Sum Score arms	
0 Normal	2 (2)
1 Minor symptoms in one or both arms	14 (16)
2 Moderate symptoms in one or both arms	54 (61)
3 Severe symptoms in one or both arms preventing at least one listed function	18 (20)
4 Severe symptoms in both arms preventing all functions listed but some purposeful movements still possible	0
5 Severe symptoms in both arms preventing all purposeful movements	0
Overall Disability Sum Score legs	
0 Walking is not affected	25 (28)
1 Walking is affected but does not look abnormal	15 (17)
2 Walks independently but gait looks abnormal	42 (48)
3 Usually uses unilateral support to walk 10 metres (single crutch or arm)	4 (5)
4 Usually uses bilateral support to walk 10 metres (crutches, two arms)	0
5 Usually uses wheelchair to travel 10 metres (25 feet)	1 (1)
6 Restricted to wheelchair, but able to make some leg movements	1 (1)
7 Restricted to wheelchair/bed most of the day, no movements	0

Data are presented as median (range) or number (%); CMAP = compound muscle action potential.

to assess demyelinative slowing. Features of demyelination at entrapment sites, which are the elbow segment of the ulnar nerve and the fibular head segment of the peroneal nerve, were not analyzed and did not contribute to the diagnosis of MMN. Conduction abnormalities were classified as: definite CB (CMAP area reduction of at least 50%); probable CB (CMAP area reduction of 30-50% in an arm nerve); demyelinative slowing (motor conduction velocity (MCV) <75% of the lower limit of normal, distal motor latency (DML) or shortest F-wave latency >130% of the upper limit of normal).

Presence of a decreased distal CMAP (distal CMAP amplitude below the lower limit of normal) was scored for the median, ulnar, radial, musculocutaneous, peroneal and tibial nerves on both sides, and was considered to reflect axon loss.[23] Needle electromyography was not performed for this study.

Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the institutional committee of the University Medical Center Utrecht. All patients gave written informed consent prior to the study.

Determination of antibodies against single gangliosides

Serum samples were collected from all 88 patients and stored at -80° C until use.

The presence of IgM, IgG and IgA antibodies against the gangliosides GM1, GM2, GD1a and GD1b was tested using the standardized enzyme-linked immunosorbent assay (ELISA) of the Inflammatory Neuropathy and Treatment (INCAT) group with minor modifications.[24] In short, 300 pmol ganglioside/well was used. Serum samples were titrated using two-fold serial dilution series starting at 1:100. The titer was defined as the highest dilution that resulted in a corrected optical density (OD) (extinctions of ganglioside coated wells minus OD of non-coated wells) higher than the cut-off value (OD > 0.3 for IgM, OD > 0.2 for IgG and OD > 0.1 for IgA). Positive control sera containing anti-GM1 IgM, IgG, IgA, anti-GM2 IgM, anti-GD1a IgM, IgG and anti-GD1b IgG, and negative control sera (without anti-ganglioside antibody activity) were included in each experiment. Assays were performed in triplicate. Sera with anti-ganglioside IgM antibody titers of 1:400, and IgA and IgG titers of 1:100 and higher were considered positive. These cut-off levels precluded the inclusion of patients with unspecific, low titer anti-ganglioside antibodies, which are occasionally detected in sera from healthy donors and disease controls.[24]

IgM, IgG and IgA antibodies against GalNAc-GD1a, GM1b, GT1a, GT1b, GQ1b, and the glycolipid SGPG were detected by an ELISA as described elsewhere.[13] For this purpose, serum samples of all 88 MMN patients were shipped deep frozen to the laboratory of the Department of Neurology and Clinical Research at Niigata University, Japan. Control sera and unmarked duplicates were included to ensure validity and reproducibility. The cut-off level for this ELISA was defined as a corrected OD of 0.1 or higher and the highest dilution that yielded this OD was defined as the titer. The assays were performed in triplicate, and repeated to ensure reproducibility.

Absorption study

To examine cross-reactivity of anti-ganglioside IgM antibodies absorption studies were performed as described previously.[25, 26] In short, 300 pmol of GM1 was used to coat individual wells. Serum samples were added to the wells at the dilution that yielded an OD between 0.5 and 2.5. After overnight incubation at 4°C, these serum samples were harvested and used to test reactivity against a second ganglioside using ELISA. Absorption rates were expressed as percentages of the OD obtained with and without absorption. All assays were performed in duplicate.

Determination of antibodies against ganglioside complexes

IgM antibody activity against ganglioside complexes (combinations of any two of GM1, GM2, GD1a, GD1b, GT1b and GQ1b) was determined using 150 pmol/well for each ganglioside, and serum dilutions of 1:100. Gangliosides were mixed for 15 minutes before they were added to the wells in the ELISA plates. Assays were performed in duplicate. Antibody activity against ganglioside complexes was arbitrarily defined as a corrected OD 0.2 higher than the corrected OD for a single ganglioside.[16] To evaluate the effect of complex inhibition, i.e. decreased antibody activity against a mixture of two gangliosides in comparison with a single ganglioside,[15, 27] OD values were expressed as percentage activity (using the corrected OD) of a mixture of gangliosides compared to OD values when using only one ganglioside (i.e. only GM1 or GD1b).

Statistical analysis

The acquired data were processed and analysed with the use of the Statistical Package for the Social Sciences (version 12.0, SPSS Inc., Chicago). Differences in patient characteristics between groups were tested with the Mann-Whitney *U* test, the χ^2 or the Fisher exact test. Two-tailed *p*-values < 0.05 were considered significant. Demyelination (the number of nerves with conduction slowing and/ or prolonged distal latencies) and axon loss (the number of nerves with a decreased distal CMAP) were calculated as continuous variables (range 0 to 12 nerves).

RESULTS

Anti-ganglioside antibodies and cross-reactivity

Anti-GM1 IgM antibodies with titers 1:400 or higher were detected in serum from 38 MMN patients (43%) (Figure 3.1). Anti-GM1 IgA antibody activity was detected in serum from four MMN patients (5%) (titers 1:100 and 1:400); three of these patients had high anti-GM1 IgM antibody titers (> 1:6400), in the remaining patient IgA was the only anti-GM1 antibody isotype (1:400). Only one patient (1%) had anti-GM1 IgG antibody activity (titer 1:100), in addition to anti-GM1 IgA (titer 1:400) and anti-GM1 IgM (titer 1:51,200).

Antibody activity against GD1b was detected in serum from eight patients (9%) (titers ranging from 1:400 to 1:6400), these eight patients had anti-GM1 IgM antibodies (titers ranging from 1:400 to 1:51,200). IgM antibody activity against GM2 was detected in serum from five MMN patients (6%) (titers ranging from 1:400 to 1:1600); two of these patients had anti-GM1 IgM antibodies (both titers 1:800). Incubation of these sera with GM1 prior to application in GM2 and GD1b ELISA significantly reduced (33-70%) GD1b and GM2 specific ELISA signals, indicating cross-reactivity. Sera with IgM antibody activity against GM2 did not bind to GalNAc-GD1a.

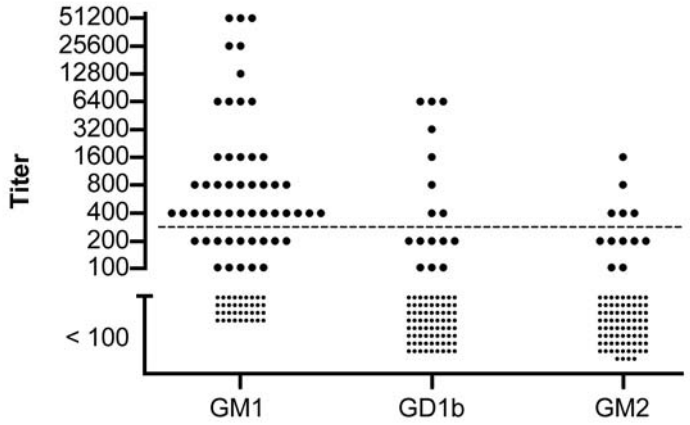

Figure 3.1 Anti-GM1, GD1b and GM2 immunoglobulin M antibody titers in serum from 88 patients with multifocal motor neuropathy.

In none of the 88 sera IgM, IgG or IgA antibody activity against GD1a, GalNAc-GD1a, GM1b, GT1a, GT1b, GQ1b or SGPG was detected.

Antibodies against ganglioside complexes and complex inhibition

Antibodies against ganglioside complexes were not detected. Complex inhibition i.e., a decreased signal of anti-GM1 IgM antibody activity in the presence of other gangliosides, was observed when GM1 was complexed with gangliosides expressing two or more sialic acids (GD1a, GD1b, GT1b and GQ1b). ELISA OD values of IgM antibodies against GM1/GD1a, GM1/GD1, GM1/GT1b and GM1/GQ1b were 65%, 77%, 62% and 76%, compared to OD values of GM1 ELISA. Addition of a second ganglioside with just one sialic acid had little effect; antibody activity to a mixture of GM1/GM2 was 93%.

Complex inhibition of anti-GD1b IgM activity was even more marked. ELISA OD values of IgM antibody activity to mixtures of GD1b/GD1a, GD1b/GT1b and GD1b/GQ1b were 55%, 61% and 63% of values against GD1b only. IgM antibody activity against the mixture of GD1b/GM2 was 87% of OD values against GD1b.

Associations with clinical features

Patients with anti-GM1 IgM antibodies had lower MRC sum scores ($p < 0.01$), more disability ($p < 0.01$) and more axon loss ($p = 0.05$) compared to patients without anti-GM1 IgM antibodies (Table 3.2). Furthermore, higher anti-GM1 IgM antibody titers were associated with lower MRC sum scores (Spearman correlation coefficient = 0.43; $p < 0.0001$) (Figure 3.2).

Table 3.2 Comparison of patients with and without anti-GM1 IgM antibodies

	Anti-GM1 IgM Negative (n = 50)	Anti-GM1 IgM Positive (n = 38)	*p* (2-tailed)
Gender (male)	37 (74)	27 (71)	0.76
Age at onset (years)	40 (22-63)	38 (23-66)	0.39
Time to treatment (years)	5 (0-16)	5 (1-22)	0.33
Duration MMN (years)	11 (2-43)	12 (2-38)	0.90
Age at inclusion (years)	52 (30-73)	55 (27-78)	0.37
MRC sum score	169 (126-179)	158 (108-179)	<0.01
Abnormal vibration sense	7 (14)	12 (32)	0.06
Maintenance IVIg	38 (76)	29 (76)	0.97
IVIg grams per week	13 (8-35)	15 (5-100)	0.09
ODSS arms and legs	3 (0-6)	4 (1-9)	<0.01
Definite motor CB	1 (0-8)	1 (0-6)	0.59
Probable motor CB	2 (0-7)	2 (0-9)	0.41
Demyelination	1 (0-6)	2 (0-9)	0.48
Axon loss	2 (0-7)	2 (0-10)	0.05

Data are presented as median (range) or number (%); MRC sum score = Medical Research Council score of 18 muscle groups (maximum score 180); IVIg = intravenous immunoglobulin; ODSS = Overall Disability Sum Score arms and legs (maximum score 12); CB = conduction block; Definite motor CB = number of definite motor conduction blocks; Probable motor CB = number of probable motor conduction blocks; Demyelination = number of nerves with conduction slowing and/ or prolonged distal latencies (maximum 12); Axon loss = number of nerves with decreased distal compound muscle action potential (maximum 12).

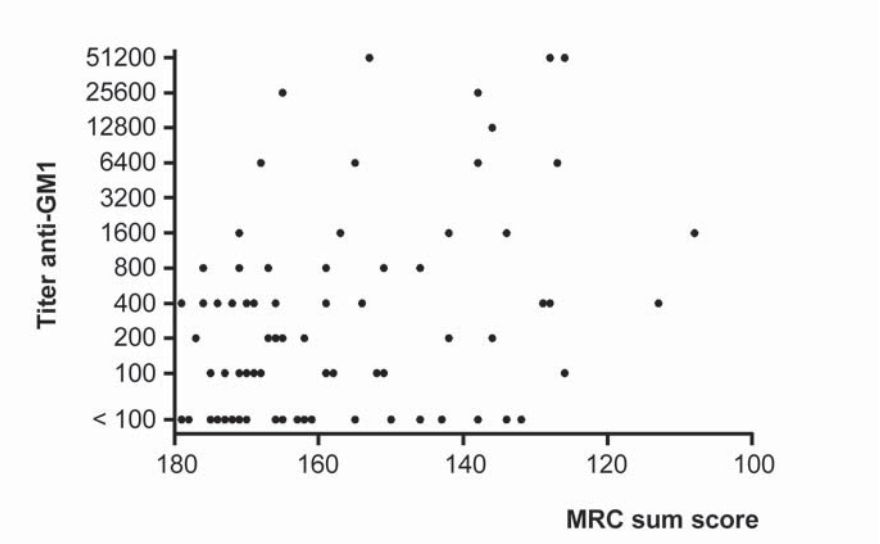

Figure 3.1 Correlation of anti-GM1 immunoglobulin M antibody titer and Medical Research Council sum score.

Abnormal vibration sense was more common in MMN patients with anti-GD1b IgM activity (n = 8) than in patients with anti-GM1 but without anti-GD1b IgM activity (n = 30, $p < 0.01$); disease duration in both groups was comparable. Patients with anti-GM1 and anti-GD1b IgM antibody activity also had more disability ($p = 0.04$) and more axon loss ($p = 0.04$) (Table 3.3).

DISCUSSION

This study of a large cohort of patients with MMN shows that the presence of anti-GM1 IgM antibodies is associated with more severe weakness and disability. Higher antibody titers correlated with more severe weakness. Axon loss, which is associated with weakness that is no longer amenable to treatment, was also more prevalent in patients with anti-GM1 IgM antibodies. The presence of anti-GM1 IgM antibodies cross-reacting with GD1b in eight patients was associated with discrete vibration sense abnormalities, which are per definition not present at onset of MMN but can develop in the course of many years in a subset of patients.[28] Sample sizes of previous studies may have been too small to show these associations.[29, 30] Results of

Table 3.3 Comparison of patients with anti-GM1 and with both anti-GM1 and anti-GD1b antibodies

	Anti-GM1 IgM Positive (n = 30)	Anti-GM1/-GD1b Positive (n = 8)	*p* (2-tailed)
Gender (male)	22 (73%)	5 (63%)	0.67
Age at onset (years)	38 (23-66)	43 (30-60)	0.42
Time to treatment (years)	5 (1-22)	4 (1-20)	0.74
Duration MMN (years)	11 (2-27)	15 (5-38)	0.18
Age at inclusion (years)	51 (26-77)	61 (42-75)	0.09
MRC sum score	162 (108-179)	135 (126-171)	0.06
Abnormal vibration sense	6 (20%)	6 (75%)	< 0.01
Maintenance IVIg	23 (77%)	6 (75%)	1.00
IVIg grams per week	15 (5-50)	19 (10-100)	0.07
ODSS arms and legs	4 (1-9)	5 (4-8)	0.04
Definite motor CB	1 (0-6)	1 (0-5)	0.27
Probable motor CB	3 (0-9)	2 (0-5)	0.52
Demyelination	2 (0-9)	1 (0-5)	0.94
Axon loss	2 (0-10)	6 (2-9)	0.04

Data are presented as median (range) or number (%); MRC sum score = Medical Research Council score of 18 muscle groups (maximum score 180); IVIg = intravenous immunoglobulin; ODSS = Overall Disability Sum Score arms and legs (maximum score 12); CB = conduction block; Definite motor CB = number of definite motor conduction blocks; Probable motor CB = number of probable motor conduction blocks; Demyelination = number of nerves with conduction slowing and/ or prolonged distal latencies (maximum 12); Axon loss = number of nerves with decreased distal compound muscle action potential (maximum 12).

this study suggest that anti-GM1 IgM antibodies may play a role in MMN pathogenesis, and that they may be a marker for a more severe disease course.

The associations of anti-GM1 IgM antibodies with more severe weakness and GD1b cross-reactivity with vibration sense abnormalities in patients with MMN may be explained by the distribution of the gangliosides in peripheral nerves and have an important analogy with variants of GBS. Pure motor forms of GBS are associated with anti-GM1 IgM and IgG antibodies[8, 9] and sensory ataxic forms with anti-GD1b IgM and IgG antibodies.[6, 14, 15] GM1 is especially highly expressed in axonal membranes of motor nerves and on the surface of Schwann cells.[6] Binding of GM1 antibodies to the axolemma at the nodes of Ranvier and Schwann cells may cause complement activation and eventually disruption of sodium channel clusters, resulting in characteristic nerve conduction abnormalities.[31, 32] GD1b has an increased expression in dorsal root ganglia and sensory nerves, and binding of antibodies may therefore explain the reduced vibration sense.[6] The majority of the patients with vibration sense abnormalities in our study had anti-GM1 IgM antibodies, often cross-reacting with GD1b. We did not find other signs of sensory involvement during clinical examination, which excludes Lewis-Sumner syndrome.[33] The slowly progressive disease course in MMN compared to GBS may be due to the fact that IgM antibodies do not pass the blood-nerve barrier as effectively as IgG.

Specificities of anti-ganglioside antibodies in sera from MMN patients are very limited. We detected antibody reactivity against GM1, GD1b and GM2, but not against any other ganglioside. We could not corroborate the finding of anti-GM1b and GalNAc-GD1a IgM antibodies reported previously in Italian and Japanese MMN patients.[34] The terminal structure of GM2 and GalNAc-GD1a is similar, but anti-GM2 IgM antibodies did not cross-react with anti-GalNAc-GD1a. Antibodies against GD1b and GM2 almost always cross-reacted with GM1, which suggests that antibodies specific for other gangliosides than GM1 are indeed very rare in MMN. This is in contrast with GBS, which is characterized by antibodies against a wide range of specificities.[35] Recently, antibodies against novel epitopes in ganglioside complexes have been detected in sera from patients with GBS, which were associated with specific clinical features.[17, 36] We hypothesized that sera from 'seronegative' MMN patients might also contain antibodies against ganglioside complexes, but we did not find evidence for this in any of our 88 patients.

Ganglioside complexes containing gangliosides with two or more sialic acid groups reduced binding to GM1. This phenomenon, known as complex inhibition, has been described previously in other inflammatory neuropathies[15] and in 11 patients with MMN,[37] and may be caused by conformational changes of ganglioside epitopes, or steric hindrance. Whatever may be the cause, these findings further emphasize the unique limited range of specificities of ganglioside-specific antibodies in MMN.

The large variability in the reported prevalence of anti-GM1 IgM antibodies in previous smaller cohorts of MMN patients is the result of differences in methodology to detect antibodies.[3-5] Low titers of anti-GM1 IgM antibodies were also found in sera from patients with motor neuron disease or other inflammatory diseases. We used INCAT methodology with a

cut-off that has been selected to include only antibody titers that are specific for inflammatory neuropathies.[24] Therefore, it seems unlikely that the associations with clinical features are the result of the inclusion of patients with an unspecific low titer of antibodies.

Results of this study show that anti-GM1 IgM antibody screening alone is the most efficient initial diagnostic serum study in the clinical evaluation of MMN, as opposed to broader arrays of anti-ganglioside antibodies at much higher costs, with small additional diagnostic yield. It remains to be established whether anti-GM1 IgM 'seronegative' patients with MMN have antibodies against other nerve constituents. The finding that MMN is characterized by a limited number of pathogenic B-cell clones may help to develop new and targeted immunotherapeutic strategies.

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4

Evidence for monoclonality of serum anti-GM1 antibodies in multifocal motor neuropathy

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In preparation.

ABSTRACT

Multifocal motor neuropathy (MMN) is an inflammatory polyneuropathy in which up to half of the patients have serum IgM antibodies against the ganglioside GM1. These antibodies usually remain present life long and show a highly restricted specificity, which might suggest a persistent proliferation of one or very few B-cell clones. To study the clonality of anti-GM1 IgM antibodies, we determined light-chains of anti-GM1 IgM antibodies in serum from 44 patient with MMN and 23 patients with Guillain-Barré syndrome (GBS). Exclusive use of either kappa or lambda light chains was found in 38 (86%) of patients with MMN. Four MMN patients had a paraprotein, two with the same light chain as theanti-GM1 antibodies. Exclusive use of a single light-chain was found in 9 (39%) patients with GBS. These results suggest that anti-ganglioside antibodies in most patients with MMN are produced by a single or very limited number of B-cell clones.

INTRODUCTION

A range of acquired polyneuropathies is associated with antibodies against glycoprotein or glycolipid constituents of peripheral nerves.[1] Specificities of these antibodies are often associated with clinical characteristics.[1-4] Antibodies against the ganglioside GM1, which is highly expressed in motor nerves, is associated with pure motor forms of Guillain-Barré syndrome (GBS) and with multifocal motor neuropathy (MMN).[1]

Molecular mimicry, a mechanism by which structural GM1 mimics in microbial lipo-oligo-saccharides provides the antigenic drive that leads to clonal proliferation of GM1-specific B-cells, underlies GBS pathogenesis.[5] Polyneuropathy with monoclonal gammopathy of undetermined significance (MGUS) is occasionally associated with anti-GM1 IgM antibodies, and may be caused by clonal proliferation of GM1-specific B-cells.[6] The pathogenic mechanism leading to anti-GM1 IgM production in patients with MMN is unknown and it is unknown whether MMN is characterized by monoclonal rather than polyclonal proliferation of B-cell clones.

We recently performed a national cross-sectional study on MMN in the Netherlands.[7] Serum samples of this relatively large cohort of MMN patients were used to characterise the anti-GM1 IgM antibody light chains and were compared with anti-GM1 IgM antibody light chain use of patients with GBS.

PATIENTS AND METHODS

Patients and serum samples

Patients were recruited through a national cross-sectional study on MMN. All MMN patients fulfilled the diagnostic criteria published previously.[8] Eighty-eight patients were included[7] and serum from all patients was tested for the presence of anti-GM1 IgM antibodies. Forty-four MMN

patients had elevated anti-GM1 IgM antibody titers and were included in the present study. Serum samples from 23 GBS patients who had participated in various treatment trials and who met the previously defined diagnostic criteria[9] with elevated anti-GM1 IgM antibody titers, but no IgG or IgA antibodies against GM1, were also tested. The study was approved by the Ethics Committees of the University Medical Center Utrecht and the Erasmus Medical Center Rotterdam and all patients gave informed consent. Serum from MMN patients receiving intravenous immunoglobulin (IVIg) maintenance treatment was obtained just before IVIg treatment and serum from GBS patients was obtained before IVIg therapy and within two weeks of onset of weakness.

Serum samples of two patients with a previously described MGUS polyneuropathy and monoclonal anti-GM1 IgM antibodies were used as positive controls.[10] Patient P1 had an IgM lambda M protein and lambda anti-GM1 antibodies (titer 3200), patient P2 had an IgM kappa M protein and kappa anti-GM1 antibodies (titer 25600). Serum samples of nine healthy controls without IgM anti-GM1 antibody activity were used as negative controls. Serum samples were stored at -80° Celsius until use.

Laboratory assays

All serum anti-GM1 IgM antibody titers were determined using the validated enzyme-linked immunosorbent assay (ELISA) of the Inflammatory Neuropathy Cause and Treatment (INCAT) group.[11] Anti-GM1 IgM antibody titers were defined as the reciprocal of the highest dilution that resulted in an optical density (OD) higher than the cut-off value of 0.3 after subtraction of the OD of non-coated wells. Positive serum samples were titrated using two-fold serial dilution series starting at 1:100.

The light chain of serum anti-GM1 IgM antibodies was assessed by using peroxidase-conjugated goat-anti-human kappa Ig (Sigma, A5175) and goat-anti-human lambda Ig (Sigma, A7164). GM1-coated plates were incubated with serum samples in a 1:100 dilution, washed, incubated for 1.5 hours at room temperature with K or L-specific antibodies and developed by adding substrate solution (citrate buffer, sodium hydrogen phosphate, milli-Q, o-phenylenediamine). Plates were read with an ELISA reader at 490 nm. The concentration of light chains of the anti-GM1 IgM antibodies was expressed as the equivalent of standard dilution series of a monoclonal IgG1 kappa and a monoclonal IgG1 lambda fraction purified from the plasma of myeloma patients with a purity of at least 95% (Sigma I5154 and Sigma I5029). Control experiments using monoclonal IgG1 kappa with anti-lambda and monoclonal IgG1 lambda with anti-kappa showed no cross-reactivity. Pilot studies showed that the detection limits were 0.013 μg/ml for IgK and 0.006 μg/ml for IgL. The antibodies were considered to be using only one light chain when the concentration of one of the light chains was below and the other above the detection limit. If both K and L signals were higher than the detection limits, results were used to calculate an IgK/IgL ratio.

Serum samples were tested for the presence of M protein by standard electrophoresis and immunofixation techniques.[12]

Statistics

Chi-square analysis using 2x2 tables was used to analyze differences in the distribution of patients with anti-GM1 IgM antibodies using a single light chain and both light chains. *P*-values < 0.05 were considered statistically significant.

RESULTS

Serum anti-GM1IgM antibody titers

The median titer of the anti-GM1 IgM antibodies in serum from MMN patients was 800 (range 100 to 51200, interquartile range (IQR) 400 to 1600) and in serum from GBS patients 800 (range 100 to 25600, IQR 200 to 3200). Serum samples from GBS patients had no detectable serum IgG or IgA antibodies to GM1; four MMN patients had IgA anti-GM1 antibody activity (titers 100 and 400) and one MMN patient had low IgG anti-GM1 antibody activity (titer 100). No anti-GM1 antibodies were found in the sera from the nine healthy controls.

Serum anti-GM1 IgM light chain concentrations

IgK or IgL light chain of anti-GM1 IgM antibodies was not detectable in serum from nine healthy controls. The anti-GM1 light chains in anti-GM1 IgM in serum of the positive controls with polyneuropathy associated with IgM monoclonal gammopathy were the same as the light chain of the M-protein: patient P1 with an IgM lambda M protein had anti-GM1 IgL concentration 0.089μg/mL and no detectable anti-GM1 IgK. Patient P2 with an IgM kappa M protein had anti-GM1 IgK concentration 0.710μg/mL and no detectable anti-GM1 IgL.

The concentration of the anti-GM1 IgK and IgL in the sera from the patients with MMN and GBS patients are shown in Figure 4.1. In 38 (86%) of 44 patients with MMN anti-GM1 antibodies of only one type of light chain were detected: exclusive IgK use was found in 20 patients (concentration range 0.089 to 2.179μg/mL), and exclusive IgL use in 18 patients (concentration range 0.010 to 0.334μg/mL). In addition, one patient had a serum IgK/IgL-ratio of > 10 (IgK 2.173μg/mL, IgL 0.010μg/mL, ratio 217.30) and two patients had a serum IgK/IgL concentration ratio of < 0.1 (IgK 0.010μg/mL, IgL 0.341μg/mL, ratio 0.03 and IgK 0.079μg/mL, IgL 0.890 μg/mL, ratio 0.09). The remaining three MMN patients had anti-GM1 antibodies with both K and L light chains with a IgK/IgL-ratio between 0.1 and 10. One of these patients had both anti-GM1 IgG and IgA antibodies.

Anti-GM1 IgM antibodies of nine (39%) out of 23 patients with GBS expressed a single light chain. The exclusive use of IgK was found in four patients (concentration range 0.216 to 1.329μg/mL) and the exclusive use of IgL in five patients (concentration range 0.010 to 0.047μg/mL). In addition, 3 patients had a serum IgK/IgL-ratio of > 10 (IgK 0.536μg/mL, IgL 0.010μg/

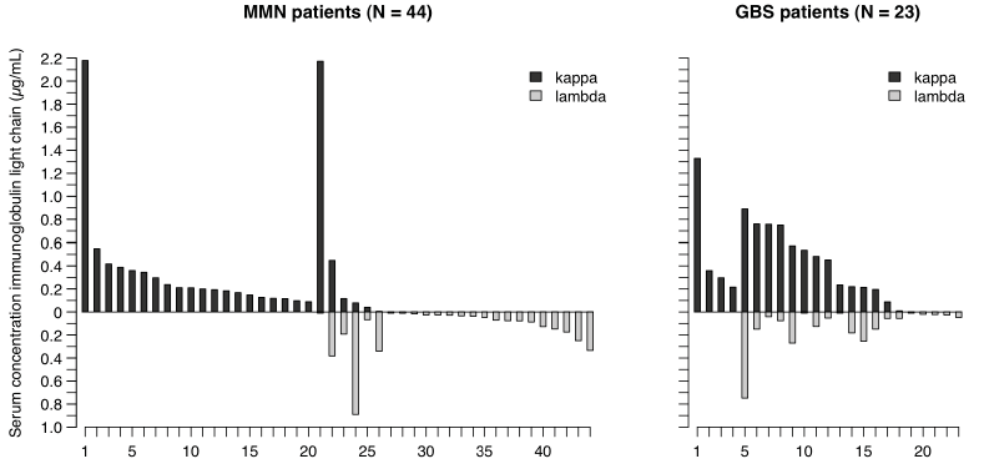

Figure 4.1 Concentration anti-GM1 IgK and IgL in sera from 44 patients with multifocal motor neuropathy and 23 patients with the Guillain-Barré syndrome.

mL, ratio 53.60; IgK 0.235µg/mL, IgL 0.010 µg/mL, ratio 23.50; IgK 0.757µg/mL, IgL 0.041 µg/mL, ratio 18.46) and no patients had a IgK/IgL-ratio of < 0.1. The remaining 11 patients had anti-GM1 IgM antibodies expressing both light chains with a IgK/IgL-ratio between 0.1 and 10. Patients with MMN were found to have anti-GM1 IgM antibodies using a single light chain more often than patients with GBS (χ^2 $p < 0.001$).

Serum M protein

An IgM M protein was identified in four MMN patients (patient 2 kappa and patient 22, 24 and 42 lambda. The light chains of the M protein and the serum IgM anti-GM1 antibodies (monoclonal) corresponded in patient 2 and 42. Patient 22 and 24 had IgM anti-GM1 antibodies with both IgL and IgK (IgL 382µg/mL, IgK 0.448µg/mL and IgL 0.890µg/mL, IgK 0.079µg/mL). Serum from GBS patients 2 and 22 contained an IgM M protein, and light chains of their M protein and serum anti-GM1 antibodies (monoclonal) were the same.

DISCUSSION

In this study we demonstrate that serum anti-GM1 IgM antibodies in the majority of patients with MMN contain either an IgL or IgK light chain. This suggests that these antibodies are monoclonal, in contrast to anti-GM1 IgM antibodies in GBS.

We developed a sensitive ELISA to detect IgL and IgK light chain use of anti-GM1 IgM antibodies. Anti-GM1 IgM antibodies with only one type of Ig light chain were found in 38 (86%) out of an unselected group of 44 patients with MMN and anti-GM1 IgM antibodies. Three more patients had an anti-GM1 IgK/IgL-ratio of > 10 or < 0.1, which also suggests a restricted use of one light chain. In contrast, we detected restricted use of only one light chain in a minority (39%) of patients with GBS.

GBS is the prototype inflammatory neuropathy associated with anti-GM1 antibodies, which are elicited by antigenic stimulation of GM1-specific B-cells by microbial GM1-mimics.[1, 13] These results indicate that GM1-specific B-cell clones in individual patients are not restricted in their use of either IgK or IgL. Moreover, frequencies of IgK and IgL in anti-GM1 IgM from patients with MMN and GBS were comparable, excluding the possibility of preferential use of one of the light chains for the production of anti-GM1 IgM.

However, anti-GM1 IgM antibodies in serum from 9 (39%) of 23 patients with GBS contained only a single light chain. This indicates that the GM1-specific B-cell repertoire recruited by strong external stimuli such as microbial GM1-mimics may be limited. Anti-GM1 IgM antibodies are part of the natural antibodies against bacterial constituents, which are produced by a limited number of B-cell clones. Although we cannot exclude the possibility that the innate B-cell repertoire for GM1 is more restricted in patients with MMN than with GBS, thus explaining the restricted use of light chains, this seems unlikely. In theory, the number of patients with a polyclonal response could be underestimated since the ELISA will not discriminate anti-GM1 antibodies derived from an oligoclonal population of B-cells using the same immunoglobulin light chain. However, the apparently random use of IgL and IgK in anti-GM1 IgM argues against this possibility.

The presence of an IgM M-protein in serum from patients with MMN would further support the monoclonality of anti-GM1 antibodies. Monoclonal anti-GM1 antibodies have previously been demonstrated in patients with chronic neuropathy and monoclonal gammopathy,[10, 14] and patients with B-cell lymphoma may develop a neuropathy similar to MMN.[15-17] However, serum samples from only two of our patients contained an IgM M protein with the same light chain as the anti-GM1 antibodies. This may suggest that the concentration of the anti-GM1 antibodies in serum was too low to be detected by electrophoresis and immunofixation. Previous studies have indeed shown that the amount of monoclonal antibodies is insufficient to produce a detectable M protein in the majority of patients with MMN. Only after purification of anti-GM1 antibodies in three MMN patients the typical monoclonal pattern of the antibodies was demonstrated.[14] The presence of monoclonal antibodies without detectable M protein in serum is not uncommon and has been found in patients with other auto-immune diseases[18] and even in healthy persons.[19]

There is convincing evidence from experimental studies that anti-GM1 antibodies interfere with nerve function and may contribute to the development of MMN.[20, 21] The monospecificity of antibodies associated with MMN2 and the restricted use of light chains

both support a monoclonal origin. Our results may indicate that single GM1-specific B-cell clones may proliferate due to unknown mechanisms of immune activation without progression to (premalignant) monoclonal gammopathy. GM1-specific B-cells are a possible target for therapy. Further characterization of GM1-specific B-cells may allow the development of selective depletion or inactivation strategies.

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5

Associated autoimmune diseases in patients with multifocal motor neuropathy and their family members

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Journal of Neurology, in press.

ABSTRACT

Multifocal motor neuropathy (MMN) is a rare immune-mediated disorder and is characterized by male predominance, the presence of serum anti-GM1 IgM antibodies in up to half of all patients, responsiveness to intravenous immunoglobulins (IVIg) and an increased frequency of HLA type HLA-DRB1*15. Aim of this study was to assess whether the frequency of autoimmune diseases (AID) is increased in patients with MMN and their first-degree family members, since this would indicate that MMN shares pathogenic mechanisms with other AID. We conducted a case-control study using questionnaires to evaluate the prevalence of AID in MMN and controls, and their first-degree relatives. Questionnaires from 81 MMN patients (417 first-degree relatives) and 438 controls (2,377 first-degree relatives) were analyzed. Overall prevalence of AID was higher in MMN patients (11%) than in controls (5%) (OR 2.4, 95% CI 1.1-5.5, $p = 0.037$). Type 1 diabetes, Hashimoto's thyroid disease, and celiac disease were significantly more prevalent in family members from patients than controls. The presence of an additional AID was not associated with age at MMN onset, disease duration, titer of serum anti-GM1 IgM antibodies or HLA type HLA-DRB1*15. The higher frequency of AID in patients with MMN indicates a common autoimmune diathesis.

INTRODUCTION

Multifocal motor neuropathy (MMN) is an immune-mediated chronic asymmetrical motor neuropathy.[1, 2] More than 90% of patients with MMN respond favourably to treatment with intravenous immunoglobulins (IVIg) and sera of up to 50% of patients with MMN contain IgM antibodies against the ganglioside GM1,[3] which is highly expressed in motor nerves.[4] MMN aetiology has not been fully elucidated. Anti-GM1 IgM antibodies probably play a pathogenic role in MMN.[5] We recently reported an association of MMN with HLA DRB1*15,[6] which might indicate that MMN shares pathogenetic pathways with other autoimmune diseases (AID). AID tend to cluster in relatives of patients with neurological AID such as multiple sclerosis (MS),[7] the Lambert-Eaton myasthenic syndrome (LEMS)[8] and myasthenia gravis (MG).[9] To assess whether patients with MMN share a common autoimmune diathesis we conducted a case-control study using questionnaires to compare frequencies of AID in controls and MMN patients, and their first-degree relatives.

MATERIALS AND METHODS

The cases were patients with MMN who participated in a national cross-sectional study on MMN conducted in the Netherlands from January until December 2007.[3] Inclusion criteria were diagnoses of definite, probable or possible MMN according to criteria published previously.[10] All MMN patients were approached by telephone and asked to complete a postal questionnaire. Non-responders were again contacted by telephone after three months.

Six hundred and fifty population-based controls from a control database[11] were approached by mail and asked to complete and return the postal questionnaire. People who returned incomplete questionnaires were contacted by telephone in order to complete the data.

The questionnaire was designed to document gender, date of birth and family history regarding first-degree relatives (parents, siblings), and the presence of the 18 most prevalent AID in the Netherlands according to the data collected by the Dutch Central Bureau for Statistics.

For each specific disease a description in layman's terms was provided and subjects were asked whether they or their parents or siblings (living and deceased) had any of the AID listed. Additional questions were asked if patients or controls reported any of the following three AID: for type 1 diabetes, age at disease onset and use of insulin were used to differentiate type 1 from type 2 diabetes.[12] Since previous studies showed that the presence of rheumatoid arthritis (RA) tended to be over-reported in a comparable study design,[7] subjects were asked if the affected individuals were being treated by a rheumatologist to differentiate between actual RA and other, non-AID with affected joints such as osteoarthritis. To differentiate between pernicious anaemia and other forms of anaemia, subjects were asked if the affected individuals were or had been treated with vitamin B12 injections.

Two diseases other than AID, i.e. myocardial infarction and asthma, were included in the questionnaire to control for reporting bias. These diseases have a high prevalence in the general population and no suspected relation with MMN or AID in general.

Both patients and controls who reported the presence of AID were contacted by phone and by a medical doctor to ensure the validity of the diagnosis.

The Medical Ethical Committee of the University Medical Center (UMC) Utrecht approved the study protocol and all participants gave written informed consent.

Prevalence of AID in patients and controls, as well as in families, was analysed with logistic regression analysis and adjusted for age. Differences between patients with and patients without an AID were tested using the Mann Whitney-*U* test and the χ^2 or Fisher's exact test when appropriate.

RESULTS

Eighty-eight MMN patients[3] and 600 potential controls were asked to participate in this study and were approached by mail. Questionnaires of 81 MMN patients (response rate 92%) and 438 (response rate 73%) controls were returned. In total, 2,794 relatives of 519 index cases and controls were included in this survey.

Characteristics of MMN patients and controls

Characteristics of MMN patients and controls are summarized in Table 5.1. The median duration of MMN at inclusion was 14 years (range 1-46 years) and the median age at onset of disease was 41 years (range 22-66 years). The control group (n = 438) included 108 women (25%), a percentage comparable to the patient group, but the median age at inclusion was higher in controls (p = 0.004). We adjusted for the difference in age in the statistical analysis.

AID in MMN patients and controls

Table 5.2 summarizes the prevalence of specific AID in MMN patients and controls. Nine MMN patients (11%) had one or more of the listed AID. One MMN patient (1.2%) had two of the listed AID: Crohn's disease and Bechterew's disease; the other seven MMN patients (8.6%) had one of the listed AID. The presence of AID was not associated with patient characteristics including age at MMN onset, disease duration, maintenance treatment with IVIg, muscle strength, presence and titer of serum anti-GM1 IgM antibodies, or the presence of HLA type HLA-DRB1*15.

Twenty-two of 438 controls (5%) had one or more of the listed AID. Four controls (0.9%) had more than one AID (psoriasis and RA; Graves' hyperthyroidism and RA; MG and RA; psoriasis and Hashimoto's thyroid disease).

Prevalence of AID was higher in MMN patients compared to controls (odds ratio (OR) 2.4, 95% confidence interval (CI) 1.1-5.5, p = 0.037). Females were more affected by AID compared to males: 50% of affected subjects in MMN patients were females (χ^2 p = 0.02) and 41% of affected indices in controls were females (χ^2 p = 0.07) while the total percentage of females in both groups was 25% (Table 5.1). Celiac disease (two MMN patients), Crohn's disease (one patient), Bechterew's disease (one MMN patient) and cutaneous lupus erythematosus (one MMN patient) were exclusively observed in MMN patients but not in controls.

Table 5.1 Characteristics of MMN patients and controls

	Patients	Controls
Total number of indices	81	438
• Number of affected indices (% of total)	9 (11%)	22 (5%)
Number of females (% of total)	20 (25%)	108 (25%)
• Number of females in affected indices (% of total)	5 (56%)	9 (41%)
Age (years) at inclusion (median, range)	55 (30-77)	60 (22-90)
• Age (years) at inclusion of affected indices (median, range)	49 (41-67)	54 (46-77)

Table 5.2 Prevalence of autoimmune diseases in patients and controls

Disease	Prevalence (%) MMN patients (n = 81)	Prevalence (%) controls (n = 438)
Diabetes type 1	0	0
Graves' hyperthyroidism	0	0.9
Hashimoto's thyroid disease	1.2	0.7
Addison's disease	0	0
Rheumatoid arthritis	0	1.8
Bechterew's disease	1.2	0
Systemic lupus erythematosus	0	0
Cutaneous lupus erythematosus	1.2	0
Sjögren's syndrome	0	0
Myasthenia gravis	0	0.2
Multiple sclerosis	0	0
Dermatomyositis	1.2	0
Psoriasis	2.5	1.1
Vitiligo	1.2	0.7
Crohn's disease	1.2	0
Ulcerative colitis	0	0.2
Celiac disease	2.5	0
Pernicious anaemia	0	0.2

AID in case and control families

Table 5.3 summarizes the prevalence of AID in relatives of MMN patients and controls. Twenty-eight out of 417 family members of patients with MMN had an AID (6.7%), whereas 157 out of 2,377 relatives from controls had an AID (6.6%) (OR 1.0, 95% CI 0.7-1.6; $p = 0.93$). Type 1 diabetes, Hashimoto's thyroid disease, and celiac disease were significantly more prevalent in family members of patients than of controls. To exclude reporting bias, patients and controls were asked whether they or family members had asthma or had experienced myocardial infarction. There was no significant difference in prevalence of asthma (patients 4.9%, controls 6.4%, $p = 0.71$; relatives of patients 2.1%, relatives of controls 2.4%, $p = 0.73$) or myocardial infarction (patients 1.2%, controls 4.7%, $p = 0.33$; relatives of patients 6.5%, relatives of controls 9.1%, $p = 0.08$).

Table 5.3 Prevalence of autoimmune diseases in relatives of MMN patients and controls

Disease	Prevalence (%) relatives MMN patients (n = 417)	Prevalence (%) relatives controls (n = 2,377)	*p*-value
Diabetes type 1	1.2	0.2	0.002
Graves' hyperthyroidism	0.7	0.8	n.s
Hashimoto's thyroid disease	1.4	0.5	0.01
Addison's disease	0	0	n.s
Rheumatoid arthritis	1.9	1.9	n.s
Bechterew's disease	0.7	0.3	n.s
Systemic lupus erythematosus	0	0	n.s
Cutaneous lupus erythematosus	0	0	n.s
Sjögren's syndrome	0	0	n.s
Myasthenia gravis	0	0.1	n.s
Multiple sclerosis	0.2	0.4	n.s
Dermatomyositis	0	0.04	n.s
Psoriasis	1.9	1.4	n.s
Vitiligo	0.7	0.2	n.s
Crohn's disease	0	0.6	n.s
Ulcerative colitis	0	0.3	n.s
Celiac disease	0.2	0	0.02
Pernicious anaemia	0	0.1	n.s

DISCUSSION

This study shows that AID may occur more frequently in patients with MMN compared to controls. This suggests that patients with MMN may have an increased risk for AID, and that MMN may share common pathogenic mechanisms such as genetic background, environmental triggers and/ or changed immune homeostasis with AID.[13, 14]

The main strengths of this study are the relatively large number of included patients with this rare neuropathy and the inclusion of relatively large numbers of gender-matched controls. Baseline characteristics of patients and controls were comparable except for age at inclusion, which was significantly higher in the control group (median age 55 versus 60 years). Recall bias or bias introduced by differences in response rates are unlikely, given the comparable prevalence of asthma and myocardial infarction in patients and controls. The prevalence of AID in controls was within the estimated range (5-9.4%) of the Dutch Central Bureau for Statistics.

Although the Dutch MMN patient cohort[3] is relatively large, the main limitation of this study remains its power. The study is clearly underpowered to detect associations of specific AID with MMN due to the rarity of both MMN and AID. In view of the association with elevated serum levels of anti-GM1 IgM antibodies, we hypothesized that B-cell dysfunction is an important underlying pathogenic mechanism in MMN. There was no co-segregation of mainly B-cell-mediated AID such as myasthenia gravis and systemic lupus erythematosus with MMN, but the numbers of patients and controls are too small to draw definite conclusions. Although the use of questionnaires may increase the risk of ascertainment bias, we feel this is unlikely. Telephone interviews were conducted to confirm the validity of reported diagnoses. The accuracy of reported diagnoses was also confirmed by the information (i.e. correspondence from medical specialists other than neurologists) available in patient files. We also chose to include only first-grade relatives to reduce the risk of including hearsay diagnoses in second- and third- degree relatives.

Frequencies of diabetes type 1, Hashimoto's thyroid disease and celiac disease were significantly increased among first-degree relatives of MMN patients as compared to controls. Celiac disease and Hashimoto's thyroid disease were also more frequent in patients with MMN than controls. Although these data should be interpreted with great care due to the small numbers, it is interesting to note that both type 1 diabetes and celiac disease,[15, 16] and type 1 diabetes and Hashimoto's thyroid disease[16, 17] co- segregate. MMN susceptibility may therefore be determined by genetic variants that are associated with these clusters, in addition to HLA- DRB1*15.[6]

The risk of additional AID was highest in female patients with MMN. This finding, combined with the predominance of men with MMN, may suggest gender differences in factors that determine susceptibility. Such differences have been identified previously in patients with other inflammatory neuropathies.[18] In concordance with the findings in control populations, the family histories of MMN patients show a predominance of women with an AID. Unlike the findings in LEMS[8] and MG,[9] we found no specific maternal transmission pattern of AID in patients with MMN.

Our data suggest that the frequency of additional AID is increased in patients with MMN. Studies with a larger sample size to ensure sufficient power are needed to detect associations with specific AID that could help to identify shared risk factors.

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6

Activity of the classical and lectin pathways in multifocal motor neuropathy

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In preparation.

ABSTRACT

Serum IgM antibodies against the ganglioside GM1 are detected in up to half of the patients with multifocal motor neuropathy (MMN). Complement activation by anti-GM1 antibodies plays an important role in the pathogenesis of the prototype antibody-mediated neuropathy acute motor axonal neuropathy, a variant of the Guillain-Barré syndrome (GBS) and in experimental models for antibody-mediated neuropathy. Single nucleotide polymorphisms of the mannose binding lectin gene (*MBL2*) are associated with severity of GBS. We hypothesized that complement activity may be a susceptibility marker or outcome marker for MMN and investigated whether the *MBL2* genotype, serum MBL concentrations and lectin and classical pathway activity as measured by enzyme-linked immunosorbent assays are associated with MMN. Seventy-nine MMN patients and 79 gender- and age-matched healthy controls participated in the study. Frequencies of *MBL2* genotypes did not differ between patients and controls. *MBL2* genotypes correlated with MBL serum concentration and lectin pathway activity in patients with MMN and controls. Classical and lectin pathway activity did not differ between patients with MMN and controls. Outcome and other patient characteristics were not associated with *MBL2* genotypes or serum MBL concentrations, or with complement pathway activity.

INTRODUCTION

Antibodies against glycolipids are associated with acute and chronic immune-mediated neuropathies.[1] Multifocal motor neuropathy (MMN) is a chronic immune-mediated neuropathy, causing asymmetric predominantly distal weakness of the limbs.[2, 3] Serum IgM antibodies against the ganglioside (glycolipid) GM1 are found in up to half of all MMN patients, and some MMN patients have additional IgA antibodies.[4]

Pathogenic properties of anti-ganglioside IgG antibodies have been shown using *in vitro* models for inflammatory neuropathy and animal models.[5-7] Anti-GM1 IgG antibodies are also associated with acute motor axonal neuropathy (AMAN), a variant of the Guillain-Barré syndrome (GBS). Complement activation by anti-ganglioside antibodies is crucial in the pathogenic cascade of GBS and complement-inhibiting drugs prevent damage to nerves.[7-9] A more severe disease course in GBS is associated with high serum concentrations of mannose binding lectin (MBL). Single nucleotide polymorphisms (SNPs) located in the promoter region and in exon 1 of the *MBL2* gene, associated with high lection pathway activity, were increased in GBS patients compared to healthy controls.[10]

The pathogenic properties of anti-GM1 IgM antibodies have been questioned, but several lines of evidence suggest that they play a role in the pathogenesis of MMN. Anti-GM1 IgM antibodies activate complement *in vitro*, in contrast to similar antibodies in serum from patients with motor neuron disease.[11] The presence of anti-GM1 IgM antibodies is associated with axonal damage and severe weakness in MMN.[4]

Anti-GM1 antibodies are natural antibodies which can activate the lectin pathway in addition to the classical pathway in some circumstances.[12-14] We hypothesized that relatively

high potency of classical and lectin pathways of complement could facilitate the pathogenic properties of anti-GM1 IgM antibodies in patients with MMN. We therefore compared activities of the classical and lectin pathways, *MBL2* haplotypes and MBL serum concentrations in patients with MMN and gender- and age-matched healthy controls.

METHODS

Patients and controls

Serum samples from 79 patients with MMN were used for the study. Patient characteristics were published previously.[15] All patients fulfilled the previously published diagnostic criteria for MMN.[16] Muscle strength was assessed bilaterally in all patients using a modified 10-grade scale of the Medical Research Council (MRC) ranging from 0 (= no movement, no contraction) to 5 (= normal). Eleven muscle groups of arms and seven muscle groups of legs were tested, and the MRC sum score was calculated (maximum 180). Functional impairment was assessed using the Overall Disability Sum Score (ODSS) ranging from 0 (normal) to 5 for the arms and to 7 for the legs.[17] Serum from 79 gender- and age-matched (+/- 5 years) healthy subjects served as controls.[18] DNA was extracted from venous blood using standard methodology. DNA samples were available for 75 MMN patients and 71 controls. All participants were Caucasian and of Dutch descent. The study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht and all subjects gave written informed consent. Since most MMN patients received intravenous immunoglobulin (IVIg) maintenance treatment at time of blood sampling, IgG levels were determined in serum of all participants by nephelometry (Beckman image 800).

Genotyping of *MBL2*

The (-221) X/Y promoter polymorphism and the three SNPs in exon 1 of the *MBL2* gene were determined using a previously described denaturing gradient gel electrophoresis (DGGE) assay[19] using a nested polymerase chain reaction (PCR) protocol.[20] For each sample, two PCR assays specific for the promoter X SNP (forward primer ATT TGT TCT CAC TGC CAC C; reverse primer GAG CTG AAT CTC TGT TTT GAG TT; annealing temperature 63°C; 25 cycles) or Y SNP (forward primer TTT GTT CTC ACT GCC ACG; same reverse primer and PCR conditions) were run. The two PCR products were diluted separately 1 : 100 in distilled water. *MBL2* exon 1 was amplified from these two dilutions with an extra GC-clamp attached to one primer to meet DGGE requirements [forward primer with 41-base pair clamp: CCG CCC GCC GCG CCC CGC GCC CGG CCC GCC GCC CCC GCC CCT CCA TCA CTC CCT CTC CTT CTC; reverse primer: GAG ACA GAA CAG CCC AAC ACG]. The amplified DNA was run overnight on a 6% polyacrylamide gel containing a denaturing gradient increasing linearly

from 35% to 55% formamide and urea. All *MBL2* exon 1 genotypes could be distinguished by their different patterns of migration. The corresponding *MBL2* X/Y promoter genotype could be inferred from the presence or absence of a product in the two nested PCR assays. Genotypes 0/0 and XA/0 were considered MBL-deficient, and genotypes YA/0, XA/XA, XA/YA and YA/YA were considered MBL-sufficient,[21, 22] with the YA/YA genotype related to the highest lectin pathway activity.

Serum concentration of MBL

Serum concentration of the multimeric MBL protein was determined in sera from patients and controls using a commercially available enzyme-linked immunosorbent assay (ELISA) (Sanquin). Briefly, serum was added to a mannan coated microtiter plate. After washing, horseradish peroxidase (HRP) conjugated antibody to MBL was added to the wells. After washing, substrate solution was added. The reaction was stopped chemically and the colour intensity was read in an ELISA reader (Bio-Rad) at 450nm. The MBL concentration of the sample was determined using a standard calibration curve.[20] The maximum concentration that could be measured with the ELISA was 4000 ng/ml.

Lectin and classical pathway activity

We determined C3 deposition via the lectin and classical pathway in serum from 79 MMN patients and 79 controls, using previously published ELISA-based methodology with minor modifications.[23, 24] In short, Nunc Maxisorb microtiterplates were coated with mannan (Sigma, M7504, 10µg/ml) for the lectin pathway and with human-IgM (Calbiochem, 3µg/ml) for the classical pathway in a 0.1M Na-carbonate buffer (pH 9.6), 50µl was added per well and incubated overnight at room temperature. One lane of wells was left uncoated as a control for aspecific complement activation. After each step plates were washed four times with phosphate buffered saline (PBS) containing 0.05% Tween-20. Residual protein binding sites were blocked with 80µl 1% bovine serum albumin (BSA) in PBS 0.05% Tween-20 for 1 hour at 37°C. Serum samples were diluted in GVBS++ (Veronal containing 0.05 mM $CaCl_2$, 0.025 mM $MgCl_2$ and 0.1% gelatine; pH 7.4-7.6). To correct for the day-to-day variation and the variation between the plates, 6 wells on each plate were incubated with pooled serum from 10 healthy donors. In order to block the contribution of the classical pathway to the complement activation by mannan, anti-human C1q (Sanquin, MW1828) was added[25] to the serum during assessment of the lectin pathway activity. All serum dilutions were added in triplicate, 50 µl per well and incubated for 1 hour at 37°C. Complement binding was detected by adding 50 µl mouse anti-C3 'WM1' digoxygenin (dig) labeled antibody (0.1µg/ml) for 1 hour at 37°C, followed by incubation with 50 µl peroxidase labeled anti-dig antibody (Roche) for 1 hour at 37°C. All detection antibodies were diluted in PBS containing 1% BSA and 0.05% Tween-20. Enzyme

activity of peroxidase was determined by adding 50 µl substrate solution (5.4ml Milli-Q, 600µl 1.1M NaAc pH6, 100 µl TMB 6mg/ml, 50µl ureumperoxidase 20mg/ml). The reaction was stopped by addition of 50 µl 1N H_2SO_4 and the optical density (OD) was measured at 450 nm using an ELISA reader (Bio-Rad).

Statistical analyses

Pearson's χ^2 test was used to verify if SNPs were in Hardy-Weinberg equilibrium and to compare the haplotype, genotype and allele frequencies between patients and controls. The independent samples T-test was used to compare age and the χ^2 test was used to compare gender between patients and controls. The Mann-Whitney *U* test was used to compare IgG levels, MBL concentration and lectin and classical pathway activity between patients and controls. Pearson Correlation was used to test for an association between complement activity, IgG level and clinical characteristics in the patient group. *P*-values < 0.05 were considered to be statistically significant.

RESULTS

Patients and controls

Median age of the 79 MMN patients was 52 years (range 27-78 years) and of the 79 controls 53 years (range 27-78 years) (p = 0.87). Sixty MMN patients and sixty-one controls were males (p = 0.85). Seventy MMN patients (89%) received maintenance IVIg treatment every few weeks. The median IgG level was 18g/L in patients (range 7-40g/L) and 11g/L in controls (range 7-28g/L) (p < 0.001).

Genotyping of *MBL2*

MBL2 gene promoter and exon 1 polymorphisms could be tested in 75 MMN patients and 71 controls. The SNPs were in Hardy-Weinberg equilibrium. Table 6.1 shows the genotype frequencies of MMN patients and controls. There were no significant differences in haplotype distributions (Pearson's χ^2 p = 0.17), genotype distributions (Pearson's χ^2 p = 0.18 for X/Y and p = 0.19 for A/0), or allele distributions (Pearson's χ^2 p = 0.45 for X/Y and p = 0.10 for A/0).

Serum concentration of MBL

The median serum concentration of MBL was 1550ng/ml (range 40-4,000ng/ml) in 79 MMN patients and 1760ng/ml (range 20-4,000ng/ml) in 79 controls (Mann-Whitney U p = 0.97). The MBL concentration correlated with the genotype in patients and controls (Table 6.1).

Table 6.1 *MBL2* genotype, MBL concentration and lectin pathway activity in MMN patients and controls

	Genotype	n (%)	MBL concentration, ng/ml (range)	Lectin pathway activity, % (range)*
Patients (n = 75)	YA/YA	29 (39)	3120 (900-4,000)	147 (0-329)
	YA/XA	18 (24)	1900 (620-3,400)	165 (0-319)
	YA/0	15 (20)	520 (140-1,300)	14 (0-176)
	XA/XA	4 (5)	580 (140-1,060)	6 (0-63)
	XA/0	4 (5)	90 (40-160)	0 (0-4)
	0/0	5 (7)	80 (60-140)	0 (0-0)
Controls (n = 71)	YA/YA	19 (27)	3540 (1,640-4,000)	206 (0-340)
	YA/XA	19 (27)	2180 (660-3,600)	178 (16-347)
	YA/0	23 (32)	580 (200-2,200)	29 (0-200)
	XA/XA	0	-	-
	XA/0	4 (6)	70 (20-410)	2 (0-6)
	0/0	6 (8)	40 (20-80)	0 (0-6)

* In relation to activity of pooled serum from 10 healthy donors (arbitrarily set at 100%).

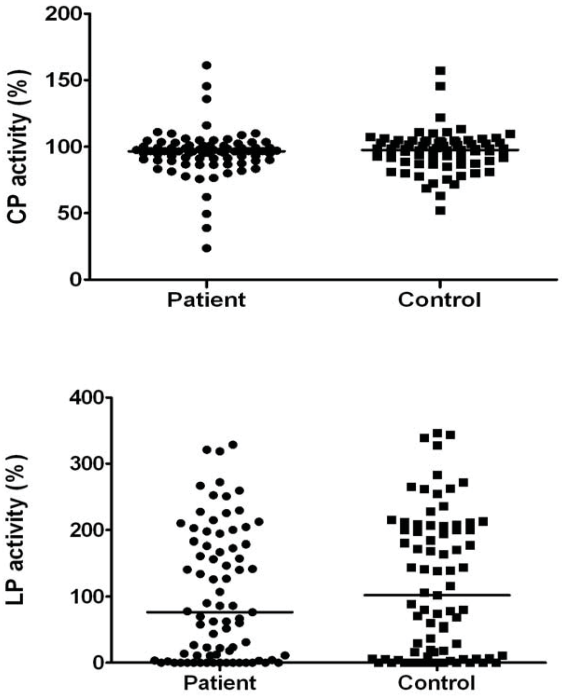

Figure 6.1 Classical pathway and lectin pathway activity in MMN patients and controls. Classical pathway (CP) and lectin pathway (LP) activity in 79 MMN patients and 79 controls relative to activity of pooled serum from 10 healthy donors (arbitrarily set at 100%). Medians are shown.

Lectin and classical pathway activity

Figure 6.1 shows the activity of the lectin and classical pathway of 79 MMN patients and 79 controls relative to the activity of pooled serum from 10 healthy donors (activity of pooled serum was arbitrarily set at 100%). The median lectin pathway activity was 77% (range 0-329%) in MMN patients and 96% (range 0-347%) in controls (Mann-Whitney *U* $p = 0.37$). The lectin pathway activity correlated with the genotype and MBL concentration in MMN patients and controls (Table 6.1).

The median classical pathway activity compared to the activity of the pooled serum from 10 healthy donors was 96% (range 24-161%) in MMN patients and 98% (range 52-157%) in controls (Mann-Whitney *U* $p = 0.32$).

There was no significant correlation in MMN patients between IgG concentration and lectin pathway activity (Pearson Correlation $p = 0.18$) or classical pathway activity ($p = 0.30$). There was no significant correlation in MMN patients between lectin pathway activity and MRC sum score ($p = 0.14$) or ODSS ($p = 0.14$) or between classical pathway activity and MRC sum score ($p = 0.16$) or ODSS ($p = 0.57$).

DISCUSSION

In this study we did not find differences in lectin and classical pathway activity, MBL concentrations or *MBL2* genotypes between patients with MMN and healthy controls, or between MMN patients with more severe or relatively mild disease course. These findings suggest that high innate lectin or classical pathway activity is not a significant risk factor for the development of MMN or FOR a more severe disease course.

This study has certain limitations. First, we assessed complement pathway activity using ELISA methodology. ELISA may not be sufficiently sensitive to detect biologically relevant differences in complement pathway activity. Since the activity of both pathways was slightly lower in patients with MMN than in controls, the chance that we missed a relevant effect seems small. The accuracy of the comparable lectin pathway activity in patients and controls was supported by the similar distributions of *MBL2* genotypes in patients and controls. Although the frequency of the YA/YA genotype was slightly higher among patients than controls, the finding that *MBL2* genotypes associated with MBL deficiency (XA/0 and 0/0)[21, 22] were found in 12% of patients indicates that MBL-deficiency does not protect against MMN. A virtual increase of the number of Dutch controls using data published previously[10, 14] would not have changed our results. Second, most of the patients with MMN in this study used IVIg maintenance therapy. This might have caused decreased complement activity,[12, 13] but IgG concentrations did not inversely correlate with complement activity. To clarify the effects of IVIg maintenance treatment, complement pathway activity should ideally also be measured in patients with MMN naïve for treatment with IVIg.

The hypothesis that anti-GM1 IgM antibodies in MMN patients are pathogenic is supported by the fact that they are more frequently observed in MMN patients than (disease) controls,[26] that they are associated with disease characteristics,[4] and by the analogy with AMAN.[1] However, data that would directly support complement activation in patients with MMN, for example the presence of complement split products or C1q-C4 or C1q-C3 complexes, have to the best of our knowledge not been published.[27, 28] Measuring complement activation products may be challenging due to the probably very localized and low-intensity complement-mediated cytotoxicity in MMN. As things stand, we cannot exclude the possibility that complement activation is less important than we previously assumed. Complement-independent inhibiting effects of anti-ganglioside antibodies on neurite outgrowth have recently been shown.[29] However, it is important to point out that the lack of an association in this study does not exclude a role of complement in MMN pathogenesis.

Our data are obviously different from findings in patients with GBS, where a clear association of *MBL2* genotype, high concentration of serum MBL and high lectin pathway activity was found with the severity of the disease.[10] Despite the fact that anti-GM1 antibodies can be found in both patients with GBS and MMN, our data suggest that the role of complement may differ between these patients. An explanation might be that high MBL concentrations and activity of the lectin pathway may facilitate the 'molecular mimicry' processes that eventually lead to the production of anti-GM1 IgG antibodies in GBS rather than antibody-mediated damage to nerves. GBS with anti-GM1 antibodies is often preceded by infections with *Campylobacter jejuni* (*C. jejuni*) strains that express GM1-mimics on their lipo-oligosaccharides,[30, 31] whereas MMN is not.[32] Further dissection of the pathways that result in the production of anti-GM1 IgM antibodies in patients with MMN may eventually explain why the antibodies in GBS and MMN are similar, but complement pathway activity is not.

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Genetics of MMN

7

Increased frequency of HLA-DRB1*15 in patients with multifocal motor neuropathy

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Neurology 2010;74(10):828-832.

ABSTRACT

Objectives: The favorable response to treatment with intravenous immunoglobulins and the presence of IgM antibodies to the glycolipid GM1 are indications that inflammation underlies multifocal motor neuropathy (MMN) pathogenesis. We investigated the association of MMN with human leukocyte antigen HLA class I and II antigens.

Methods: HLA class I and II antigens of 74 Dutch patients with MMN and 700 controls were determined in a case-control study. Associations of HLA types with MMN disease characteristics were investigated.

Results: Compared with controls, patients with MMN had higher frequencies of HLA-DRB1*15 (41 vs 24%, p = 0.0017). Disease characteristics were not associated with specific HLA types.

Conclusions: Similar associations were found in patients with multiple sclerosis (MS) and women with chronic immune-mediated demyelinating neuropathy (CIDP) which may suggest that these demyelinating disorders share pathogenic mechanisms.

INTRODUCTION

Multifocal motor neuropathy (MMN) is characterized by slowly progressive asymmetrical limb weakness, predominantly in the distal muscle groups of the arm, with onset usually between the third and fifth decade of life and a higher prevalence in men.[1-4] The favorable response to treatment with intravenously applied immunoglobulins combined with the frequent presence of antibodies to the glycolipid GM1, which is expressed in peripheral motor nerves, suggest a role for immune-mediated mechanisms in MMN pathogenesis.[5-10]

The highly polymorphic human leukocyte antigen (HLA) system is crucial for antigen presentation of derived peptides to T-cells and the adaptive immune response. Many autoimmune diseases are associated with specific HLA alleles.[11] Associations of neurological disorders with HLA types may be unique, such as the association of multiple sclerosis with DR15 and DQ6,[12] but may also be a shared characteristic, such as the association of both acetylcholine receptor antibody positive myasthenia gravis and Lambert-Eaton myasthenic syndrome with DR3 and B8, suggesting similar pathogenic mechanisms.[13]

We assessed whether HLA class I and II antigens are associated with increased susceptibility to MMN or influence MMN disease course.

METHODS

Study population

From 2002-2007, 74 patients with a diagnosis of definite or probable MMN according to previously published criteria, were recruited at the University Medical Center Utrecht, a tertiary

referral clinic for patients with neuromuscular disorders in The Netherlands.[1] All patients had a clinical phenotype compatible with a diagnosis of MMN and definite (i.e. segmental CMAP area reduction of at least 50%, or of at least 30% over 2.5 cm on inching) or probable (i.e. segmental CMAP amplitude reduction of at least 30% in an arm nerve) conduction block in the median (recording m. abductor pollicis brevis and m. flexor carpi radialis), ulnar (recording m. abductor digiti V), radial (recording m. extensor carpi ulnaris) and musculocutaneous (recording m. biceps brachii) nerves, which were investigated up to Erb's point, or the peroneal (recording m. extensor digitorum brevis) and tibial (recording m. abductor hallucis) nerves, which were investigated up to the popliteal fossa.

Decreased distal CMAP (distal CMAP amplitude below the lower limit of normal) was scored for the median, ulnar, radial, musculocutaneous, peroneal and tibial nerves on both sides, and was considered to reflect axon loss.[14, 15]

Sex, age at onset, site at onset, the presence of anti-GM1 IgM antibodies, abnormal MRI of the brachial plexus, response to IVIg therapy, disability and muscle strength were documented. Onset of disease was defined as onset of first weakness. Serum anti-GM1 IgM antibody titers were assessed by a validated enzyme-linked immunosorbent assay (ELISA). Sera were considered positive if anti-GM1 IgM titers were ≥ 1:400, since this cut-off allowed distinction between patients with inflammatory neuropathy and unspecific antibody titers in healthy and disease (i.e. motor neuron disease) controls.[16] MRI of the brachial plexus was performed according to a protocol described previously.[17] Abnormal MRI signal of the brachial plexus was defined as swelling and/or increased signal intensity on T2-weighted magnetic resonance imaging.[17] Response to therapy was defined according to criteria published elsewhere.[18] Disability was scored according to 1) the Modified Rankin Scale, ranging from 0 = no symptoms at all to 5 = severe disability, requiring constant nursing care and attention, and 2) the Overall Disability Sum Score which ranks functional impairment in limbs from 0 (normal) to 5 for the arms (severe symptoms in both arms preventing all purposeful movements) and to 7 for the legs (restricted to wheelchair or bed most of the day, preventing all purposeful movements).[19] Muscle strength was assessed bilaterally by one investigator (E.A.C.) using a modified 10-grade scale of the Medical Research Council (MRC) ranging from MRC 0 (= no movement, no contraction) to MRC 5 (= normal strength). MRC grading was performed for shoulder abduction, elbow flexion, elbow extension, wrist extension, wrist flexion, finger extension, finger flexion, finger spreading, thumb abduction, thumb adduction, thumb opposition, hip flexion, knee flexion, knee extension, foot dorsal flexion, foot plantar flexion, toe extension and toe flexion. The MRC sum scores were calculated by summation of all MRC values (maximum score 180).

Seven hundred Dutch blood bank donors were included as controls.

HLA typing

In the 74 patients with MMN and 700 population-based controls, typing for HLA class I (HLA-A and HLA-B) was performed by serology using conventional complement-dependent cytotoxicity assays and for HLA class II (HLA-DRB1 and HLA-DQB1) by PCR amplification with sequence-specific primers. We used WHO nomenclature for each HLA type, including splits and broads for HLA class I serology (for example, HLA-A9 encompasses A24).

Statistical analysis

Twenty-seven HLA types (6 HLA-A broad, 1 HLA-A split, 7 HLA-B broad, 2 HLA-B split, 7 HLA-DR, 4 HLA-DQ) had frequencies > 15% in either patients or controls. Only these antigens were analyzed for association with MMN susceptibility using the χ^2 test or Fisher's exact tests. Because 27 HLA types were considered in the analysis, a Bonferroni correction factor of 27 was applied and a p-value < 0.00185 was considered significant. Possible associations with age at onset, elevated titers of anti-GM1 IgM antibodies (≥ 1:400), abnormal MRI of the brachial plexus, response to IVIg therapy, numbers of definite and probable CB, numbers of nerves with decreased CMAP amplitudes, and disability measured by the Rankin scale, the ODSS (arm and leg) and the MRC sum score were analyzed by the χ^2 test or Fisher's exact tests.

Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the institutional ethical committee of the University Medical Center Utrecht. All patients gave written informed consent prior to the study.

RESULTS

Characteristics of 74 patients with MMN and 700 controls are shown in Table 7.1 Clinical characteristics of patients with MMN were similar to those reported in previous studies.[20]

Table 7.2 shows the HLA types with frequency > 15% in either patients or controls. Of these 27 antigens, significantly increased frequencies of HLA-DRB1*15 (41% vs 24%; OR 2.2, 95% CI 1.3 to 3.6; $p = 0.0017$) were observed in patients with MMN.

Median age at onset, elevated titers of anti-GM1 IgM antibodies in serum, response to IVIg therapy, disability scores and MRC sum score, numbers of definite and probable CB, and numbers of nerves with decreased CMAP amplitudes, were not significantly associated with HLA-DRB1*15.

Table 7.1 Characteristics of patients and controls

	MMN, n = 74	Controls, n = 700
Male, n (%)	54 (73)	419 (60)
Median age at examination, yr (range)	52 (27-78)	50 (23-70)
Median age at onset, yr (range)	38 (22-66)	–
Anti-GM1 antibodies, titers ≥ 1:400, n (%)	30 (41)	–
Abnormal MRI brachial plexus, n (%)	23 (31)	–
Response to therapy	65 (88)	–
Median MRC sum score (range)	166 (108-179)	–
Definite MMN, n (%)	60 (81)	–
Probable MMN, n (%)	14 (19)	–
Definite CB in arm nerves, n (%)	59 (80)	–
Probable CB in arm nerves, n (%)	65 (88)	–
Definite CB in leg nerves, n (%)	15 (20)	–
Probable CB in leg nerves, n (%)	2 (3)	–

CB = conduction block; MMN = multifocal motor neuropathy; MRC = Medical Research Council. MRI brachial plexus was performed in 51of 74 patients. Response to therapy was measured in 68 of 74 patients.

DISCUSSION

In this study, HLA-DRB1*15 was more prevalent among patients with MMN than in population-based controls. Interestingly, the HLA-DRB1*15 haplotype is a consistent genetic risk factor for MS,[12] and an increased frequency has also been observed among women with CIDP as compared to controls.[21] This suggests that MMN etiology may share pathogenic pathways with MS and CIDP.

To ensure diagnostic accuracy we only included patients with a diagnosis of probable or definite MMN according to previously defined criteria (Supplementary Table). Thus, we exclude the possibility of including patients with an MMN-mimic. Our cohort of 74 patients is one of the largest published from a single center, and all patients were Caucasian and from Dutch ancestry.

DRB1*15 may increase the risk of MMN by facilitating pathogenic pathways. T-cells are thought to play an important role in the pathogenesis of both MS and CIDP, and in both diseases T-cell infiltrates have been observed at sites of demyelination.[22-25] HLA-DRB1*15 may facilitate antigen presentation of specific fragments of myelin, and thereby contribute to the adaptive immune response. There is as yet no solid proof of a role of T cells in MMN pathogenesis. The glycolipid GM1 is the only known target for antibodies in MMN, but an additional role of T-cells has not been excluded. MMN is a pure motor nerve neuropathy, which limits the possibility of taking nerve biopsies. Pathological studies in MMN are, therefore, few and insufficient to document the presence and characteristics of leukocyte infiltrates in nerves.[1, 26, 27]

Table 7.2 HLA class I and II in patients with multifocal motor neuropathy and controls

	MMN, n = 74		Controls, n = 700				
HLA	n	(%)	n	(%)	OR	(95 % CI)	*p*
Class I							
A1	18	(24)	242	(35)	0.6	(0.4-1.1)	0.1
A2	35	(47)	350	(50)	0.9	(0.6-1.5)	0.7
A3	21	(28)	200	(29)	1.0	(0.6-1.7)	1.0
A9	20	(27)	129	(18)	1.6	(0.95-2.8)	0.1
A24 (9)	17	(23)	70	(10)	2.7	(1.5-4.9)	0.01
A11	11	(15)	65	(9)	1.7	(0.9-3.4)	0.1
A19	19	(26)	187	(27)	0.9	(0.5-1.6)	0.8
B5	11	(15)	76	(11)	1.5	(0.7-2.9)	0.3
B51 (5)	11	(15)	62	(9)	1.8	(0.9-3.6)	0.1
B7	23	(32)	189	(27)	1.2	(0.7-2.1)	0.4
B8	16	(22)	200	(29)	0.7	(0.4-1.3)	0.2
B12	19	(26)	186	(27)	1.0	(0.6-1.7)	0.9
B44 (12)	18	(25)	146	(21)	1.2	(0.7-2.2)	0.5
B15	12	(16)	106	(15)	1.1	(0.6-2.1)	0.8
B35	17	(23)	109	(16)	1.6	(0.9-2.9)	0.1
B40	10	(14)	116	(17)	0.8	(0.4-1.6)	0.5
Class II							
DRB1*01	17	(23)	161	(23)	1.0	(0.6-1.8)	1.0
DRB1*03	13	(18)	198	(28)	0.5	(0.3-1.0)	0.05
DRB1*04	15	(20)	176	(25)	0.8	(0.4-1.4)	0.4
DRB1*07	19	(26)	144	(21)	1.3	(0.8-2.3)	0.3
DRB1*11	9	(12)	126	(18)	0.6	(0.3-1.3)	0.2
DRB1*13	14	(19)	169	(24)	0.7	(0.4-1.3)	0.3
DRB1*15	30	(41)	167	(24)	2.2	(1.3-3.6)	0.0017*
DQB1*02	26	(36)	280	(40)	0.8	(0.5-1.4)	0.5
DQB1*03	34	(47)	357	(51)	0.8	(0.5-1.4)	0.5
DQB1*05	28	(38)	233	(33)	1.2	(0.8-2.1)	0.4
DQB1*06	38	(52)	306	(44)	1.4	(0.9-2.3)	0.2

CI = confidence interval; HLA = human leukocyte antigen; MMN = multifocal motor neuropathy; OR = odds ratio. Set of alleles with frequency > 15 % in either patients or controls were analyzed by χ^2 test or Fisher's exact test. * $p < 0.00185$ was considered as significant using the Bonferroni correction for n = 27 comparisons.

Alternatively, the presence of HLA-DRB1*15 could increase the risk of MMN synergistically with other, as yet, unknown risk factors. The presence of HLA-DRB1*15 and elevated antibody titers to Epstein-Barr virus independently increase the risk of MS, but their combined presence increases the risk by more than their sum.[28] Just like MS, MMN is probably a multifactorial disease with a complex etiology, and genetic and environmental factors that trigger pathogenic pathways remain to be elucidated.[29] Although infections may have a similar role in MMN as EBV has in MS,[30] preliminary studies failed to find an association with selected pathogens.[31]

Myasthenia gravis is a prototypical antibody-mediated autoimmune disease and is associated with specific HLA molecules.[13, 32-34] It seems unlikely that HLA-DRB1*15 facilitates the production of IgM antibodies to GM1 in patients with MMN by similar mechanisms as anti-acetylcholine receptor antibodies are produced in patients with myasthenia gravis.[1, 6] GM1 is a glycolipid and is a T-cell independent antigen. Antigen presentation of GM1 does occur in the context of the CD1 cluster of HLA-like molecules rather than classical HLA alleles.[35, 36] The distribution of HLA class I and II alleles did not differ between patients with and those without anti-GM1 antibodies.

Finally, the observed association with HLA-DRB1*15 may indicate linkage disequilibrium with other immune-modulating genes that influence susceptibility to MMN. The HLA locus contains a large number of genes that contribute to the immune response, including tumor necrosis factor (TNF) and complement components.

In Guillain-Barre syndrome (GBS),[37] inclusion body myositis (IBM),[38] Lambert-Eaton myasthenic syndrome[39] and myasthenia gravis,[13] HLA-alleles are associated with earlier age at onset of disease and other clinical characteristics. The present study failed, however, to show an association with clinical characteristics such as age at onset, response to therapy, disability and conduction block or axonal damage. The HLA-DRB1*15 haplotype is not associated with age at onset, clinical course or disease severity in MS either.[12] Future studies on the role of the HLA-DRB1*15 haplotype and other (genetic) factors in susceptibility of MMN may help to further elucidate the immunopathogenesis of MMN.

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8

Multifocal motor neuropathy is not associated with genetic variation in *PTPN22*, *BANK1*, *Blk*, *FCGR2B*, *CD1A/E* and *TAG-1* genes

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Journal of the Peripheral Nervous System
(accepted).

ABSTRACT

Multifocal motor neuropathy (MMN) is a rare immune-mediated neuropathy characterized by asymmetric limb weakness. IgM antibodies to the ganglioside GM1, which is abundantly expressed in motor nerves, are found in serum of approximately half of all patients with MMN. Treatment with intravenous immunoglobulin (IVIg) or subcutaneous immunoglobulin improves muscle strength. HLA-DRB1*15 haplotype is associated with MMN, suggesting that genetic heterogeneity underlying autoimmune disease (AID) may be important for MMN pathogenesis. We, therefore, investigated frequencies of single nucleotide polymorphisms (SNPs) in the candidate genes; *PTPN22*, *BANK1*, *Blk*, and *FCGR2B*, which have been found to be associated with other AIDs, *CD1A* and *CD1E*, important for antigen presentation of glycolipids, and *TAG-1*, which is associated with responsiveness to IVIg in patients with chronic inflammatory demyelinating polyneuropathy. SNP frequencies were determined by means of TaqMan SNP genotyping assay and direct sequencing of candidate genes in 92 Dutch patients with MMN and 1152 healthy controls. SNP frequencies did not differ between patients and controls (all p-values > 0.15) and disease characteristics were not associated with SNP genotypes. Our results suggest that allelic variation of these candidate genes does not play a major role in determining MMN susceptibility.

INTRODUCTION

Multifocal motor neuropathy (MMN) is a rare immune-mediated neuropathy, characterized by slowly progressive, asymmetric, predominantly distal limb weakness. Conduction block (CB), the failure of a nerve impulse to propagate through a structurally intact axon, is the electrophysiological hallmark of MMN.[1,2] The favorable response to treatment with intravenous immunoglobulin (IVIg) and the presence of IgM antibodies to the ganglioside GM1 in serum from approximately half of all patients[3] support the hypothesis that MMN is at least in part a B cell-mediated inflammatory disorder. We recently found an increased frequency of the HLA-DRB1*15 haplotype in patients with MMN,[4] the haplotype previously shown to be associated with multiple sclerosis.[5] Only one other study has addressed whether single nucleotide polymorphisms (SNPs) in candidate genes increase MMN susceptibility.[6] In this pilot study including 13 Italian patients, polymorphisms of CD1 molecules were not found to be associated with MMN, but the limited number of patients precluded definite conclusions.

Autoimmune diseases (AIDs) share genetic characteristics, as was shown by the identification of common susceptibility genes.[7,8] SNPs in protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*), B-cell scaffold protein with ankyrin repeats (*BANK1*) and B lymphocyte kinase (*Blk*) genes influence B-cell functioning and are associated with B-cell mediated and other AIDs, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, type 1 diabetes and autoimmune thyroid disease.[9] Promoter polymorphisms in the Fc gamma receptor class IIb (*FCGR2B*) gene, which encodes an inhibitory IgG receptor expressed on B-cells, lead to distinct haplotypes. One of the haplotypes is found to be associated with SLE

and chronic inflammatory demyelinating polyneuropathy (CIDP).[10-12] Polymorphisms of the CD1 cluster of genes, i.e. *CD1A* and *CD1E*, may determine efficacy of antigen presentation of glycolipids such as GM1, and in one study were found to be associated with Guillain-Barré syndrome (GBS),[13] although this was not corroborated in a second study.[14]

Patients with MMN are generally responsive to IVIg treatment, with a few exceptions. A recent study in patients with CIDP suggested that IVIg responsiveness may be determined by SNPs in the transient axonal glycoprotein 1 (*TAG-1*) gene, which encodes a protein important for axon-Schwann cell interactions.[15]

To study the contribution of SNPs in genes involved in antigen presentation and B-cell function to MMN susceptibility, presence of serum anti-GM1 IgM antibodies, IVIg responsiveness and outcome, we performed a genetic association study in a large cohort of well-defined MMN patients.

MATERIALS AND METHODS

Study population

Ninety-two patients with a diagnosis of probable or definite MMN according to previously published criteria were included in the study.[2] Briefly, all patients had slow or stepwise progressive limb weakness, no objective sensory abnormalities, no bulbar signs, no upper motor neuron signs, and no other cause of neuropathy. Patients with definite MMN had at least one definite motor CB and patients with probable MMN had at least one probable motor CB. Patients had normal sensory nerve conduction in segments with motor CB and normal sensory nerve action potential amplitudes on distal stimulation at the time of the first nerve conduction study. All patients were Caucasian and of Dutch descent. Clinical characteristics of all but four patients have been reported previously.[16] To summarize gender, age at onset, muscle strength, disability, magnetic resonance imaging (MRI) results of the brachial plexus, electrophysiological findings, response to IVIg therapy, and presence of serum anti-GM1 IgM antibodies have been documented. Serum anti-GM1 IgM antibody titers were assessed by enzyme-linked immunosorbent assay (ELISA),[17] and considered positive at the highly specific cut-off titer ≥1:400. Disability was scored according to the modified Rankin Scale and the Overall Disability Sum Score (ODSS). Muscle strength was assessed bilaterally in 18 muscle groups using the Medical Research Council (MRC) scale ranging from 0 to 5, and added resulting in a MRC sum score with a maximum of 180. Response to IVIg therapy was defined as an increase of ≥1 MRC grade in at least two muscle groups without a decrease in other muscle groups.[18] Electrophysiological characteristics included definite or probable CB and evidence of axonal loss.[16]

A total of 1152 healthy Dutch subjects were used as controls.[19]

The Medical Ethical Committee of the University Medical Center Utrecht approved the study protocol and all patients gave written informed consent.

Genetic analysis

Genomic DNA was extracted from venous blood using standard methodology. DNA samples were used for SNP analysis in *CD1A* (rs2269714, rs2269715), CD1E (rs1065457), TAG-1 (rs895932, rs2275697, rs2229866) and FCGR2B (rs3219018, rs34701572) by direct sequencing. Primers have already been described previously.[11, 13, 15] Polymerase chain reaction (PCR) amplifications were carried out on 50 ng genomic DNA in a total volume of 10 µl. Reaction mixture contained 0.2 mM of each primer, 200 µM of each dinucleotide triphosphate (dNTP), PCR buffer and Taq Polymerase. The PCR was performed using a touchdown thermocycling program, with the following conditions: 95°C for 3 min; 92°C for 60 s; 15 cycles of 92°C for 30 s, 65°C for 30 s with a decrement of 0.5°C per cycle, 72°C for 60 s; followed by 30 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 60 s; 72°C for 20 min (GeneAmp9700, Applied Biosystems, Foster City, California). After thermocycling, the PCR reactions were diluted in 25 µl H2O and 1 µl was used as template for sequencing reactions which were performed using BigDye Terminator 3.1 chemistry (Applied Biosystems) in a total volume of 5 µl. After thermocycling, sequencing products were purified by ethanol precipitation and analyzed on a 96-capillary 3730XL DNA analyzer (Applied Biosystems), as described previously.[20] Sequence data were analyzed in PolyPhred.[21]

For *PTPN22* (rs2476601), *BANK-1* (rs17266594) and *Blk* (rs13277113) we used a pre-designed TaqMan SNP Genotyping Assay (Applied Biosystems). The PCR reaction was carried out in a total reaction volume of 5 µl containing 1x TaqMan PCR master mix and 1x assay mix. The following amplification protocol was used: 95°C for 10 min; 40 cycles of 92°C for 15 s and 60°C for 60 s. Allelic PCR products were analyzed on an ABI Prism 7900 HT sequence detection system using SDS 2.3 software.

Genetic and statistical analysis

SNP frequencies in patients and controls were compared by χ^2 testing using PLINK program v 1.07.[22] Variables that differed significantly between patients and controls were included in the analysis as covariates. The Bonferroni adjustment was used to correct for multiple comparisons. A *p*-value of 0.0045 (0.05/11) was considered statistically significant.

Possible associations of SNPs with MMN disease characteristics were analyzed by Fisher's exact test for categorical variables and by One-Way Anova for continuous variables.

To estimate the degree of linkage disequilibrium (LD) in the *TAG-1* and *FCGR2B* genes, the standardized disequilibrium coefficient (D') was calculated using Haploview (version 4.1, Broad Institute, Cambridge, MA, USA).[23] Significance for *TAG-1* and *FCGR2B* haplotypes between patients and controls, as well as haplotype analysis for *TAG-1* SNPs between IVIg responders and non-responders, were computed through permutation testing (100000 permutations) by Haploview.

RESULTS

Patients

Patient and control characteristics are shown in Table 8.1. Ninety-two MMN patients and 1152 controls were enrolled in the study. Sixteen patients preferred not to use IVIg because of mild symptoms or side-effects. Seventy (92%) of the 76 patients treated with IVIg had responded to treatment. Since age and gender differed significantly between patients and controls, the odds ratios and *p*-values were adjusted for these variables.

Genotype and allele frequencies in patients and controls

The results, expressed as genotype and allele frequencies, in both patients and controls are shown in Table 8.2. Genotype distributions were in Hardy–Weinberg equilibrium, with the exception of the *FCGR2B* promoter SNPs (rs3219018 *p*-value 2.9E-07, rs34701572 *p*-value 5.6E-06). Genotype and allele frequencies did not differ between MMN patients and controls.

LD analysis of *TAG-1* and *FCGR2B* showed strong linkage (*TAG-1* D' value > 0.8, *FCGR2B* = 0.99). *TAG-1* and *FCGR2B* haplotypes between patients and controls, and haplotype frequencies of the *TAG-1* gene between responders and non-responders to IVIg therapy, did not differ after correction with 100,000 permutations (data not shown).

Table 8.1 Characteristics of patients and controls

	No. of subjects	Patients	No. of subjects	Controls
Male, n (%)	92	65 (71)	1152	592 (51)
Median age at inclusion (range), y	92	51 (26-76)	1152	62 (22-96)
Median age at onset (range), y	92	40 (22-66)		-
Definite MMN, n (%)	92	73 (79)		-
Probable MMN, n (%)	92	19 (21)		-
Axonal damage, n (%)	92	78 (85)		-
Median MRC sum score (range)	87	166 (108-179)		-
Median ODSS (range)	87	4 (2-9)		-
Median Rankin Scale (range)	87	2 (0-4)		-
Anti-GM1 IgM antibodies, titers ≥1:400, n (%)	88	39 (44)		-
Response to IVIg therapy, n (%)	76	70 (92)		-
Median IVIg dose (range), gram/week	69	14 (5-100)		-
HLA-DRB1*15 positive, n (%)	71	29 (41)		-
Abnormal MRI brachial plexus, n (%)	55	25 (45)		-

y = years; MMN = multifocal motor neuropathy; MRC = Medical Research Council; ODSS = Overall Disability Sum Score; IVIg = intravenous immunoglobulin; MRI =, magnetic resonance imaging.

Table 8.2 Genotypic and allelic frequencies in patients and controls

		Genotype frequency, n (%)			Allele frequency, n (%)			
Gene (SNP)	N	AA	AB	BB	A	B	OR (95% CI)*	*p*-value*
PTPN22 (rs2476601)								
Patients	88	75 (85)	11 (13)	2 (2)	161(91)	15 (9)	0.96 (0.56-1.65)	0.881
Controls	1080	888 (82)	178 (17)	14 (1)	1954 (90)	206 (10)		
BANK1 (rs17266594)								
Patients	88	44 (50)	41 (47)	3 (3)	129 (73)	47 (27)	0.89 (0.62-1.27)	0.516
Controls	998	496 (50)	420 (42)	82 (8)	1412 (71)	584 (29)		
BLK (rs13277113)								
Patients	91	52 (57)	33 (36)	6 (7)	137 (75)	45 (25)	0.96 (0.67-1.37)	0.807
Controls	1078	612 (57)	398 (37)	68 (6)	1622 (75)	534 (25)		
CD1A (rs2269714)								
Patients	92	79 (86)	12 (13)	1 (1)	170 (92)	14 (8)	1.35 (0.76-2.42)	0.308
Controls	978	862 (88)	112 (11)	4 (1)	1836 (94)	120 (6)		
CD1A (rs2269715)								
Patients	92	79 (86)	12 (13)	1 (1)	170 (92)	14 (8)	1.52 (0.84-2.75)	0.171
Controls	875	779 (89)	94 (11)	2 (0)	1652 (94)	98 (6)		
CD1E (rs1065457)								
Patients	88	39 (44)	35 (40)	14 (16)	133 (64)	71 (36)	1.20 (0.86-1.66)	0.281
Controls	634	305 (48)	256 (40)	73 (12)	866 (68)	402 (32)		

TAG-1 (rs895932)								
Patients	92	24 (26)	47 (51)	21 (23)	95 (52)	89 (48)	0.97 (0.71-1.33)	0.865
Controls	937	261 (28)	447 (48)	229 (24)	969 (52)	905 (48)		
TAG-1 (rs2275697)								
Patients	92	61 (67)	27 (29)	4 (4)	149 (81)	39 (19)	0.96 (0.65-1.42)	0.845
Controls	873	577 (66)	253 (29)	43 (5)	1407 (81)	339 (19)		
TAG-1 (rs2229866)								
Patients	92	38 (41)	40 (44)	14 (15)	116 (63)	68 (37)	0.86 (0.63-1.18)	0.358
Controls	982	315 (36)	367 (42)	185 (21)	997 (57)	737 (43)		
FCGR2B (rs3219018)								
Patients	91	62 (68)	29 (32)	0 (0)	153 (84)	29 (16)	0.81 (0.49-1.34)	0.412
Controls	1106	723 (65)	373 (34)	10 (1)	1819 (82)	393 (18)		
FCGR2B (rs34701572)								
Patients	91	72 (79)	19 (21)	0 (0)	163 (90)	19 (10)	0.75 (0.41-1.36)	0.340
Controls	1093	829 (76)	264 (24)	0 (0)	1922 (88)	264 (12)		

*Odds ratio and *p*-value for allelic association.
SNP = single nucleotide polymorphism; N = number of subjects.

There was no association of SNPs with age at onset, muscle strength, disability, abnormal MRI of the brachial plexus, electrophysiological findings, response to IVIg therapy, increased titers of anti-GM1 IgM antibodies or presence of the HLA-DRB1*15 allele.

DISCUSSION

Our results do not suggest that polymorphisms of candidate genes that influence antigen presentation, B-cell function and susceptibility to AIDs, or responsiveness to IVIg treatment play an important role in increasing susceptibility to MMN, nor do they modify its disease course.

MMN is presumably an immune-mediated neuropathy, as suggested by the presence of serum anti-GM1 IgM antibodies in up to half of all patients, and the responsiveness to IVIg. Pathogenicity of serum anti-GM1 IgM antibodies is suggested by the fact that higher titers are associated with more weakness, axon loss and higher disability scores,[3] results from *in vivo* and *in vitro* studies,[24, 25] and the analogy with axonal variants of GBS, which are also associated with serum anti-GM1 antibodies.[26] Serum anti-GM1 antibodies are thought to bind to GM1 in peripheral nerves, activate complement, and interfere with function of sodium channels.[27-29] The possible relevance of genetic heterogeneity for susceptibility to MMN was recently shown by the association of MMN with the HLA-DRB1*15 allele.[4] We, therefore, selected genes that might be involved in downstream events that lead to serum anti-GM1 IgM production as candidate genes for this study.

Antigen presentation of glycolipids by antigen-presenting cells (APC) probably represents the first step that leads to activation of B-cells that produce anti-GM1 IgM. Glycolipid structures are processed and presented by APC through CD1 molecules, which constitute a conserved family of HLA-like molecules. CD1B is the key CD1 molecule for presentation of GM1, but is not polymorphic; the polymorphic CD1E may play a secondary role.[30] Polymorphisms of *CD1A* and *CD1E* genes were associated with GBS in one study,[13] but not in a second larger study[14] nor in a pilot study of 13 Italian patients with MMN.[6] Our data did not show an association of *CD1A* or *CD1E* SNPs with MMN, nor the presence of serum anti-GM1 IgM antibodies. Pooling of our data with those from the previous Italian study did not change the results. Although our study is underpowered for detecting small effects, these data exclude a major role of CD1A and CD1E SNPs as susceptibility markers for MMN.

A primary B-cell defect, leading to hyperactivity of B cells or a decreased ability to respond to inhibitory signals may represent pathogenic mechanisms that lead to serum anti-GM1 antibody production.[31] SNPs in genes that regulate B-cell function are common risk factors for several AID.[32, 33] *PTPN22* and *BANK1* play a role in B-cell receptor signalling, *Blk* and *BANK1* in B-cell activation, and *FCGR2B* is an important inhibitory receptor of B-cell functions. SNPs in these genes are associated with AIDs in which B-cells contribute to pathogenesis, including systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes mellitus, autoimmune thyroid disease and CIDP.[9-12] We assessed whether heterogeneity of *PTPN22*, *BANK1*, *Blk* and

FCGR2B is associated with MMN. The allele frequencies of these genes were, however, very similar for patients with MMN and controls. It is not clear why the distribution of *FCGR2B* genotypes was not in Hardy-Weinberg equilibrium. The higher than expected number of heterozygotes may be explained by variation in copy numbers of *FCGR2B*.[34] The FCGR locus is characterized by a high degree of homology caused by gene duplication and recombination.[35] Our data do not support the hypothesis that MMN shares some of the common genetic risk markers with other AIDs. Furthermore, none of the SNPs were associated with specific MMN disease characteristics. This is similar to the HLA-DRB1*15 haplotype, which was found to be enriched in patients with MMN, but which was also not associated with disease characteristics.[4]

Most patients with MMN respond to treatment with IVIg. Lack of responsiveness was associated with the degree of axonal damage and the time that lapsed without treatment.[16] A recent study in Japan showed that *TAG-1* SNPs may predict responsiveness in CIDP.[15] Lack of IVIg responsiveness in MMN patients is probably not explained by specific *TAG-1* haplotypes as reported in the Japanese patients with CIDP. The association of IVIg responsiveness and *TAG-1* heterogeneity may be exclusive to CIDP. There may also be ethnic differences in the contribution of *TAG-1* polymorphisms to IVIg responsiveness, something which is further supported by the fact that allelic and genotype frequencies differed significantly between Japanese and Dutch populations.

This study failed to find evidence for other genetic markers that predispose to MMN in addition to the HLA-DRB1*15 haplotype.

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Treatment in MMN

9

New liquid intravenous immunoglobulin (10% IVIg) for treatment of multifocal motor neuropathy: a prospective study of efficacy, safety and tolerability

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Journal of Neurology 2008;255(10):1598-1599.

ABSTRACT

Background: Maintenance treatment with intravenous immunoglobulins (IVIg) may attenuate muscle weakness and delay the permanent nerve damage associated with multifocal motor neuropathy (MMN), but this treatment can be time consuming. This study compared the safety, efficacy and convenience of a 10% ready-to-use liquid IVIg preparation with a freeze-dried 5% IVIg preparation.

Methods: A prospective open-label, non-controlled study was performed in 20 adults with MMN over 12 months. After 3 months of treatment with their regular maintenance dose of freeze-dried 5% IVIg, patients were treated for a further 9 months with an equivalent dose of 10% liquid IVIg. Muscle strength was measured using the MRC scale and muscle dynamometry.

Results: At inclusion, the mean IVIg regimen was 18.8 g/week (range 10-45 g/week) given every 3 weeks (range 1-4 weeks) and was similar with the 10% liquid IVIg preparation. Muscle strength was similar in the period in which patients used 5% IVIg compared with the new 10% liquid IVIg ($p = 0.126$). With 10% liquid IVIg, infusion time did not change in 3 patients (15%), was reduced up to twofold in 12 patients (60%) and more than twofold in 5 patients (25%). Mean infusion time with 10% liquid IVIg was 2.5 hours compared to 4.5 hours with 5% IVIg. Mild drug-related adverse effects occurred in 35% of patients receiving 10% liquid IVIg compared to 40% with 5% IVIg.

Conclusion: Ready-to-use 10% liquid IVIg was well tolerated in patients with MMN and was associated with substantial reductions in infusion times in 85% of patients.

INTRODUCTION

Multifocal motor neuropathy (MMN) is an immune-mediated demyelinating neuropathy characterized by a slowly progressive, asymmetric weakness of the limbs without sensory loss. Conduction studies show that motor conduction blocks occur outside of common nerve entrapment sites. The pathogenesis of MMN has not been elucidated, but it is assumed that IgM antibodies may bind to glycolipids expressed in nerves and initiate inflammatory changes in nerves or myelin sheets.[1] Steroids, plasma exchange and mycophenolate mofetil are not effective treatment for MMN.[2-6] Cyclophosphamide may be effective but its side-effects restrict its use in patients with MMN.[7]

Open-label and placebo-controlled studies have shown that treatment with intravenous immunoglobulin (IVIg) improves muscle strength in patients with MMN.[8-10] Maintenance treatment with IVIg is necessary because the beneficial effects only last for a few weeks. Also, MMN onset occurs at a relatively young age (20-50 years) and IVIg treatment must be administered for periods over many years. The infusion time of freeze-dried 5% IVIg products, which require reconstitution prior to use, depends on the dose administered but is usually time consuming, taking up to 8 hours. Liquid and concentrated (10%) IVIg products have recently been introduced on the market, but their efficacy, tolerability and safety have not been studied

in patients with MMN. It has been reported that the ready-to-use 10% liquid IVIg preparation used in the current study was well tolerated and significantly reduced infusion time in patients with primary immunodeficiency.[11] The aim of this study was to evaluate efficacy, safety and tolerability of the ready-to-use 10% liquid IVIg preparation in patients with MMN.

MATERIALS AND METHODS

Patients

Twenty adult patients with MMN were included. Inclusion criteria were the following: a diagnosis of 'definite MMN' or 'probable MMN' according to the criteria published previously;[1] patients had to be responsive to IVIg treatment according to published criteria;[10, 12, 13] patients had to have received maintenance treatment with freeze-dried 5% IVIg for a period of at least one year prior to inclusion.

Exclusion criteria were treatment with other immunosuppressive drugs in the three months preceding the study; a history of severe adverse reactions to immunoglobulin treatment; IgA deficiency; congestive heart failure or renal dysfunction; pregnancy or breast-feeding. Patients gave written informed consent prior to enrolment.

Study design

The study design was a single centre, prospective, open-label, non-controlled crossover study with a total duration of 12 months. After enrolment, patients were treated with their regular maintenance treatment of freeze-dried 5% (50 g/L) IVIg (Gammagard, Baxter BV, Austria) for 3 months. During this time, patients visited the outpatient clinic three times: at baseline (t = -2), after 6 weeks (t = -1) and after 12 weeks, at which time treatment with 10% liquid IVIg was initiated (t = 0). Patients were then treated for a period of 9 months with 10% (100g/L) liquid IVIg (Kiovig, Baxter BV, Austria) at an equivalent dose to their previous 5% IVIg. There were 3 visits to the outpatient clinic during this 9-month treatment period: after 6 weeks (t = 1), after 18 weeks (t = 2) and after 36 weeks (t = 3). Infusions with 10% liquid IVIg were started at 0.5 mL/kg/h. Infusion rates did not exceed 8 mL/kg/h, and were reduced if patients experienced side effects.

Patients received an extra 10% liquid IVIg dose (maximum 150g in 5 days) when either the upper or lower limb score of the Guy's Neurological Disability Scale, or the score of two or more motor activities on the self evaluation scale had increased 1 point and if muscle strength worsened one grade on the MRC scale or 50% measured in Newton by hand-held dynamometry in at least two affected muscles or muscle groups (see Evaluation). If patients did not improve after an extra dose with 10% liquid IVIg, the maintenance dose could be increased or treatment could be switched to IVIg 5%.

Evaluation

Patients were evaluated by the same investigator (EAC) at all visits.

Muscle strength was documented using a modified 10-grade scale of the Medical Research Council (MRC).[14, 15] The strength of five muscles or muscle groups of the arm (shoulder abduction, elbow flexion, elbow extension, wrist flexion and wrist extension) and of five muscles or muscle groups of the leg (hip flexion, knee flexion, knee extension, foot dorsiflexion and foot plantar flexion) were measured bilaterally. MRC sum score of these ten muscles or muscle groups were calculated (maximum score 100). Hand-held dynamometry (measurements in Newton) was performed in those muscle groups with an MRC score between 4- and 5-.

Functional impairment was assessed using the Guy's Neurological Disability Scale with functional impairment of the arms scored as follows: 0 = no impairment, 1 = impairment in one or both arms, not affecting functions such as fastening zips or buttons, tying a bow in laces or strings, washing or brushing hair and feeding, 2 = impairment in one or both arms affecting some but not preventing any of the functions listed, 3 = impairment in one or both arms, affecting all or preventing one or two of the functions listed, 4 = impairment in one or both arms preventing three of the functions listed, 5 = unable to use either arm for any purposeful movements. Functional impairment of the legs was scored as follows: 0 = walking is not affected, 1 = walking is affected but patient is able to walk independently, 2 = usually uses unilateral support (stick, ankle-foot orthosis to walk outdoors but walks independently indoors, 3 = usually uses bilateral support to walk outdoors, or unilateral support to walk indoors, 4 = usually uses wheelchair to travel outdoors, or bilateral support to walk indoors, 5 = usually uses a wheelchair indoors.[16]

A Self Evaluation Scale was scored from 0 (normal) to 5 (impossible) for five motor activities of daily life (worst score 25) chosen by the investigator and the patient at baseline.

Adverse events

The occurrence of adverse events was documented at every visit, including common IVIg related adverse events (headache, chills, fever, fatigue, nausea, vomiting, dizziness, arthralgia, allergic skin reactions), and serious adverse events (low blood pressure, anaphylactic shock, thromboembolic events, aseptic meningitis, haemolysis and acute renal failure).

Statistical analysis

To analyze if there is a difference in muscle strength after changing IVIg preparations the Wilcoxon signed-rank test for two related samples was used. Significance was assumed at $p < 0.05$.

RESULTS

Twenty patients with MMN were treated with IVIg. Patient characteristics are summarized in Table 9.1.

Dose and infusion times

During the 3 months of 5% IVIg treatment, the mean dose was 18.8 g/week (range 10-45 g/week) and mean infusion frequency was once every 3 weeks (range 1-4 weeks), and this did not change after switching to 10% liquid IVIg. Nine patients needed an extra dose of IVIg in the nine months they used the 10% liquid IVIg compared to 10 patients that needed an extra dose of 5% IVIg in the preceding year.

In comparison with the infusion times for the 5% IVIg treatment, infusion time for 10% liquid IVIg remained the same in 3 patients (15%), was reduced up to twofold in 12 patients (60%) and more than twofold in 5 patients (25%). Mean infusion time with 10% liquid IVIg was 2.5 hours compared to 4.5 hours with 5% IVIg (Table 9.2).

Efficacy

Muscle strength documented using the MRC scale was comparable between the period in which patients used freeze-dried 5% IVIg (time points t = -2, -1, 0) and the period with 10% liquid IVIg (t = 1, 2, 3) (Wilcoxon signed-rank test for two related samples, two-tailed, $p = 0.126$.)

Table 9.1 Patient characteristics

Patient characteristics	
Age (years)	
Mean	53
Range	36-67
M:F (n)	16:4
Age at onset of MMN (years)	
Mean	38
Range	22-54
Age at first IVIg infusion (years)	
Mean	45
Range	26-59
IVIg (g/week)	
Mean	18.8
Range	10-45
Frequency infusion (weeks)	
Mean	3
Range	1-4

Table 9.2 Infusion data and adverse events

Pt no	Treatment dose	Extra dose 5% IVIg	Extra dose 10% liquid IVIg	Infusion time 5% IVIg (hours)	Infusion time 10% liquid IVIg (hours)	% time reduction	Adverse events 5% IVIg	Adverse events 10% liquid IVIg
1	30g/2wks	90 g	90 g	3	2	33%	none	none
2	35g/1wk	90 g	90 g	1.5	1.5	0%	none	none
3	40g/4wks	No	No	6	1.5	75%	none	none
4	35g/3wks	No	90 g	5	2.5	50%	skin reaction	skin reaction
5♀	45g/3wks	90 g	90 g	6	3	50%	headache	headache
6	40g/2wks	90 g	90 g	2.5	1.5	40%	headache	none
7	40g/2wks	120 g	No	4	2	50%	none	none
8	30g/2wks	No	No	3	3	0%	none	none
9	35g/3wks	No	No	1.5	1	33%	none	none
10	40g/3wks	No	No	5	5	0%	none	dizziness
11	45g/2wks	No	No	2.5	1.5	40%	none	none
12	50g/4wks	150 g	150 g	5	2.5	50%	none	none
13	50g/2.5wks	No	No	5	2	60%	none	none
14♀	30g/3wks	No	No	7.5	4	47%	headache	headache
15♀	40g/3wks	90 g	No	6	2.5	58%	none	none
16	45g/1wk	120 g	150 g	6	4	33%	headache	headache
17	30g/1wk	100 g	100 g	3	1.5	50%	headache	headache
18	45g/2wks	No	No	7.5	4	47%	skin reaction	skin reaction
19♀	45g/3wks	90 g	90 g	7.5	2.5	67%	headache	none
20	40g/2wks	No	No	4.5	2	56%	none	none

Guy's Neurological Disability score for functional impairment of the arms was identical in all patients at t = 0 (prior to 10% liquid IVIg treatment) and t = 3 (after 9 months of 10% liquid IVIg treatment). A deterioration in Guy's Neurological Disability score for the legs was recorded in one patient whose score increased from a 1 at t = 0 to a 2 at t = 3.

Changes in the scores for the Self Evaluation Scale were recorded in two patients who improved by one point, and in one patient who deteriorated by one point (Table 9.3).

Adverse events

Prior to treatment with 10% liquid IVIg, 8 out of 20 patients (40%) reported side effects with 5% IVIg. Seven out of 20 patients (35%) reported mild side effects with 10% liquid IVIg (Table 9.2): four times headache, two times mild allergic skin reaction, one time dizziness.

Headache was experienced within 72 hours after infusion. Three patients mentioned the headache was less severe with 10% liquid IVIg compared to 5% IVIg. Two patients experienced headache with 5% IVIg, but no headache at all with 10% liquid IVIg.

Two patients reported mild allergic skin reaction following 10% liquid IVIg treatment: in one patient this was more severe and in the other it was less severe compared to 5% IVIg.

Table 9.3 Muscle strength and functional impairment

Pt No	MRC Sum Score (t = -2, -1, 0)	MRC Sum Score (t = 1, 2, 3)	Guy arm t = 0/t = 3	Guy leg t = 0/t = 3	SES t = 0/t = 3
1	87.55	88.67	2/2	3/3	9/9
2	82.78	86.85	2/2	3/3	11/11
3	97.62	98.01	2/2	0/0	9/8
4	94.98	96.23	2/2	0/0	9/9
5♀	94.72	97.23	2/2	1/1	11/12
6	90.84	93.39	2/2	2/2	15/15
7	96.17	96.12	2/2	0/0	7/7
8	92.12	92.90	2/2	2/2	11/10
9	83.05	84.48	2/2	4/4	15/15
10	85.56	84.67	3/3	0/0	12/12
11	98.38	99.45	1/1	1/1	6/6
12	97.45	97.67	2/2	1/1	14/14
13	91.24	91.79	2/2	2/2	11/11
14♀	87.15	86.22	2/2	1/1	13/13
15♀	97.45	97.89	1/1	0/0	2/2
16	88.23	88.07	3/3	1/1	19/19
17	81.06	79.56	3/3	4/4	15/15
18	86.17	83.46	3/3	2/2	15/15
19♀	95.78	94.84	1/1	0/0	9/9
20	92.11	94.50	2/2	1/2	10/10

Guy arm = Guy's Neurological Disability score arms; Guy leg = Guy's Neurological Disability score legs; SES = Self Evaluation Scale.

One patient experienced dizziness after 2 months with 10% IVIg treatment. This dizziness appeared even after infusion of 10% liquid IVIg at a low infusion rate. This male patient had never experienced adverse events with 5% IVIg, and treatment was continued with the 5% preparation, the dizziness disappeared.

DISCUSSION

Most patients with MMN need lifelong treatment with IVIg. In this study we treated patients with MMN with a ready-to-use 10% IVIg. This liquid formulation does not have to be dissolved prior to administration which means that pharmacy preparation time and associated costs are greatly reduced. Also, the 10% concentration results in reduced infusion times compared to products with lower concentrations. Pathogen safety is increased by the inclusion of three virus inactivation and removal steps. The efficacy, tolerability and safety of this 10% IVIg solution has been demonstrated in preceding clinical studies but not in patients with a demyelinating neuropathy like MMN.[11, 17, 18]

The effects on muscle strength of treatment with 10% liquid IVIg and with the comparator freeze-dried 5% IVIg were similar. Functional impairment was assessed by Guy's Neurological

Disability Scale (of the arms and legs) and a Self Evaluation Scale, before and after the switch from 5% IVIg to 10% liquid IVIg. No differences in functional impairment of the arms occurred and only 1 patient of the total 20 reported a minor reduction, of one point, in leg impairment. Similarly, minor changes of no more than a single point with the Self Evaluation Scale were recorded in three patients. These data suggest that 10% liquid IVIg treatment was at least as effective as treatment with the freeze-dried 5% IVIg product.

The overall percentage of reported adverse events of IVIg in patients with neurological diseases is approximately 40-80%, with severe adverse events reported in 2-4.5%.[19-21] In our study, 7 of 20 patients (35%) experienced mild side effects with the 10% liquid IVIg compared to 8 of 20 (40%) with 5% IVIg. Three patients mentioned reduced headache intensity with 10% liquid IVIg compared to 5% IVIg and, in two patients, headache completely disappeared when treatment with 10% liquid IVIg was initiated. One patient was switched back to IVIg 5% because he experienced dizziness after infusion of 10% liquid IVIg.

The long-lasting frequent infusions are burdensome for MMN patients. This study demonstrated that infusion time can be reduced with 10% liquid IVIg with no increase in adverse effects. Substantial reductions in infusion times were recorded in 17 out of 20 (85%) patients. Indeed, the mean infusion time was reduced from 4.5 hours to 2.5 hours. Since IVIg treatment of MMN must be administered every few weeks and over a period of years, these reductions in infusion times will lead directly to large reductions in the time that the patient needs to spend in the hospital or at home while receiving intravenous therapy. This in turn is known to have beneficial effects both on quality of life and cost-effectiveness of treatment.[11]

CONCLUSION

This study shows a good tolerability of the new 10% liquid IVIg in patients with MMN. Muscle strength and functional impairment after administration of 10% liquid IVIg are comparable with that observed with 5% IVIg treatment. In addition, switching to 10% liquid IVIg significantly reduced infusion time in most patients.

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10

Home-based IVIg treatment is convenient and time-saving in patients with multifocal motor neuropathy

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Journal of the Peripheral Nervous System
2011;16(2):147–149.

ABSTRACT

Maintenance IVIg treatment attenuates muscle weakness and may delay nerve damage in patients with multifocal motor neuropathy (MMN). IVIg infusions are time-consuming and the frequent hospital visits can be burdensome for patients. We performed a cross-sectional descriptive study; fifty-two MMN patients receiving home-based treatment and 15 MMN patients receiving hospital-based treatment completed a questionnaire documenting time, dose, adverse events, advantages and disadvantages of IVIg treatment. Median duration of one dose of IVIg (median dose 40g at home, 35g in hospital) was shorter at home than in hospital: 2h 45min (range 1-8h) versus 4h (range 1-7h) ($p = 0.04$). The main reason for treatment in hospital was difficult intravenous access. Thirty-two percent of home-treated patients missed from half-a-day up to two days of work per month compared to 86% of hospital-treated patients ($p = 0.03$). Occurrence of mild adverse events was comparable (home- and hospital-treated patients 13%). Two serious adverse events occurred at home; one systemic reaction and one pulmonary embolism in a patient who exceeded the maximum allowed infusion rate. Home-based IVIg treatment is safe when maximum infusion rates are taken into account and the presence of an anaphylactic kit at home is ensured. Home-based IVIg treatment is convenient and time-saving for MMN patients.

INTRODUCTION

Multifocal motor neuropathy (MMN) is a chronic immune-mediated neuropathy characterized by slowly progressive, asymmetric weakness of the limbs and characteristic motor conduction blocks (CB) outside of common nerve entrapment sites.[1] Steroids, plasma exchange and mycophenolate mofetil are not effective[2-5] and the side-effects of cyclophosphamide restrict its use to selected patients.[6] At present, intravenous immunoglobulin (IVIg) treatment is the only intervention known to improve muscle strength; it may prevent axonal degeneration and an unfavorable outcome.[7-10]

Long-term maintenance treatment with IVIg in MMN is necessary because the beneficial effects last for only a few weeks.[11, 12] Moreover, MMN onset occurs at a relatively young age and IVIg treatment must be administered for periods of many years. The IVIg infusions are time-consuming and expensive,[13] and the frequent visits to the hospital may be burdensome for patients.

In 1994, home-based maintenance IVIg treatment became available in The Netherlands for patients with MMN. We recently performed a national study on MMN in order to identify all patients with MMN in The Netherlands. This enabled us to study the safety, advantages and disadvantages of home-based maintenance IVIg treatment compared to IVIg maintenance treatment in hospital.

MATERIALS AND METHODS

Patients

This study had a cross-sectional descriptive design and was conducted from July to December 2009. Eighty-eight patients with MMN who had participated in the national study on MMN in 2007 and six additional patients diagnosed since then were approached by telephone. All patients had a diagnosis of MMN according to criteria published previously.[14] In summary, all patients had slow or stepwise progressive limb weakness, no objective sensory abnormalities except for vibration sense abnormalities at onset, no bulbar signs, no upper motor neuron signs, and no other cause of neuropathy. Patients had normal sensory nerve conduction in segments with motor CB and normal distal sensory nerve action potential amplitudes at the first nerve conduction study.

During the interview, patients were asked if IVIg had been administered in the past and whether they received maintenance IVIg treatment. All patients receiving maintenance IVIg treatment, either at home or in hospital, were requested to complete a questionnaire documenting time, dose, adverse events and experienced advantages and disadvantages of home-based and hospital treatment. Response to treatment, defined as an increase of ≥1 MRC grade in at least two muscle groups without a decrease in other muscle groups, was documented.[10] The study was approved by the institutional committee of the University Medical Center Utrecht and informed consent was obtained from all study participants.

Practice of home-based IVIg treatment in The Netherlands

IVIg treatment at home is organized and provided by several home-care agencies. To be eligible, patients have to be treated in hospital at least once, usually with a cumulative dose of 2 g/kg. If IVIg therapy does not have major side-effects, the possibility of therapy at home is discussed with the patient by the treating physician. If patients agree to be treated at home, their physician contacts the home-care agency. Prior to the first IVIg infusion a specialized nurse from the home-care agency explains the procedures, checks whether patients understand and whether an anaphylactic kit (containing epinephrine, prednisone and antihistamines) is available, measures blood pressure and ensures IV access. The nurse remains in the patients' home during treatment to monitor vital signs, blood pressure and document any adverse event. The initial IVIg infusion rate is 0.5 ml/kg/hour, using different IVIg brands of concentration ranging between 50 g/L and 100 g/L. If the patient can tolerate a higher infusion rate i.e., has a stable blood pressure and experiences no adverse reactions, the infusion rate can be titrated to a maximum of 8 ml/kg/hour. The general practitioner of the patient is informed about the home-based treatment and can be contacted in case of an adverse event.

Statistics

Differences between patients receiving home-based and patients receiving hospital-based maintenance IVIg treatment were tested with the Mann-Whitney *U* test and the χ^2 test.

RESULTS

Patients

Table 10.1 presents characteristics of all patients who received maintenance IVIg treatment and who were included in the study. The first attempt to administer IVIg treatment (to a patient) at home was in 1994. To date, 59 patients with MMN have received maintenance IVIg treatment at home. Of these 59 patients, 52 (88%) participated in the present study. The characteristics of the seven patients who did not participate did not differ significantly from those included. Table 10.2 lists specific data of the patients receiving maintenance IVIg treatment at home.

Fifteen MMN patients have received maintenance IVIg treatment in hospital; all 15 participated and completed the questionnaire. Reasons for preference for maintenance IVIg treatment in hospital were: difficult IV access (six patients), better monitoring by medical personnel (eight patients) or concomitant disease (heart disease). None of the hospital-treated patients had ever used IVIg at home.

Duration of IVIg treatment

All 52 home-treated patients had received at least one IVIg course at a cumulative dose of 2 g/kg in hospital before home-treatment was initiated. The median IVIg dose was 40 g (range 5 g to 60 g) with a median frequency of once per three weeks (range one to six weeks). The median IVIg dose for the 15 patients who received hospital-based maintenance IVIg treatment was 35 g (range 25 g to 45 g) with a median infusion frequency of once per three weeks (range one to seven weeks). Duration of IVIg treatment at home was shorter compared to treatment in hospital ($p = 0.04$) (Table 10.1).

Twenty-eight of 52 home-treated patients were in paid employment; the other twenty-four patients did not have paid work or were retired. Nine patients in paid employment (32%) had to miss work because of the IVIg infusions (five patients, half-a-day per month, three patients, one day per month and one patient, two days per month). Patients who received maintenance IVIg treatment in hospital missed work more often compared to patients who received maintenance IVIg treatment at home ($p = 0.03$). Seven patients treated in hospital had paid work; the other eight patients did not or were retired. Six patients in paid employment (86%) missed work because of the IVIg infusions (two patients, half-a-day per month, one patient, one day per month and three patients, two days per month).

Table 10.1 Differences between patients receiving IVIg treatment at home and in hospital

	IVIg at home (n = 52)	IVIg in hospital (n = 15)	*p*-value
Gender (male)	37 (71)	14 (93)	0.09
Age at symptom onset (years) (median, range)	43 (21-63)	49 (25-66)	0.08
Disease duration at inclusion (years) (median, range)	11 (2-26)	9 (3-27)	0.62
Time from onset to first IVIg treatment (years) (median, range)	5 (0-22)	5 (1-20)	0.97
Median IVIg dose (g/kg/week)	0.19 (0.07-0.72)	0.13 (0.06-0.53)	0.58
Duration of one IVIg treatment (hours.minutes)*	2.45 (1-8)	4 (1-7)	0.04
Frequency of mild adverse events	7 (13)	2 (13)	1.00
Number of patients missing work for more than half-a-day per month	9 (32) **	6 (86) ***	0.03

Data are presented as numbers (%), unless otherwise specified; g/kg/week = Median IVIg dose converted to grams per kilogram body weight per week; * = Cut-off at quarters; mild adverse events = headache, chills or and mild skin rash; ** = total of home-treated patients in paid employment n = 28; *** = total of hospital-treated patients in paid employment n = 7.

Satisfaction scored by patients

Thirty of 52 patients had received maintenance IVIg treatment in the hospital prior to treatment at home; these thirty patients scored 'satisfaction' of home-treatment higher on a zero to ten scale (median score 9; range 6 to 10) compared to hospital-treatment (median score 7; range 1 to 10).

Advantages of treatment at home as indicated by patients were: convenience (92%), time gain (63%) or other (no need to travel, presence of family, more opportunities for distraction, no hospital atmosphere) (25%). The reported disadvantages were: absence of medical staff in case of an adverse event (27%), problems with IV access or the infusion system (10%), not being taken care of by the same nurse or other logistic problems (8%).

Advantages of treatment in hospital indicated by patients were: more permanent presence of medical staff in case of an adverse event or difficulties finding IV access (47%) and convenience (20%). The reported disadvantages were the following: time-consuming (40%) and travelling costs (7%).

Minor adverse events

Seven home-treated patients (13%) reported mild adverse events following IVIg infusions: headache (three patients), chills (three patients) and mild skin rash (one patient). Twelve patients used prophylactic drugs prior to the IVIg infusions at the time of this study because they had experienced mild adverse events in the past (Table 10.1).

Two hospital-treated patients (13%) reported mild skin rash following IVIg infusions; no other adverse events were reported. None of these patients had used prophylactic drugs prior to the IVIg infusions.

Table 10.2 Data of 52 MMN patients on home-based maintenance IVIg treatment

	Maintenance IVIg treatment at home (n = 52)
Start home-based treatment	
1994 – 1999	5 (10)
2000 – 2005	24 (46)
2006 – 2010	23 (44)
IVIg treatment prior to home-based treatment	
One 5-day IVIg course (cumulative dose 2g/kg) in hospital	10 (19)
More than one 5-day IVIg course in hospital	12 (23)
Maintenance IVIg treatment (0.4-1.0g/kg) in hospital	30 (58)
Person responsible for administration of IVIg	
Nurse from home-care agency	43 (83)
Self-infusion	7 (13)
Partner	1 (2)
General practitioner	1 (2)
IVIg preparations used	
Liquid preparation	41 (79)
Lyophilyzed product	11 (21)
Need for use of prophylactic drugs due to side-effects	
No	40 (77)
Acetaminophen	8 (15)
Non-steroidal anti-inflammatory drugs	2 (4)
Antihistamines	2 (4)
Initiative for selection moment of infusion	
Patient*	50 (96)
Home-care agency**	2 (4)
Initiative for ordering IVIg at local pharmacy	
Patient	24 (46)
Home-care nurse	28 (54)
Picking up IVIg at local pharmacy	
Patient	38 (73)
Home-care nurse	14 (27)

Data are presented as numbers (%).* The patient can choose the day and time of the IVIg infusion. ** The home-care agency creates a schedule for patients who receive IVIg infusions.

Serious adverse events

Nine patients who received maintenance IVIg treatment at home had a portacath. Two of these experienced a thromboembolic event after the portacath had been put in place. One patient experienced jugular vein thrombosis before IVIg was administered; the portacath was removed and was not replaced. A second patient had a thrombus in the vena cava superior after using the portacath for IVg infusions for two years. The portacath was removed and placed in the leg (great saphenous vein).

Two patients who received maintenance IVIg treatment at home experienced a serious adverse event. One had a systemic reaction consisting of drowsiness and a generalised rash

during the IVIg infusion. The nurse from the home-care agency stopped the IVIg infusion and administered antihistamines and epinephrine from the anaphylactic kit. Although the patient felt better, he was taken to hospital but could be discharged the same day. This serious adverse event occurred during infusion of a brand of IVIg liquid that he had already used three times before (twice in hospital and once at home). After the adverse event he switched to a lyophilized product. This was administered in hospital (40 g every three weeks) for three months before the patient resumed his treatment at home. He has not experienced an adverse reaction since.

One wheelchair-bound, cigarette-smoking patient had a pulmonary embolism, diagnosed five days after infusion of a lyophilized IVIg brand which he had (already) used for seven years. He exceeded the maximum allowed infusion rate of 8 ml/kg/hour which was explicitly stated in the protocols of the hospital and the home-care agency. The infusion rate used was 11.5 ml/kg/hour. He went to hospital because of pain on the left side of his chest five days after the IVIg infusion and was treated with anticoagulants for six months. He continued his maintenance IVIg treatment at home at a lower infusion rate (1 ml/kg/hour) and did not experience any adverse event in the subsequent three years.

DISCUSSION

This study shows that IVIg treatment at home is feasible and safe for the majority of patients with MMN. Not only is it preferred by patients, it also reduces the number of days patients are unable to work.

Safety is a major issue when considering treatment at home. Mild adverse effects include headache, chills and fever, and skin rash. Rare severe side-effects of IVIg include meningismus, severe anaphylactic reactions, thrombo-embolism, cardiac and kidney failure, and stroke.[15] The frequency of side-effects of IVIg infusions has been extensively studied in patients with primary antibody deficiencies, mild adverse events occurring in less than five percent to 42.7% of patients, while the percentage of serious adverse events was zero to 4.5%.[15-18] These frequencies are similar to those reported by patients with MMN in this study. Thirteen percent of patients with MMN experienced mild side-effects and two patients (3%) a serious complication. A pulmonary embolism occurred in a patient with other established risk factors (i.e. smoking and immobility). The maximum allowed infusion rate was exceeded in this case. IVIg treatment at home can be supervised by a specialized nurse or the general practitioner, but some experienced patients chose self-infusion or infusion by a trained partner.[19] Although the association of pulmonary embolism and the rapid infusion of IVIg may be incidental, this case illustrates the need for continued feed-back and advice to patients who prefer self-infusion. The importance of compliance with protocols, in particular maximum infusion rates, should be explicitly mentioned to patients at the start of treatment and during follow-up. The presence of a kit with drugs to treat anaphylactic reactions is another essential element to ensure patient safety.

IVIg treatment is never initiated at home. The current protocol demands that the first IVIg course with a cumulative dose of 2 g/kg IVIg, usually administered in five consecutive days, always takes place in a hospital in order to evaluate possible infusion reactions and to ascertain tolerance. In most cases, this is followed by a second course of five days of IVIg treatment in hospital. The possibility of home-treatment is only discussed with patients provided they have no relevant medical history or IgA deficiency. In the present study, patient satisfaction with treatment at home was good. None of the patients on maintenance IVIg home-treatment had returned to treatment in hospital. All MMN patients in this study visited their treating neurologist as an out-patient at least once per year for evaluation of muscle strength and disability, and dose and side-effects of IVIg. Furthermore, patients expressed their satisfaction with the fact that they did not need to spend time travelling to hospital. Significantly fewer patients in the home-based maintenance IVIg treatment group had days off work. This, combined with the fact that admission costs for treatment in Dutch hospitals in The Netherlands are approximately 4 times higher than treatment at home with the help of a specialized nurse, clearly indicates that society as a whole benefits from IVIg treatment at home.

Difficult IV access was the most important reason for treatment in hospital. The use of a portacath can be complicated by thrombosis and infection.[20] An alternative approach might consist of subcutaneous infusion of immunoglobulins (scIg). Small scale studies suggest that scIg is efficacious for sustaining muscle strength in MMN patients,[21, 22] at least in the first year. Long-term follow-up studies of MMN patients on the use of scIg in MMN patients are needed in order to evaluate whether the long-term effects on muscle strength and disability are comparable to IVIg.

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11

IVIg inhibits classical pathway activity and anti-GM1 IgM mediated complement deposition in MMN

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Journal of Neuroimmunology 2010;
229(1-2):256-262.

ABSTRACT

The effects of intravenous immunoglobulins (IVIg) on anti-GM1 IgM titer and function, classical complement pathway activity, and antibody-complement interaction were investigated in 62 patients with multifocal motor neuropathy (MMN). *In vitro*, IVIg decreased complement deposition by anti-GM1 IgM antibodies. First IVIg treatment (2 g/kg) decreased C1q and C4 concentrations and classical pathway activity in serum. In sera from patients receiving IVIg maintenance therapy (0.4 g/kg) C4 concentrations and classical pathway activity were generally lower at higher IgG concentrations. The beneficial effects of IVIg in MMN may be explained by reduced antibody-mediated complement deposition in nerves amplified by a systemically attenuated classical pathway.

INTRODUCTION

Multifocal motor neuropathy (MMN) is characterized by slowly progressive, predominantly distal, asymmetrical limb weakness.[1] Motor nerve conduction studies show characteristic conduction block outside common nerve compression sites.[2, 3] Treatment with intravenously administered immunoglobulins (IVIg) improves muscle strength, although beneficial effects are short-lived.[4, 5] Plasmapheresis and corticosteroids are not effective and may even worsen disease course.[6-9] Cyclophosphamide may be useful in refractory cases.[10-12]

MMN pathogenesis remains largely elusive. Sera from a substantial subgroup of MMN patients contain IgM antibodies against the ganglioside GM1. GM1 is abundantly expressed in peripheral motor nerves.[13] Anti-GM1 antibodies are thought to cause weakness by interfering with motor nerve function at the nodes of Ranvier, myelin sheath and the neuromuscular endplate in patients with acute motor axonal neuropathy (AMAN).[13-16] There is evidence that similar mechanisms may underlie MMN pathogenesis. Injection of sera from MMN patients containing anti-GM1 antibodies into rat tibial nerves caused conduction block *in vivo* and *in vitro*, and serum from MMN patients blocked nerve conduction in the mouse phrenic nerve-diaphragm preparations.[17-19] Pathogenic mechanisms triggered by ganglioside-specific antibodies have been recently elucidated. Binding of ganglioside-specific antibodies followed by complement activation and deposition of the membrane attack complex alters expression of sodium channels, which are essential for nerve conduction.[20-25]

It is not known how IVIg administration results in improved function of motor nerves in MMN patients.[4, 5] Experimental models for inflammatory neuropathy do, however, suggest that the complement system, anti-GM1 IgM antibodies and their interaction play an important role in MMN pathogenesis.[23, 24, 26] In this study, we investigated the effects of IVIg on the classical pathway of the complement system in patients with MMN receiving IVIg for the first time or as maintenance treatment, and on the interaction of anti-GM1 IgM antibodies and complement.

PATIENTS, MATERIALS AND METHODS

Patients

Serum samples from 62 patients fulfilling the diagnostic criteria for MMN, as described previously, were used for this study.[27, 28] Six patients with newly diagnosed MMN received a first course of IVIg (Gammagard, Baxter, cumulative dose 2.0 g/kg, administered in 5 days). Serum samples were collected before and after each IVIg dose. Serum samples from an additional 56 patients with MMN, treated with maintenance doses of IVIg (0.2-0.4 g/kg) at intervals ranging from 2 to 6 weeks, were collected during regular visits to the outpatient clinic of the Department of Neurology of the University Medical Centre Utrecht. Patient characteristics are summarized in Table 11.1. All patients showed clinical improvement after treatment with IVIg. Blood was collected in clotting tubes, centrifuged and the serum was aliquoted and stored at -80°C. Serum samples from 20 patients with amyotrophic lateral sclerosis (ALS) and 18 healthy volunteers were used as (disease) controls. Pooled healthy donor serum from 10 individuals without detectable anti-GM1 IgM antibody titers was used as negative control for complement activation assays and was used as a complement source in select experiments. All patients gave written informed consent, and the local ethics committee approved the study.

ELISA to detect anti-GM1 IgM antibodies

Anti-GM1 IgM antibody titers were assessed using enzyme-linked immunosorbent assay (ELISA) as described before with minor modifications.[29] In short, 100 µl of methanol, containing 0.25 µg GM1 (Calbiochem, San Diego, CA), was added to 96-well plates (NUNC, Polysorp®, Roskilde, Denmark), and left to evaporate at room temperature. Wells were blocked with 200 µl phosphate buffered saline (PBS) 1% bovine serum albumin (BSA) for 4 h at room temperature. Wells saturated with PBS 1% BSA served as control for non-specific binding. Human sera (diluted 1:100 in PBS 1% BSA) were serially diluted in triplicate and incubated overnight at 4°C. After washing six times with PBS, peroxidase-conjugated rabbit anti-human IgM antisera (DAKO Cytomation, Glostrup,

Table 11.1 Characteristics of MMN patients

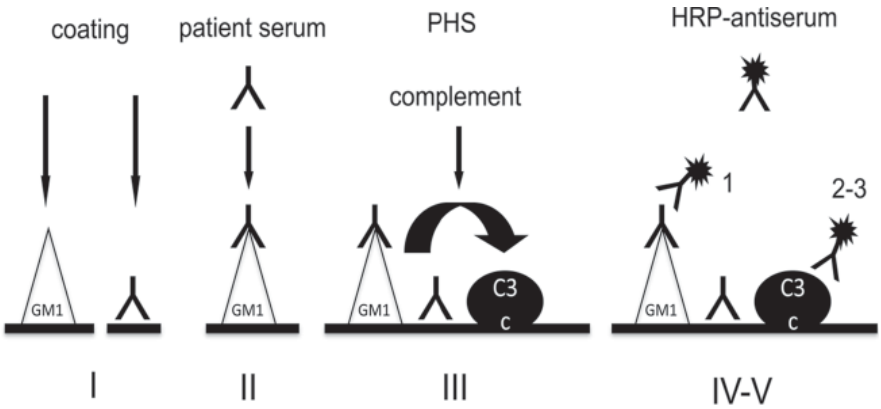
Characteristics	Newly diagnosed N = 6	Other N = 56
Age at disease onset, years	36 (28-53)	37 (22-52)
Treatment duration, months	Not applicable	56 (1-93)
Male	5 (83)	53 (95)
Disease duration until start treatment, months	6 (1-19)	12 (6-48)

Data are presented as median (range) or number (%).

Denmark), diluted 1:1000 in PBS 1% BSA, were added and incubated for 3 h at room temperature. Plates were developed using 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS®, Roche Diagnostics, Indianapolis, USA), after washing six times with PBS, and read at 405 nm using an ELISA reader (Multiscan RC, Thermo Labsystems). Anti-GM1 IgM titers were defined as the highest dilution yielding an OD of ≥ 0.05 after subtraction of background values (Figure 11.1).[30]

ELISA to quantify complement activation by anti-GM1 IgM antibodies

Methanol 100 µl containing 2.5 µg GM1 (Calbiochem, San Diego, CA) was added to 96-well plates (NUNC, Polysorb®, Roskilde, Denmark) and left to evaporate for 4 h at room temperature. Wells coated with purified IgM (Pierce, Rockford, IL) served as a positive control. The remaining wells and GM1-coated wells were blocked using PBS 1% BSA (200 µl/well) for 4 h at room



Assay	I Coating	II Serum	III Complement	IV-V Readout
1. Anti-GM1 IgM titer	GM1	+	-	IgM titer
2. Complement activation by anti-GM1 IgM	GM1	+	PHS	C3c
3. Intrinsic classical	IgG	-	Patient or control	C3c

Figure 11.1 Schematic presentation of assays used to determine anti-GM1 IgM antibody titre, activation of complement by anti-GM1 IgM, and intrinsic activity of the classical pathway of complement in serum from MMN patients and controls. (I) 96-well plates were coated with either GM1 or human IgG. (II) In select experiments, (heat-inactivated) serum from MMN patients and controls is added to GM1-coated wells, allowing anti-GM1 IgM to bind to GM1. (III) A complement source, either pooled serum from healthy donors (PHS), or serum from MMN patients and controls is added. If the classical complement pathway is activated by either anti-GM1 IgM or IgG directly coated in wells, this causes C3 deposition. (IV) Readout. HRP-labelled antibodies to human IgM or C3c are added to detect the presence of anti-GM1 IgM or C3c, respectively. (V) HRP is used to generate the detection signal. HRP, Horseradish Peroxidase.

temperature. Wells saturated with PBS 1% BSA served as a control for non-specific binding. Serum aliquots from MMN patients and controls were heat-inactivated (to abrogate intrinsic complement activity) for 30 minutes at 56 °C, serially diluted in PBS 1% BSA in triplicate and incubated overnight at 4 °C. After washing six times with PBS, 50 µl of 1% pooled healthy donor serum in PBS (or PBS in control wells) was added as a complement source and incubated at 37°C for 30 minutes. In select experiments, IVIg preparations (Gammagard, Baxter, Deerfield, USA) or albumin (Sigma, St. Louis, USA) at a final concentration of 1 g/L, or EDTA (25mM) were added in addition to the complement source. Plates were washed 6 times and wells were incubated with 50 µl of rabbit anti-human-complement factor 3c (Nordic, Tilburg, the Netherlands) diluted 1:7500 in PBS 1% BSA for 1 h at room temperature. This antibody reacts with C3c, C3b, C3bi and smaller fragments. After washing six times with PBS, 50 µl of goat anti-rabbit IgG-HRP (Pierce, Rockford, USA) diluted 1:1000 in PBS 1% BSA was added and incubated for 1 h at room temperature. Plates were developed using ABTS® (Roche Diagnostics, Indianapolis, USA), after washing six times with PBS, and read at 405 nm using an ELISA reader (Multiscan RC, Thermo Labsystems) (Figure 11.1). Pilot studies showed that patient serum dilutions of 1:200 gave optimal and reproducible optical density values of complement factor 3 deposition in GM1-coated wells (within the linear range after subtraction of background values). OD values for complement activation were considered positive when they were at least ≥ 2 SD higher than background signals obtained with pooled healthy donor serum (Figure 11.1).[30]

Immunoglobulin, C1, C3, and C4 concentrations in patient serum samples before and after IVIg treatment

IgM, IgG and complement C1q, C3, and C4 concentrations in serum were determined using standard nephelometric techniques (Beckman Coulter Image). Complement C3 and C4 concentrations were determined according to the manufacturer's instructions (Beckman Complement C3, C4). To quantify C1q serum levels, polyclonal rabbit anti-human C1q complement (DAKO Cytomation) in combination with calibrator 1 (Diasorin, Saluggia, Italy) was used. With this method, normal C1q, C3, and C4 serum levels ranged from 220 to 300 mg/L, 0.9-1.8 g/L, and 0.10-0.47 g/L, respectively.

Activity of the classical pathway of complement (CH50) in serum before and after IVIg treatment

CH50 reflects the erythrocyte lysing capacity of complement. A standard preparation of sheep red blood cells coated with anti-sheep erythrocyte antibody is incubated with several dilutions of patient serum. The reciprocal of the dilution of serum that lyses 50% of the erythrocytes reflects activity of the classical pathway of complement and is expressed as percentage of values obtained with a reference serum (normal range 75-125%).

ELISA to quantify intrinsic classical complement pathway activity in patient serum samples

An ELISA-based complement deposition assay was used to quantify intrinsic classical complement pathway activity in sera from patients with MMN and controls, drawn before and on average 12 days (SD 10 days) after treatment with IVIg. 25 μg/ml immunoglobulins (Gammagard, Baxter, Deerfield, USA) was coated to 96-well plates (NUNC, Polysorb®, Roskilde, Denmark) by overnight incubation at 4°C. After washing six times, plates were blocked with 200 μl PBS 1% BSA for 3 h at room temperature. After washing six times with PBS, 50 μl of 1% serum from MMN patients or controls in PBS (or PBS without serum as negative control) was added and incubated at 37°C for 30 minutes. Plates were washed six times and wells were incubated with 50 μl of rabbit anti-human-complement factor 3c (Nordic, Tilburg, the Netherlands) diluted 1:7500 in PBS 1% BSA for 1 h at room temperature. After washing six times with PBS, 50 μl of goat anti-rabbit IgG-HRP, diluted 1:1000 in PBS 1% BSA was added and incubated for 1 h at room temperature. Plates were developed using ABTS (Roche Diagnostics), after washing six times with PBS, and read at 405 nm using an ELISA reader (Multiscan RC, Thermo Labsystems) (Figure 11.1).

Statistical analysis

Anti-GM1 IgM titers before and after treatment with a cumulative dose of 2.0 g/kg IVIg were analyzed using χ^2 test. The effect of anti-GM1 IgM titers on complement activation, and of IVIg on complement component concentrations and CH50 values was determined using linear regression analysis. The differences in anti-GM1 IgM-induced complement deposition in MMN patients and disease controls were analyzed using an unpaired *t* test. Differences in intrinsic complement activity in sera from MMN patients, disease and healthy controls were analyzed using one-way ANOVA. Correlations between CH50, IgG concentrations and complement deposition were analyzed using Pearson's correlation coefficient. To determine the correlation between anti-GM1 IgM titers and complement activation, Spearman's log rank test was used. A p-value < 0.05 was considered significant.

RESULTS

Immunoglobulin concentrations and anti-GM1 IgM antibody titers before and after IVIg

In serum samples from six newly diagnosed MMN patients IgG, IgM and IgA concentrations were within the normal range before the start of IVIg. During IVIg treatment (2.0 g/kg, administered in 5 consecutive days), IgG levels increased on average 3-fold from 13.2 g/L (± 1.78

g/L) to 34.9 g/L (± 2.67 g/L, normal value 7-16 g/L ($p = 0.0001$)). IgA and IgM concentrations did not change. Anti-GM1 IgM antibodies could be detected in five of the newly diagnosed MMN patients and ranged from 1:100 to 1:6400 (Table 11.2). Anti-GM1 IgM antibody titers did not change during treatment with a cumulative IVIg dose of 2.0 g/kg, administered on 5 consecutive days ($p = 0.8$).

Anti-GM1 IgM antibodies were detected in 43 out of 56 MMN patients who received IVIg maintenance treatment (Table 11.2). In 36 of these patients, anti-GM1 IgM antibodies could be determined before the start of IVIg treatment, and in another 20 patients who already had received IVIg maintenance treatment at the time of inclusion. Paired serum samples obtained before and during maintenance treatment were available from 16 patients with MMN. Anti-GM1 IgM titers did not change during maintenance treatment ($p = 0.9$). Anti-GM1 IgM antibodies were also found in one healthy individual (1:200) and in 4 out of 20 disease controls (1:100 in 2 patients, 1:200 in 1 patient and 1:400 in 1 patient).

Complement-activating capacity of anti-GM1 IgM antibodies

Thirty-one out of 40 (78%) sera from MMN patients containing anti-GM1 IgM activated the complement system, as reflected by the deposition of C3 (Figure 11.2A). In contrast, pooled healthy donor serum did not activate complement (Figure 11.2B). C3 deposition was not detected when C1q deficient (Figure 11.2C) or EDTA (Figure 11.2D) containing sera from healthy donors were added as a complement source, suggesting that anti-GM1 IgM

Table 11.2 Anti-GM1 IgM titres

	MMN patients		Healthy controls
Titre	Newly diagnosed	Other	
Negative	1 (17)	13 (23)	17 (94)
1:100	1 (17)	11 (20)	0
1:200	1 (17)	11 (20)	1 (6)
1:400	2 (33)	10 (18)	0
1:800	0	3 (5)	0
1:1600	0	2 (4)	0
1:3200	0	4 (7)	0
1:6400	1 (17)	2 (4)	0
Total	6 (100)	56 (100)	18 (100)

Data are presented as numbers (%). Due to rounding, the sum of % is not always 100. Data are presented as values from three representative experiments.

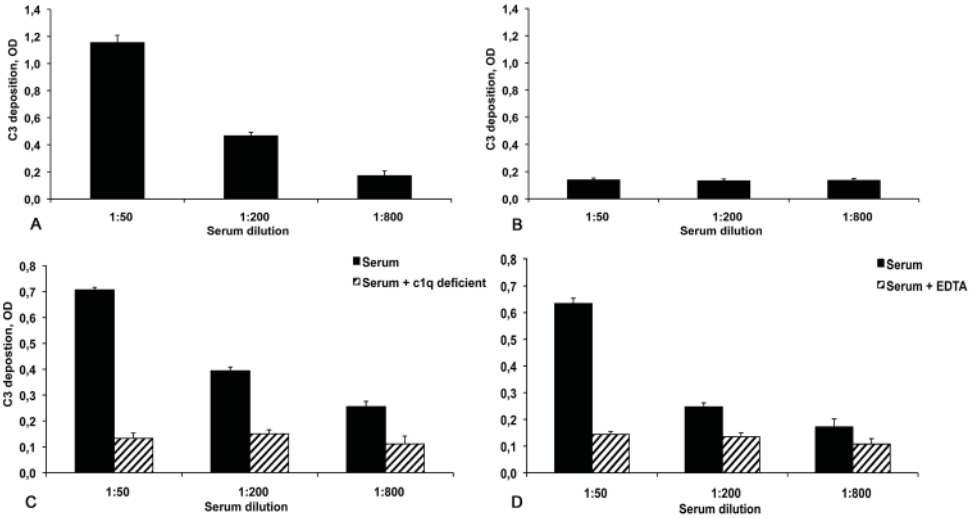


Figure 11.2 Anti-GM1 IgM antibodies in sera from patients with MMN activate complement via the classical pathway. (A) Serum of patients with MMN containing anti-GM1 IgM antibodies was added to GM1-coated ELISA plates. After binding, pooled healthy donor serum (PHS) was added as a complement source. C3 deposition was used as a read-out for complement activation. (B) Serum from healthy donors without detectable anti-GM1 IgM antibodies does not activate complement. (C) Addition of C1q deficient serum as a complement source to ELISA plates containing anti-GM1 IgM from MMN sera does not lead to complement deposition. (D) Addition of EDTA, a Ca^{2+} scavenger, to the complement source abrogates complement deposition.

activated the classical pathway of complement. In serum from patients without complement activation, anti-GM1 IgM titers were not higher than 1:100, except for one patient with a titer of 1:400. Complement deposition correlated significantly with anti-GM1 IgM titers ($p < 0.001$) (Figure 11.3A). One serum sample from a patient without detectable anti-GM1 IgM titers activated complement. Complement deposition slightly higher than background levels could be detected in sera of only two disease controls with anti-GM1 IgM titers of 1:100 and 1:200. Complement activation by anti-GM1 IgM antibody containing sera was significantly higher when using serum of MMN patients compared to serum of disease controls ($p = 0.001$) (Figure 11.3B).

The effect of IVIg therapy on the complement activating capacity of anti-GM1 IgM was determined using paired serum samples taken before and during treatment from 16 MMN patients. Complement activation before and during IVIg maintenance treatment was similar ($p = 0.8$), indicating that IVIg maintenance treatment does not alter the complement activating properties of anti-GM1 IgM antibodies.

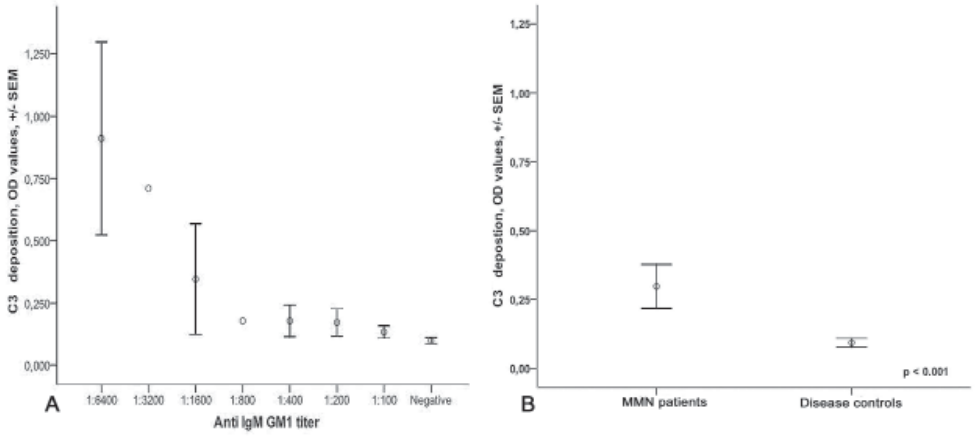


Figure 11.3 (A) Complement deposition was significantly higher in MMN patients with high anti-GM1 IgM titers (serum dilution 1:200). (B) Complement deposition was significantly higher when using sera with anti-GM1 IgM antibodies from MMN patients than from disease controls (serum dilution 1:200).
Data are presented as mean values from three independent experiments and after subtraction of background signals. MMN = multifocal motor neuropathy; OD = optical density; ELISA = enzyme-linked immuno-assay.

In vitro modulation of complement activity by IVIg

We next evaluated whether the addition of IVIg interferes with anti-GM1 IgM-mediated complement deposition *in vitro*. Complement deposition was reduced in the presence of IVIg when using MMN patient sera with anti-GM1 IgM titers of 1:6400 (Figure 11.4A) and 1:400 (Figure 11.4B).

IVIg reduces concentrations of complement factors C1q and C4, and classical pathway activity in serum

IVIg administration significantly reduced C1q ($p = 0.01$) (Figure 11.5A) and C4 concentrations ($p < 0.001$) (Figure 11.5B) as well as classical pathway activity ($p = 0.02$) (Figure 11.5D) in serum samples from six patients with MMN who received IVIg for the first time (cumulative dose 2.0 g/kg). In contrast, C3 concentrations decreased only slightly after the first day of treatment, but remained unchanged thereafter (Figure 11.5C).

IgG concentrations were determined in serum from 3 patients during the first 5-day course of IVIg. There was a significant and dose-dependent inverse correlation of IgG concentrations with C4 levels ($p = 0.015$), but not with C1q and C3 concentrations or CH50 values.

In 53 serum samples from 39 MMN patients receiving IVIg maintenance therapy, C1q, C3 and C4 concentrations were within the normal range. Median IgG concentration was 18.7 g/L

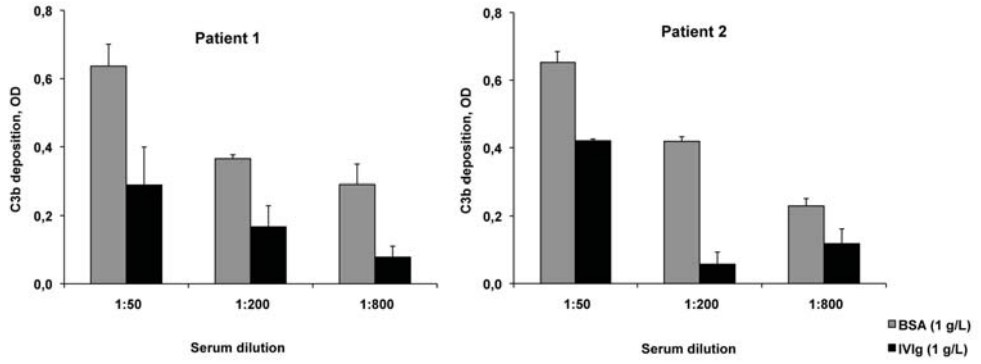


Figure 11.4 The presence of IVIg attenuates complement deposition in ELISA plates. 1g/L IVIg or BSA (control samples) was added to GM1-coated ELISA plates after incubation with MMN patient sera with anti-GM1 IgM titers of 1:6400 (A), 1:400 (B) respectively. Data are presented as mean values from three independent experiments after subtraction of background signals. OD = optical density; IVIg = intravenous immunoglobulins; BSA = bovine serum albumin; ELISA = enzyme-linked immuno-assay.

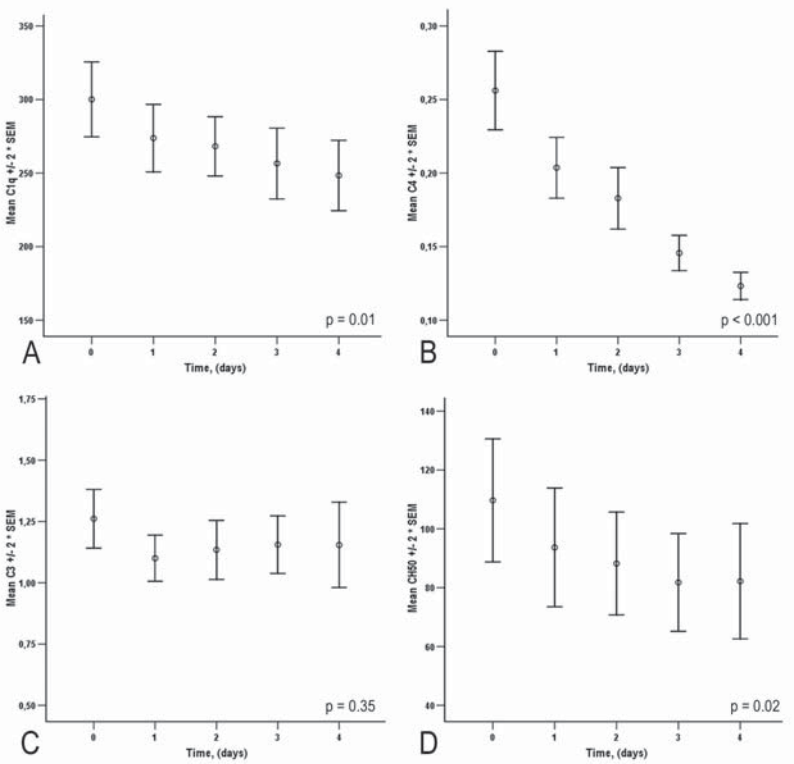


Figure 11.5 IVIg administration on 5 consecutive days reduces C1q concentration (A), C4 concentration (B) and classical pathway activity, as reflected by reduced hemolytic activity (D), in serum from MMN patients. C3 concentrations remained unchanged (C). Data are presented as mean values from three independent experiments. IVIg = intravenous immunoglobulins; MMN = multifocal motor neuropathy.

(range 12.6 – 35.6 g/L, normal values 7-16 g/L). Intrinsic activity of the classical pathway of complement in serum from patients with MMN on maintenance treatment was slightly lower (median optical density (OD) value 0.503, range 0.120 – 0.980) than in sera from healthy controls (median OD value 0.591, range 0.167 – 0.960), but this failed to reach statistical significance (p = 0.7). Intrinsic classical pathway of complement activity was lower in sera from MMN patients with higher IgG concentrations, as reflected by the inverse correlation with C3 deposition (p = 0.01, R^2 = 0.2) and C4 concentrations (p = 0.001, R^2 = 0.2).

DISCUSSION

The reported immune-modulating effects of IVIg are numerous, but it is not known which of these effects causes improvement of muscle strength in patients with MMN.[4, 31] The present study shows that IVIg attenuates the deposition of C3 fragments after complement activation by anti-GM1 IgM antibodies *in vitro*. This local effect may be further amplified by a systemic decrease of serum concentrations of complement factors C1q and C4, but not C3, and of classical pathway activity. Experimental models have shown that complement deposition is crucial in the pathogenesis of neuropathies caused by glycolipid-specific antibodies.[23, 24, 32, 33] Complement-induced damage to the axon, myelin sheath or Schwann cells can be prevented by drugs that interfere with complement deposition.[25, 34] IVIg may represent a potent modulator of complement by exerting both systemic and local (i.e. in nerves) effects. It is tempting to speculate that the crucial role of complement in MMN pathogenesis is reflected by the poor results obtained with plasmapheresis in comparison with IVIg treatment. In contrast to IVIg, plasmapheresis may replenish rather than reduce concentrations of complement factors, thus allowing continuing deposition of complement factors and clinical deterioration.[8, 9]

In the mouse phrenic nerve-diaphragm model, the addition of IVIg reduced binding of anti-GQ1b IgG and, as a result, decreased deposition of the complement membrane attack complex.[35] Although we also observed that the addition of IVIg attenuated complement deposition by anti-GM1 IgM, antibody binding to GM1 did not change significantly. We cannot exclude that IVIg causes a crucial alteration of the binding kinetics of anti-GM1 IgM antibody for its antigen that is not detected by ELISA. Since antibody affinity is an important determinant for activation of complement,[36] changes in binding kinetics may result in reduced levels of complement activation. Alternatively, IVIg may decrease the deposition of membrane attack complex (MAC) by the formation of complexes between IVIg and C3b, thereby preventing the incorporation of activated C3 molecules into C5 convertases.[37]

We detected anti-GM1 IgM antibodies in sera of 77% of the MMN patients on IVIg maintenance treatment, a relatively high percentage in comparison with other studies. Differences in ELISA protocols and cut-off values probably cause the wide range of anti-GM1 IgM positive MMN patients (25% up to 85%) in the literature.[11, 38-42] Using a higher cut-off value of 1:400, 44% of the sera of MMN patients contained anti-GM1 IgM antibodies. However,

complement activation by anti-GM1 IgM antibodies was significantly higher using sera with low anti-GM1 IgM titers from MMN patients as compared to controls with similar titers. Only 2 sera from disease controls induced complement activation, but only slightly higher than background levels. These results indicate that titers and complement-activating properties of anti-GM1 IgM are generally higher in patients with MMN than controls. We recently found a moderate but significant correlation between anti-GM1 IgM titers and the degree of weakness, disability and axon loss in patients with MMN.[43] Combined, these data suggest that anti-GM1 IgM antibodies play a role in MMN pathogenesis.

Treatment with IVIg at a cumulative dose of 2.0 g/kg also causes a rapid decrease of C1q and C4 concentrations in blood, and a reduction of the hemolytic activity of the classical complement pathway. It is striking that, with the exception of a slight decrease on the first day of treatment, C3 concentrations remain virtually unchanged. These findings suggest that, despite IVIg-induced activation of the classical pathway with C1q and C4 consumption, a functional C3 convertase is not formed.[44] We observed a similar depletion of C1q and C4, but not C3, in patients with IgG deficiency who were treated with IVIg, suggesting that attenuation of the classical pathway is common after IVIg administration, and is not confined to inflammatory disorders. IVIg administration did not reduce anti-GM1 IgM titers or complement-activating properties of these antibodies in serum of MMN patients treated with IVIg. We, therefore, feel that it is unlikely that IVIg has an additional anti-idiotypic effect in MMN. Others have reported similar findings.[45-48]

The improvement in muscle strength after IVIg treatment wears off after several weeks and most patients require maintenance treatment, which generally consists of infusion of 0.4-1.0 g/kg every 2 to 6 weeks. Although infusion of 0.4 g/kg IVIg reduced concentrations of C1q, C4 and haemolytic activity of the classical pathway of complement (Figure 11.4), C1q, C3 and C4 concentrations and the activity of the classical pathway of complement in serum from patients on maintenance treatment were within the normal range. Apparently, concentrations of complement factors normalize in the days to weeks after IVIg maintenance treatment. However, the inverse correlations of intrinsic activity of the classical complement pathway with IgG and C4 concentrations in serum seem to suggest that IVIg-induced modulation of complement function occurs to some extent even at normal concentrations of complement factors. The long-lasting effects of IVIg therapy may also be explained by prolonged reduction of concentrations of complex mediators of complement amplification, such as C3b2-containing complexes.[44]

Experimental drugs, which prevent complement deposition, are effective in animal models of antibody-mediated neuropathy.[34, 49, 50] Although these experimental treatments remain to be tested, further attenuation of complement activation, or techniques such as immunoadsorption that specifically reduce levels of circulating anti-GM1 IgM antibodies, in addition to IVIg, may prove effective treatment strategies for patients with MMN.[51]

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12

General discussion

Based on:
van der Pol WL, Cats EA, van den Berg LH. Intravenous immunoglobulin treatment in multifocal motor neuropathy. Journal of Clinical Immunology 2010;30 S79-83.
Vlam L, van der Pol WL, Cats EA, Straver DCG, Piepers S, Franssen H, van den Berg LH. Multifocal motor neuropathy: clinical features, pathophysiology and treatment. Nature Reviews Neurology, in press.

CLINICAL FEATURES OF MMN

At the start of this research project, several small and medium-sized cohort studies and a few elaborate reviews highlighting the clinical features of multifocal motor neuropathy (MMN) had been published,[1-6] but the relative rarity had precluded detailed studies of the phenotype and correlates of outcome in larger numbers of patients. Therefore, we aimed at identifying and examining all patients with MMN in the Netherlands. In a cross-sectional study we identified 97 patients with MMN in The Netherlands, which corresponds to a prevalence of at least 0.6 per 100,000 inhabitants. This prevalence is similar to prevalence estimates in previous studies.[3] Investigation of 88 MMN patients, which is the largest group of MMN patients in the world at this moment, allowed us to describe the clinical phenotype in detail.[7] MMN is, probably due to its occasional similarity to early stages of amyotrophic lateral sclerosis (ALS), often considered a benign disease. Nevertheless, functional impairment can be quite serious. One-fifth of our patients reported severe disability of the arms, and more than half of MMN patients reported severe fatigue, a feature that also accompanies other immune-mediated disorders of the peripheral nervous system such as the Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP).[8] To analyze determinants of outcome in MMN patients, we used multivariate analysis, which showed that axon loss and years without treatment with intravenously administered immunoglobulins (IVIg) were associated with more severe weakness and disability (*chapter 2*).

PATHOGENESIS: NERVE CONDUCTION ABNORMALITIES AND ANTIBODIES

Although axonal degeneration is the most important determinant of muscle weakness,[7, 9] conduction block (CB) in motor nerves is the characteristic finding that distinguishes MMN from ALS and lower motor neuron disorders with a comparable clinical phenotype.[3, 5] CB is defined as an inability of motor nerves to propagate action potentials. The mechanisms causing CB are incompletely understood. Both dysfunction of the axon and myelin sheath may underlie CB. Whether CB precedes axon loss, whether CB and axon loss occur in parallel and are caused by different pathogenic mechanisms, or whether they are distinct phenomena of similar pathogenic processes, is unknown. The few pathological studies of motor nerves from patients with MMN have shown diverging results. Both demyelination[10] and axonal degeneration were reported.[11] Sodium channel dysfunction may play an important role in MMN pathogenesis.[12]

Presence of IgM antibodies against the ganglioside GM1 is a second characteristic feature of MMN. It is tempting to speculate on the possible connection between the presence of anti-GM1 IgM antibodies, sodium channel dysfunction and electrophysiological abnormalities. Experimental studies have provided the first evidence that sodium channel dysfunction and the presence of anti-GM1 antibodies may be linked.[13] Although anti-GM1 antibodies have also

been detected in serum from patients with other neurological disorders than inflammatory neuropathies,[14] complement and leukocyte activating properties of anti-GM1 antibodies are probably unique to GBS and MMN.[15] Anti-GM1 antibodies may therefore cause complement deposition after binding to GM1 where it is highly expressed, such as the paranodal myelin or possibly the axolemma at the nodes of Ranvier.[16] The deposition of the membrane attack complex in paranodal myelin and the axolemma would explain the occurrence of demyelination and axonal damage as seen in motor nerves from patients with MMN. Recent studies have also provided insight how sodium channels are affected by the binding of anti-GM1 antibodies. Using the rabbit model of acute motor axonal neuropathy (AMAN), it has been shown *in vivo* that antibodies against the ganglioside GM1 disrupt the sodium channel clusters at the nodes of Ranvier.[13] This study has therefore provided important clues how immunological and electrophysiological features of MMN may be linked.

PATHOGENESIS: ANTIBODY SPECIFICITY AND ORIGIN

The presence of serum anti-ganglioside IgM antibodies against GM1 was already acknowledged in the early descriptions of MMN. The reported prevalence of anti-GM1 IgM antibodies in MMN patients varied widely probably due to differences in laboratory assays.[16-19] IgM antibody activity against gangliosides other than GM1 was also reported,[16, 20] but this had not been investigated in detail. In patients with GBS, specificity of anti-ganglioside antibodies is associated with clinical features.[16, 21, 22] In *chapter 3* we studied the prevalence and specificity of antibodies against single gangliosides and complexes of gangliosides and the association with clinical features in MMN patients. Specificities of anti-ganglioside antibodies are very limited. We detected almost exclusively IgM antibody reactivity against GM1 (43% of patients), GD1b (9%) and GM2 (6%), but not against any other ganglioside. Antibodies against GD1b and GM2 almost always cross-reacted with GM1, which suggests that antibodies specific for other gangliosides than GM1 are indeed very rare in MMN. The presence of anti-GM1 IgM antibodies was associated with more severe weakness and disability and higher antibody titers were generally accompanied by more severe weakness. Axon loss was also more prevalent in patients with anti-GM1 IgM antibodies. These results mimic findings in AMAN. It is interesting to note that GM1 is also expressed in dorsal root ganglia, and that it is unknown why motor deficits predominate in neuropathies associated with anti-GM1 antibodies.[16] We more often found vibration sense abnormalities in patients with anti-GM1 IgM antibodies than those without, and there was a significant relation with vibration sense abnormalities in patients with antibodies against GM1/GD1b. These findings might suggest that longstanding exposure to anti-GM1 antibodies may lead to decreased function of dorsal root ganglia, and decreased amplitudes of sensory nerve action potentials (SNAPs).[23, 24] Taken together, the results of this study lend further support to the hypothesis that anti-GM1 IgM antibodies play a role in MMN pathogenesis.[25]

We used a very specific cut-off titer to define 'positive' anti-GM1 IgM titers. Although we may thus have underestimated the percentage of patients with anti-GM1 IgM antibodies, it is clear that a significant fraction of patients is 'seronegative'. We initially hypothesized that sera from anti-GM1 seronegative MMN patients contains antibodies against ganglioside complexes, similar to patients with GBS.[26] However, we did not find evidence for this in any of our 88 patients. Since clinical phenotypes of seropositive and seronegative patients with MMN are largely comparable, it seems plausible that seronegative patients have antibodies against at present unidentified glycolipids or other motor nerve constituents with expression profiles comparable to GM1, or with a similar function. It was recently shown that sera from seronegative patients with unspecified motor neuropathy contain antibodies against NS6S heparin disaccharide.[27] This antigen was identified after the authors noticed an increased background signal in enzyme-linked immunosorbent assay (ELISA) for myelin-associated-glycoprotein (MAG). The relevance of this candidate antigen for MMN needs to be further explored.

Assuming that anti-GM1 IgM antibodies are important in the pathogenesis of MMN, we speculated which mechanisms could cause increased titers in almost half of all patients with MMN. GM1-specific B-cells are part of the natural antibody repertoire of the immune system. Stimulation of these B-cells would cause increased titers, and could be triggered by exposure to microbes expressing GM1-mimicking epitopes, i.e. 'molecular mimicry', a mechanism that causes GBS.[28] Monoclonal proliferation of GM1-specific abnormal B-cell clones (monoclonal gammopathy), or monoclonal or oligoclonal proliferation due to autoimmunity are alternative explanations. Case reports have suggested that MMN and acute neuropathies with CB may be preceded by infection with *Campylobacter jejuni* (*C. jejuni*), similar to GBS.[29-31] *C. jejuni* strains often express GM1-mimics on their lipo-oligosaccharides (LOS).[16, 32] Patients with GBS many times recall gastro-enteritis preceding weakness, and serological studies have confirmed the association of *C. jejuni* infection and GBS.[21, 28] Although case reports suggested that MMN may be preceded by *C. jejuni* infection, a more systematic analysis showed that only one out of 20 MMN patients had high anti-*C. jejuni* antibody titers.[33] In our group of 88 MMN patients anti-*C. jejuni* IgM and IgA/IgG antibody titers were comparable to those in the normal Dutch population (5-10%);[34] one MMN patient had high anti-*C. jejuni* IgM antibody activity (cut off optical density (OD) 1.0), six patients high anti-*C. jejuni* IgG antibody activity (cut off OD 10.0) and eight patients high anti-*C. jejuni* IgA antibody activity (cut off OD 0.6) (unpublished observation). It is important to note that virtually all patients with MMN use maintenance IVIg treatment, which complicates the interpretation of titers of anti-*C. jejuni* IgG antibodies. Furthermore, the average disease duration precluded that patients would recall an episode of gastro-enteritis prior to MMN onset. These and previous serological results do not support the hypothesis that MMN is a post- or parainfectious disorder caused by *C. jejuni*, similar to GBS. We also hypothesized that colonization with bacteria previously unknown to express GM1 might cause increased anti-GM1 IgM titers. We therefore screened a 'bacterial library' for species

expressing GM1 using flowcytometry and FITC-labeled choleratoxin, a high-affinity ligand for GM1. This approach did not lead to identification of new species expressing GM1-like mimics.

Monoclonal gammopathy of GM1-specific B-cells may be another cause of increased anti-GM1 IgM titers. Serum samples from approximately 15% of patients with polyneuropathy associated with IgM monoclonal gammopathy contain antibodies against gangliosides.[35] We therefore evaluated whether monoclonal gammopathy is associated with MMN. IgM monoclonal gammopathy was detected by immunofixation and immunoelectrophoresis[36] in six out of 88 patients with MMN (7%) and 9 out of 430 (2%) healthy controls (OR 6.5, 95% confidence interval 2-21, $p = 0.002$). Since we did not perform bone marrow biopsies in these patients, we do not know whether this is explained by abnormal monoclonal proliferation of plasma cells. One of our patients without anti-GM1 IgM antibodies but with monoclonal gammopathy developed Waldenstrom's disease, but this might be coincidental. To further investigate clonality of anti-GM1 IgM antibodies we assessed light chain use of anti-GM1 IgM antibodies with ELISA-methodology. In the large majority of patients with MMN only one light chain was used, which suggests that these antibodies are monoclonal (*chapter 4*). We identified two patients with an IgM M-protein using the same light chain as the anti-GM1 IgM antibodies, but also two patients with an M-protein and anti-GM1 IgM antibodies using both IgL and IgK. Furthermore, the patient with Waldenstrom's disease and one other patient with an M-protein did not have anti-GM1 IgM antibodies. It remains to be established whether the M-protein reflects the increased titer of anti-GM1 IgM antibodies or possibly antibodies against other motor nerve constituents. Nevertheless, the findings in *chapter 4* seem to support the hypothesis that the immune response to GM1 in MMN is usually restricted to a very limited number of B-cell clones.

Common pathogenic pathways underlying autoimmune disease as an explanation for MMN was the third hypothesis we explored. In *chapter 5* we describe that autoimmune diseases (AID) occur at a higher frequency in patients with MMN compared to controls. We did not find an association of specific AID with MMN, and the co-occurrence of AID was not associated with MMN disease characteristics. In family members of patients with MMN the prevalence of type 1 diabetes, celiac disease and Hashimoto's thyroid disease were increased. The study was underpowered, but the higher frequency of AID in MMN patients (11%) compared to controls (5%) suggests that MMN may represent an AID sharing genetic risk factors with other AID.[37] Candidate genes include genes that have also been shown to influence susceptibility to the clusters of type 1 diabetes and celiac disease or Hashimoto's thyroid disease.[37-39]

PATHOGENESIS: ANTIBODIES AND COMPLEMENT

Experimental models of inflammatory neuropathy have shown that anti-ganglioside antibodies exert pathogenic effects by activating the complement cascade.[13, 40] Anti-ganglioside antibodies with complement-activating properties elicit neuropathy in rabbits whereas inert antibodies do not.[15] Anti-GM1 IgM antibodies in sera from MMN patients, but not in sera

from patients with motor neuron disease, have been shown to activate complement.[41] It was also shown that genetic heterogeneity of mannose binding lectin (MBL) is associated with severity of GBS. We therefore investigated classical and lectin pathway activity, MBL serum concentration and *MBL2* haplotype in patients with MMN and controls. Complement activity was assessed using pathway-specific ELISA. Classical and lectin pathway activity did not differ between patients and controls, and was not associated with outcome of MMN. Similarly, frequencies of *MBL2* haplotypes were comparable in patients with MMN and in healthy controls. We could not show that innate differences in complement pathway activity are associated with MMN or its disease course. Complement presumably plays an important role in MMN pathogenesis, although anti-ganglioside antibodies might also exert pathogenic effects via complement-independent pathways.[42] The methodology we used may also have important limitations. ELISA methodology may not be sufficiently sensitive to detect biologically relevant differences in complement pathway activity. Moreover, complement pathway activity in patients with MMN may have been reduced by frequent infusions of IVIg (*chapter 6*).

GENETICS

To explore the role of genetic heterogeneity for MMN susceptibility and outcome, we evaluated the relevance of polymorphic candidate genes in genetic association studies. In *chapter 7* we describe the association of MMN with HLA-DRB1*15.[43] This specific association has also been described in patients with chronic inflammatory demyelinating neuropathy (CIDP)[44] and multiple sclerosis (MS).[45] In those disorders, there is evidence of T-cell involvement, and HLA-DRB1*15 may facilitate antigen presentation of specific peptides by antigen presenting cells (APC) to autoreactive lymphocytes. However, glycolipids like GM1 are not presented by classical HLA-molecules, but by molecules from the cluster of differentiation 1 (CD1) family. Polymorphisms of *CD1* were not associated with MMN (*chapter 8*).[46] The association with HLA-DRB1*15 with MMN was independent of presence of anti-GM1 IgM antibodies. We cannot exclude the possibility that this association is an indication that other unidentified auto-antigens (peptides) than anti-GM1 antibodies play a role in MMN pathogenesis. Alternatively, other genetic risk factors than HLA-DRB1*15 may be in linkage disequilibrium with HLA-DRB1*15. This latter possibility is supported by the fact that pathological and serological studies have not given any indication for T-cell involvement in MMN.[10, 11, 47] In our MMN patients we did not find an association of other genetic risk factors influencing B-cell function, that are shared by a number of AID (*chapter 8*). The increased frequency of type 1 diabetes, celiac disease and Hashimoto's thyreoid disease in family members from patients with MMN suggests that shared genetic risk markers (like single-nucleotide polymorphisms (SNPs) *CTLA4*, *RGS1*, *IL18RAP*, *TAGAP*, *PTPN2*, *CTLA4*, *SH2B3*)[37, 39] for these disorders may also be relevant for MMN pathogenesis.

TREATMENT

The responsiveness to treatment with IVIg[48-51] and cyclophosphamide[52] and the lack of beneficial effects of steroids and plasma exchange[53-55] has been used as another line of evidence that MMN is caused by an interplay of B-cells, (auto-)antibodies and complement activity. Long-term use of cyclophosphamide is restricted by its side-effects.[52] At the time of our cross-sectional study, 76% of our MMN patients received regular IVIg infusions. The fact that this treatment is time-consuming is a major disadvantage. In *chapter 9* we describe the efficacy, safety and tolerability of a new, more concentrated IVIg product. Infusion times of this IVIg preparation were substantially shorter than those of the lyophilized product. The findings in *chapter 10* show that IVIg treatment at home is time-saving and reduces the number of days missed at work. These data suggest that the additional costs of IVIg treatment (i.e. absence at work, hospital admission) can be minimized by home treatment with liquid 10% IVIg preparations.

It is unknown why IVIg exerts a beneficial effect in patients with MMN. The immune modulating effects of IVIg are multiple. In *chapter 11* we analyzed the effects of IVIg on anti-GM1 IgM antibody titers, complement-activating properties of these antibodies and complement function.[56] IVIg infusion reduces concentrations of critical components of the classical/ lectin pathways, in particular C4. In contrast, C3 levels are unchanged. This is reflected in decreased functionality of the classical pathway of complement after IVIg infusion, but normal alternative pathway activity. *In vitro* studies showed that IVIg may also inhibit complement activation or deposition by anti-GM1 IgM antibodies. Inhibiting the interaction of antibodies and complement may therefore be a relevant mechanism by which IVIg exerts its effects in patients with MMN. These findings, in combination with results from experimental studies which showed that complement inhibitors can be effective in avoiding nerve damage,[40, 57] suggested that trials with complement-inhibiting drugs might be tried. A recent open label study with eculizumab, a monoclonal antibody preventing cleavage of C5 and activation of the terminal pathway of complement, suggested some additional benefit, although most patients required continuation of IVIg therapy.[58] We cannot exclude the possibility that IVIg is a very effective inhibitor of complement,[59] even in comparison with modern biologicals. Alternatively, IVIg may exert other immunemodulatory effects than complement inhibition that cause improvement in MMN.

The results from this thesis also suggest that MMN is caused by a limited number of pathogenic B-cell clones. Elimination of these clones might be another attractive treatment strategy. A few case reports have suggested that treatment with anti-CD20 monoclonal antibody (rituximab)[60, 61] may be effective, but this has not been corroborated by others. A randomized controlled trial with rituximab is needed to evaluate its efficacy.

DIRECTIONS FOR FUTURE RESEARCH

For the development of more effective treatment strategies, the following questions need to be answered:

1. Does serum from anti-GM1 IgM 'seronegative' patients with MMN contain antibodies against other motor nerve constituents? Are these antibodies directed against glycolipids/ glycoproteins or against peptides? Is their function and expression profile similar to GM1?
2. What causes the increase in anti-GM1 IgM antibody titer in anti-GM1 IgM 'seropositive' patients?
3. Are there specific phenotypes of B-cell clones that underlie MMN pathogenesis? Can these phenotypes be used to design strategies for specific B-cell elimination?
4. Which mechanisms cause axon loss? Are anti-GM1 IgM antibody induced pathogenic effects always complement dependent?
5. Do anti-GM1 IgM antibodies cause conduction block and how does this work?
6. How often and with which dose of IVIg should patients with MMN be treated to avoid or postpone axon loss?
7. Can we identify additional genetic risk factors that determine susceptibility to MMN?

We expect that answers to these questions will contribute to the development of targeted immunotherapeutic strategies.

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Summary

Multifocal motor neuropathy (MMN) is a pure motor neuropathy with conduction block (CB) characterized by slowly progressive, asymmetrical weakness of limbs. It was first described approximately 20 years ago. MMN is an important mimic of motor neuron disease (MND), but is amenable to treatment and has a more favorable prognosis. The relative rarity of MMN has precluded detailed studies of the phenotype and the pathogenesis of MMN is incompletely understood.

Aims of this thesis were to document clinical phenotypes of MMN, to study the immune pathogenesis and genetics of MMN and to describe the correlates of outcome and response to intravenous immunoglobulin (IVIg).

PART I – CLINICAL FEATURES OF MMN

We performed a national study on MMN and identified 97 patients, which corresponds to a prevalence of at least 0.6 per 100,000 inhabitants. All participating patients were examined. We did not only confirm that MMN is more prevalent in men than women, but also showed that symptom onset was at a younger age in males. Clinical presentation and patterns of weakness were fairly homogeneous, with onset of weakness in the distal arm or distal leg, and occasionally in the upper arm. In one-third of patients the initial diagnosis was MND. Brisk, but not pathological, reflexes in weakened muscles were not uncommon. Ulnar, median, radial and tibial nerves were most commonly affected, with striking differences in weakness of muscles innervated by a common terminal nerve. Ninety-four percent of patients responded to IVIg therapy: non-responders had longer disease duration before the first treatment. Multivariate analysis showed that axon loss and longer disease duration without IVIg were independent determinants of more severe weakness and disability (*chapter 2*).

PART II – IMMUNE PATHOGENESIS OF MMN

Prevalence and specificity of antibodies against single gangliosides and ganglioside complexes in serum from 88 MMN patients were investigated. Anti-ganglioside IgM antibodies in MMN had a surprisingly limited specificity (against GM1, and occasionally against GD1b and GM2) (*chapter 3*). Therefore, we investigated whether anti-ganglioside antibodies also showed limited clonality. Using light chain analysis of anti-GM1 IgM antibodies, we demonstrated that serum anti-GM1 IgM antibodies in the majority of patients with MMN have the same Ig light chain, suggesting that these antibodies are monoclonal. This is a further indication of the highly selective fine-specificity of these antibodies (*chapter 4*). The presence of anti-GM1 IgM antibodies was associated with a relatively poor outcome. Patients with anti-GM1 IgM antibodies had more disability, more axon loss and more severe weakness compared to patients without anti-GM1 IgM antibodies (*chapter 3*). These findings support the assumption that anti-ganglioside IgM antibodies play a role in MMN pathogenesis, but also suggest that a limited number of B-cell clones is activated, even in patients with longstanding disease.

Although it seems generally accepted that MMN is an inflammatory disorder, it is not clear whether MMN is a 'classic' autoimmune disease (AID). The male predominance is uncommon for AID, which tend to have a higher incidence in women. Since different AID often co-occur within patients and their families, we studied the prevalence of AID among MMN patients and their families. In a case-control study encompassing 81 MMN patients and 417 first-degree relatives, and 438 controls and 2,377 first-degree relatives we found that AID are more common in MMN patients (11%) compared to controls (5%) (*chapter 5*). This finding might suggest that MMN is not only an inflammatory disorder, but may represent an AID sharing genetic risk factors with other AID.

Because of the importance of the complement system in the pathogenesis of experimental models for antibody-mediated neuropathy, we studied variation in the activity of the classical and lectin pathway of the complement system in MMN patients and controls. We found no difference in activity of both pathways between patients and controls, and complement activity was not associated with outcome of MMN (*chapter 6*).

PART III – GENETICS OF MMN

The human leukocyte antigen (HLA) locus is highly heterogeneous, and several HLA alleles have been found associated with AID. A case-control study showed that HLA-DRB1*15 is associated with MMN (*chapter 7*). Although this finding may support the hypothesis that MMN is a classic AID, we did not find increased frequencies of single nucleotide polymorphisms (SNPs) in genes that are common in a number of other AID (*chapter 8*).

PART IV – MMN TREATMENT

Treatment with IVIg is the only intervention known to improve muscle strength in MMN patients. It has been shown to postpone axonal degeneration. Maintenance treatment with IVIg every few weeks is necessary because the beneficial effects of IVIg infusions only last a few weeks. IVIg infusions are time-consuming and the frequent visits to the hospital may be burdensome for patients. In *chapter 9* we compared the efficacy, safety and tolerability of the 10% ready-to-use liquid IVIg preparation with the less concentrated (5%) freeze-dried preparation in 20 patients with MMN and showed that the 10% IVIg preparation was well tolerated in patients with MMN. Muscle strength and disability scores remained stable. Infusion times were substantially reduced in 85% of patients. A cross-sectional descriptive study of 52 MMN patients receiving home-based IVIg treatment and 15 MMN patients receiving hospital-based IVIg treatment showed that IVIg treatment at home is time-saving and reduces the number of days missed at work. Home-treatment is safe when maximum infusion rates are respected and the presence of an anaphylactic kit at home is ensured. Home-based IVIg treatment is more convenient for most MMN patients (*chapter 10*). Despite its use, the mechanisms of IVIg that underlie its efficacy in MMN have not been studied in detail. Relevant effector mechanisms of

IVIg include anti-idiotype effects, and modulation of B-cell and complement function, among others. In *chapter 11* we compared the complement-activating properties of anti-GM1 IgM antibodies in sera from MMN patients and disease controls. We showed that anti-GM1 IgM antibodies in sera from patients with MMN efficiently activate complement in comparison with disease controls. The addition of IVIg reduced complement deposition significantly. IVIg infusions also reduced concentrations of crucial classical pathway components including C1q in sera of MMN patients. IVIg may thus exert both local and systemic effects on the classical route of the complement system, which may contribute to reduced complement deposition in nerves.

Samenvatting
(Summary in Dutch)

Multifocale motorische neuropathie (MMN) is een puur motorische polyneuropathie. Hiermee wordt bedoeld dat alleen motorische zenuwen (en dus niet de gevoelszenuwen) minder goed gaan functioneren. Het is een langzaam progressieve aandoening waarbij asymmetrische spierzwakte van armen en/of benen ontstaat. Zenuwgeleidingsonderzoek laat vrijwel altijd de voor MMN karakteristieke geleidingsblokkade zien. Geleidingsblokkade betekent dat een actiepotentiaal in een bepaald deel van de zenuw niet (optimaal) kan worden doorgegeven en de spier daarom niet of slechts ten dele bereikt. In het bloed van patiënten met MMN worden opvallend vaak IgM antistoffen tegen het glycolipide GM1, dat in zenuwen tot expressie komt, aangetoond. MMN werd ongeveer 20 jaar geleden voor het eerst beschreven.

MMN geeft dezelfde symptomen (spierzwakte) als amyotrofische laterale sclerose (ALS). Het is van belang MMN van ALS te onderscheiden omdat het een behandelbare aandoening is en een betere prognose heeft. MMN is geen 'goedaardige' aandoening omdat er in het beloop van de ziekte permanente zwakte van ledematen en forse handicaps kunnen ontstaan. Het is daarom belangrijk om de effectiviteit van de behandeling te verbeteren. Het is tot op heden moeilijk geweest gedetailleerde studies uit te voeren naar het klinische fenotype, het beloop en de pathogenese van MMN omdat het een zeldzame ziekte is.

Het doel van de studies in dit proefschrift was om het klinische fenotype nauwkeurig te beschrijven aan de hand van een grote groep patiënten met MMN, de immuunpathogenese van MMN te bestuderen en de bijdrage van genetische variatie aan het ontstaan van MMN te onderzoeken. Kennis over determinanten voor het beloop en de uitkomst van MMN en over de pathogenese kan bijdragen aan het optimaliseren van bestaande therapieën of de ontwikkeling van nieuwe behandelingsvormen.

DEEL I – KLINISCHE KARAKTERISTIEKEN VAN MMN

In Nederland zijn er tenminste 97 patiënten met MMN. Dat komt overeen met een prevalentie van tenminste 0,6 per 100.000 inwoners. Alle patiënten met MMN werden uitgenodigd om deel te nemen aan het landelijke onderzoek naar MMN. Achtentachtig patiënten werden lichamelijk en elektrofysiologisch onderzocht in het UMCU. MMN komt vaker voor bij mannen dan bij vrouwen. De ziekte ontstaat bovendien op een jongere leeftijd dan bij vrouwen. Vrijwel altijd wordt spierzwakte het eerst bemerkt in het distale deel van een arm of een been en slechts sporadisch in het proximale deel van een arm. Er zijn vaak opvallende verschillen in zwakte tussen spieren die geïnnerveerd worden door dezelfde zenuw. Met elektrofysiologisch onderzoek werd aangetoond dat de nervus ulnaris, medianus, radialis en tibialis het meest frequent zijn aangedaan. Levendige, maar niet pathologische, reflexen werden regelmatig gevonden, ook in verzwakte spiergroepen. Vierennegentig procent van de patiënten reageerde goed op therapie met intraveneuze immunglobulines (IVIg). Patiënten die niet goed reageerden op deze therapie hadden een langere ziekteduur voordat zij hun eerste behandeling met IVIg kregen of hadden veel permanente ('axonale') schade aan hun zenuwen (*hoofdstuk 2*).

DEEL II – IMMUUNPATHOGENESE VAN MMN

In het bloed van patiënten met MMN kunnen vaak IgM antistoffen tegen het glycolipide GM1 worden aangetoond. In de literatuur wordt bovendien sporadisch melding gemaakt van het voorkomen van antistoffen tegen andere glycolipiden. Er is veel discussie over het voorkomen van antistoffen bij MMN – dit wisselt van 20 tot 80%, waarschijnlijk door het gebruik van verschillende technieken in laboratoria. De prevalentie en specificiteit van antistoffen tegen gangliosiden en complexen van gangliosiden werden daarom onderzocht in het serum van 88 Nederlandse MMN patiënten. IgM antistoffen met een titer ≥ 1:400 (een zeer specifieke waarde) tegen GM1 werden gevonden in het bloed van 38 (43%) patiënten. In 5% van de patiënten werden IgA antistoffen gevonden tegen GM1. De anti-ganglioside IgM antistoffen bleken bovendien een zeer beperkte specificiteit (tegen GM1 en sporadisch tegen GD1d en GM2) te hebben (*hoofdstuk 3*). Anti-GD1b IgM en anti-GM2 IgM antistoffen werden gevonden bij respectievelijk 8 en 5 patiënten. De GD1b en GM2 specificiteit kon grotendeels worden verklaard door kruisreactiviteit met GM1. Analyse van de lichte ketens in de anti-GM1 IgM antistoffen toonde in de meeste patiënten met MMN het gebruik van slechts één lichte keten, in tegenstelling tot soortgelijke antistoffen in het bloed van patiënten met het Guillain-Barré syndroom (*hoofdstuk 4*). Deze gegevens suggereren niet alleen dat de antistof respons erg specifiek is, maar mogelijk ook door een zeer beperkt aantal B-cel klonen wordt geproduceerd. Patiënten met anti-GM1 IgM antistoffen bleken meer beperkingen, meer axonale schade en ernstiger spierzwakte te hebben dan de patiënten zonder anti-GM1 IgM antistoffen (*hoofdstuk 3*). Deze bevindingen lijken de gedachte te ondersteunen dat anti-ganglioside IgM antistoffen een rol spelen in de pathogenese van MMN. De resultaten suggereren ook dat de antistoffen zelfs bij patiënten die al langere tijd de ziekte hebben worden geproduceerd door een klein aantal B-cel klonen.

Hoewel het zeer waarschijnlijk is dat MMN een inflammatoire ziekte is, is het onduidelijk of MMN een 'klassieke' auto-immuunziekte is. Auto-immuun ziektes (AIZ) clusteren vaak in families en patiënten. Een verhoogde prevalentie van AIZ bij patiënten met MMN en hun familieleden zou de hypothese ondersteunen dat MMN een AIZ is. Een case-control studie met 81 MMN patiënten en 417 van hun eerstegraads familieleden en 438 controles met 2377 eerstegraads familieleden vonden we inderdaad dat AIZ iets vaker bij MMN patiënten (11%) dan bij controles (5%) voorkomen (*hoofdstuk 5*). MMN zou daarom genetische (zie deel III) of omgevingsrisicofactoren kunnen delen met andere auto-immuunziekten.

Tot slot onderzochten wij het complementsysteem. IgM auto-antistoffen kunnen de klassieke en mogelijk de lectine route van het complementsysteem activeren en zo schade aan weefsels veroorzaken. Het is bekend dat het complementsysteem een belangrijke rol speelt in de pathogenese van experimentele modellen voor antistof-gemedieerde neuropathieën. Polymorfismen in het mannose-binding lectin (MBL) gen zijn bovendien geassocieerd met het Guillain-Barré syndroom. Wij onderzochten mogelijke verschillen in activiteit van de klassieke en lectine routes van het complementsysteem in het bloed en het voorkomen van

MBL polymorfismen bij patiënten met MMN en controles. Het complementsysteem van patiënten was niet 'actiever' dan dat van controles, de mate van complementactivatie was niet geassocieerd met de ernst van de ziekte, en er waren geen verschillen in de frequenties van MBL polymorfismen tussen patiënten en controles (*hoofdstuk 6*).

DEEL III – GENETISCHE RISICOFACTOREN VOOR MMN

Specifieke HLA-allelen zijn geassocieerd met AIZ. Wij onderzochten daarom het voorkomen van HLA I en II allelen bij patiënten met MMN. Een case-control studie toonde aan dat HLA-DRB1*15 vaker voorkomt bij patiënten met MMN dan bij controles, maar dat dit allel niet geassocieerd is met het ziektebeloop (*hoofdstuk 7*). Wij onderzochten ook single nucleotide polymorphisms (SNPs) in PTPN22, BANK1, Blk, en FcγRIIB, genen die geassocieerd zijn met AIZ, maar vonden we geen bewijs voor een andere genetische predispositie dan de HLA-DRB1*15 bij Nederlandse MMN patiënten (*hoofdstuk 8*).

DEEL IV – BEHANDELING VAN MMN

Behandeling met IVIg is de enige bewezen therapie voor MMN. IVIg zorgt voor kortdurende verbetering van de spierkracht en onderhoudsbehandeling stelt permanente axonale schade uit. Onderhoudsbehandeling met een frequentie van eens per 1-6 weken is nodig omdat het positieve effect slechts enkele weken aanhoudt. De IVIg infusies zijn tijdrovend en de regelmatige bezoeken aan het ziekenhuis kunnen belastend zijn voor patiënten. In *hoofdstuk 9* vergeleken we de effectiviteit, veiligheid en tolerantie van een 10% vloeibaar IVIg product met het minder geconcentreerde (5%) gevriesdroogde (nog niet opgeloste) product bij 20 MMN patiënten. Het 10% IVIg product werd door patiënten goed verdragen en kon sneller dan het 5% product worden toegediend. De spierkracht en beperkingen van patiënten veranderden niet na de het stoppen van het 5% en de start van het 10% product. In Nederland worden veel patiënten thuis met IVIg behandeld. De veiligheid van en de ervaringen van patiënten met thuisbehandeling werden onderzocht in een cross-sectionele beschrijvende studie van 52 MMN patiënten die thuis en 15 patiënten die in het ziekenhuis met IVIg behandeld worden. Thuisbehandeling met IVIg is veilig als de maximale infusiesnelheden niet worden overschreden en er medicijnen tegen anafylactische reacties aanwezig zijn. Thuisbehandeling met IVIg leidt tot minder verzuim van werk dan behandeling in het ziekenhuizen de behandelingstijden zijn korter. De meeste patiënten geven de voorkeur aan thuisbehandeling (*hoofdstuk 10*).

IVIg toediening heeft zeer veel effecten op de werking van het immuunsysteem. Het werkingsmechanisme van IVIg bij MMN patiënten is niet gedetailleerd onderzocht. Anti-idiotype effecten, effecten op B-cel functie en remming van het complementsysteem zijn mogelijke werkingsmechanismen. Wij vonden geen aanwijzingen voor anti-idiotype effecten na behandeling van patiënten met MMN. IVIg infusie veroorzaakte een daling van de

complementfactoren C1q en C4 en een verminderde activiteit van de klassieke route. *In vitro* activatie van de klassieke route van het complementsysteem door anti-GM1 IgM antistoffen werd ook geremd door toevoeging van IVIg. IVIg heeft dus mogelijk zowel lokale als systemische effecten op het complementsysteem, wat zou kunnen leiden tot een reductie van complement depositie op motorische zenuwen (*hoofdstuk 11*).

Dankwoord
(Acknowledgements)

Graag wil ik alle patiënten met MMN bedanken die aan ons onderzoek hebben deelgenomen. Van Groningen tot Maastricht zijn zij bereid geweest steeds weer naar Utrecht af te reizen. Velen van hen zie ik nog steeds op mijn polikliniek en dat is prettig en leerzaam.

Prof. dr. L.H. van den Berg, beste Leonard, ik wil je bedanken voor de mogelijkheid die je mij hebt gegeven te promoveren. Ik bewonder je manier van wetenschappelijk onderzoek doen. Je bent een inspirerende kritische begeleider met veel humor en dat maakt het interessant én ook heel leuk om onderzoek onder jouw supervisie te doen.

Dr. W.L. van der Pol, beste Ludo, je hebt me ontzettend veel geleerd over neurologie en immunologie. Als ik iets niet snapte tekende je het. Zo heb ik in mijn bureau vele schetsen van ELISAs, het complementsysteem en de werking van B- en T-cellen kunnen verzamelen. Ik ben je dankbaar voor al je uitgebreide duidelijke uitleg, je betrokkenheid en je hulp bij het schrijven van de artikelen.

Prof. dr. J. van Gijn, professor van Gijn, u bent de eerste jaren mijn opleider geweest. Het is voor mij een eer door u te zijn opgeleid. Ik hoop nog vaak de kans te krijgen samen met u patiënten te zien op de polikliniek.

Prof. dr. J.H.J. Wokke, professor Wokke, ik wil u bedanken voor al het goede onderwijs dat u organiseert tijdens onze opleiding tot neuroloog. Ook wil ik u bedanken voor de leuke momenten die u met de assistenten weet te creëren, zoals de jaarlijkse barbecue bij u thuis.

De leden van de beoordelingscommissie, prof. dr. C.E. Hack, prof. dr. I.N. van Schaik, prof. dr. J.A. van Strijp, prof. dr. J.H.J. Wokke en dr. B.C. Jacobs wil ik bedanken voor de deskundige beoordeling van het proefschrift.

Prof. dr. P.J.M. Helders, beste Paul. Dat jij de voorzitter van mijn promotie bent vind ik bijzonder leuk. Het heeft voor mij een emotionele waarde omdat jij goed bevriend was met papa en veel met hem gepubliceerd hebt over onderwerpen in de neonatologie. Als hij dit had kunnen meemaken zou hij zó trots geweest zijn.

Dr. H. Franssen, beste Hessel, dank voor al je hulp met de klinisch-neurofysiologische onderdelen van mijn proefschrift. Het was prettig dat jij op buitenlandse congressen op de eerste rij ging zitten als ik een voordracht mocht houden en dat ik op jou mocht rekenen als één van de MMN-iconen uit de wereld een moeilijke klinisch-neurofysiologische vraag stelde die ik niet zo goed kon beantwoorden als jij.

Dr. J.H. Veldink, beste Jan, dank voor al je hulp en uitleg bij de statistische analyses en voor de snelle en goede beoordeling van de artikelen.

Dr. N.C. Notermans, beste Nicolette, dank voor je begeleiding en voor het organiseren van jaarlijkse neuromusculaire borrel- en bowlingavonden.

Nienke de Goeijen, bedankt voor je betrokkenheid bij de MMN-patiënten en je altijd positieve instelling. Jij zorgt ervoor dat de IVIg-toedieningen soepel verlopen en bent altijd bereikbaar als een collega of patiënt iets wil vragen. Inge van Beilen, dank voor al je hulp bij de studie naar de vloeibare, meer geconcentreerde IVIg.

Graag wil ik de Vereniging Spierziekten Nederland (VSN) bedanken voor de hulp bij het identificeren van MMN-patienten voor de nationale MMN-studie.

Lieve labmannen en genetische experts Paul, Michael, Hylke, Frank, Ewout en Christiaan. Het was mooi om te merken dat ik in jullie achting steeg toen ik de WK-poule 2010 won en vanaf toen ook echt meedeed met jullie. Dank voor alle leuke koffiemomenten en labborrels. Peter Sodaar, bedankt voor het verwerken en opslaan van een heleboel serumsamples.

Lieve labvrouwen, Lotte, Esther, Renske, Femke. Ik was toch wel blij dat ik na een paar jaar alleen maar met mannen op het lab te hebben gezeten ook weer wat vrouwelijke collega's had met wie ik het over de labmannen en andere zaken kon hebben. Dank voor jullie gezelligheid.

Sanne Piepers, jij was één van de labvrouwen voordat ik er kwam. Jij was mijn voorganger in het onderzoek naar de immuun-pathogenese van MMN en je bent een hele leuke collega. Bedankt voor je betrokkenheid en hulp bij het onderzoek.

Renske van den Berg, mijn andere voorganger in het MMN-onderzoek. Dank je wel voor je hulp bij het eerste deel van het onderzoek. Jij hebt me geleerd hoe ik het spierkrachtonderzoek en de dynamometrie goed moet uitvoeren en je hebt me geïntroduceerd bij het grootste deel van de MMN-patienten die aan het onderzoek meededen.

Lotte Vlam, jij zet het onderzoek naar de immuun-pathogenese van MMN voort. We hebben samen heel veel tijd doorgebracht met het uitvoeren van de complement-ELISA's en onder andere daar heb ik gezien dat je een gedreven en enthousiaste onderzoeker bent. Ik hoop dat we nog regelmatig gaan samenwerken en ik heb er alle vertrouwen in dat jij een mooie promotie tegemoet gaat.

Lieve Esther Verstraete, jij bent een leuke collega en een van mijn paranimfen, daar ben ik erg blij mee. Bedankt voor de goede samenwerking op het lab en nu in de Tergooiziekenhuizen, voor alle koffie&kletsmomenten en voor je betrokkenheid bij alles.

Voor het lab was er de kopkamer. Lieve kopkamergenootjes Tom, Nadia, Frans, Jan S, Sonja en JikkeMien: om bij 40° Celsius op 4 m^2 met zijn vieren onderzoek te doen was een prestatie op zich, maar het is ons gelukt! Bedankt voor jullie tips bij het schrijven van mijn eerste artikelen, voor de IT-hulp, vrolijkheid en goede grappen.

Alle arts-assistenten wil ik bedanken voor de leuke sfeer in onze assistentengroep, voor de mooie congressen samen en de Babinski's. Dennis Nieuwkamp, een dag na mijn promotie Dr. Dennis Nieuwkamp, dat we maar een mooi feest samen voor de collega's mogen organiseren!

De studenten Anne-Suzanne Bertens, Meinie Seelen en Bram Sikking wil ik bedanken voor hun hulp bij het onderzoek en hun enthousiasme. Meinie, wat goed dat je nu bij de 'groep van Leonard' hoort en promotieonderzoek gaat verrichten. Anne-Suzanne, jij bent een gedreven student, we hebben inmiddels al twee artikelen samen gepubliceerd en over een paar jaar ben jij vast ook een collega in de neurologie.

Jos van Strijp, Erik Heezius, Suzanne Rooijakkers, Maartje Ruyken en Marc Janssen, heel erg bedankt voor jullie gastvrijheid op het laboratorium medische microbiologie en voor jullie hulp bij het maken van de protocollen voor de complement- en bacterieproeven en het uitvoeren daarvan.

Mensen van het laboratorium immunologie in het Erasmus MC. Ik wil jullie bedanken voor de ontzettend leuke en leerzame tijd die ik bij jullie heb door mogen brengen. Bart Jacobs, de labbesprekingen waren inspirerend. Er kwamen steeds weer nieuwe en interessante ideeën voor onderzoek naar voren waardoor we het oorspronkelijke plan dat ik drie maanden bij jullie zou werken ongeveer een jaar hebben verlengd en er hele mooie resultaten uit zijn gekomen. Ik heb veel van je geleerd, dank daarvoor.

Lieve Anne Tio-Gillen, jouw precieze manier van werken en proeven uitvoeren spreken me aan en zijn erg belangrijk geweest voor het onderzoek, dank daarvoor. Dank ook voor al je persoonlijke interesse en gezelligheid. Prof. dr. Jon Laman, Wouter van Rijs, Ruth Huizinga, Marie-José Melief en Karin Geleijns wil ik zeker ook bedanken voor de gastvrijheid en hulp bij de proeven.

I would like to thank you professor Nobuhiro Yuki for your help and all the ganglioside ELISAs you performed in Japan. It was a pleasure to meet you in Wurzburg in 2009.

Hans Carpay en andere neurologen in de Tergooiziekenhuizen, bedankt voor de leuke en goede stage bij jullie en jullie meeleven met mijn promotieonderzoek.

Lieve jaarclub, nog steeds ondanks alle drukte van werk, mannen en kinderen eten we iedere donderdag samen. We hebben de afgelopen jaren vakanties, weekenden, gala's, huwelijken en andere feesten meegemaakt en ik hoop dat we nog heel veel meer met elkaar gaan beleven. Jullie zijn me heel veel waard. Drie mensen uit de jaarclub wil ik apart noemen:

Mere, onze huiskunstenares, na drie schilderijen en een geboortekaartje heb je nu de mooie illustraties voor mijn proefschrift gemaakt.

Eva, met jou deel ik alles. Ik ben er dankbaar voor al bijna twintig jaar zo'n lieve goede vriendin te hebben.

Nelleke, wij hebben vanaf jaar één van de studie geneeskunde zo goed als alles samen gedaan. We zaten in hetzelfde practicumgroepje, dezelfde jaarclub, gingen met z'n tweeën op reis, schreven ons eerste wetenschappelijk artikel samen en nog veel meer. Nu ben je mijn paranimf en ik ben blij dat jij naast me staat. Je bent een supervriendin.

Lieve Robert, Judith, Dana, Aron en Lian, wat was het leuk om laatst weer met alle Cats-en in Nederland te zijn. Volgende keer met zijn allen in Israël lijkt me een mooi plan.

Prof. dr. A. Cats, lieve oom Nol, wat speciaal dat u achter de tafel zit bij mijn promotie.

Lieve Edith en Johan, Marion en Jels, dank voor jullie belangstelling in mijn werk, promotie en persoonlijke zaken. Nu heb ik weer meer tijd voor Amsterdamse bezoeken en weekendjes met jullie.

Lieve Theo, Wilma en Laura, dank jullie wel voor de gezellige momenten, lekkere diners en jullie betrokkenheid bij Eva en Sara.

Lieve papa, wat zou je hiervan hebben genoten. Jij had het liefst een heleboel Cats and Cats et al., of liever nog Cats and Cats-artikelen met mij gepubliceerd. Daar is het helaas niet van gekomen. Op de dag van mijn promotie zal ik veel aan je denken.

Lieve mama , jij bent mijn grote voorbeeld. Jij hebt me laten zien dat je de liefste moeder (en oma) kunt zijn en tegelijkertijd een geweldige arts. Ik hoop dat ik het net zo ga doen als jij. Je hebt me een heerlijke jeugd bezorgd en ik ben je dankbaar voor alles.

Eva en Sara, mijn fantastische prachtige dochters. Ik ben stapelgek op jullie!

Lieve Peter, al sinds de vonk oversloeg op de snijzaal elf jaar geleden zijn we samen. Dank je wel voor alle mooie momenten. Ik houd van je en ik hoop dat we samen met onze heerlijke dochters nog heel veel meer moois mogen beleven.

About the author

CURRICULUM VITAE

Elisabeth Aviva Cats werd geboren op 31 oktober 1979 te Utrecht. In 1998 behaalde zij haar VWO-diploma aan het Minkema College te Woerden. In september 1998 begon zij aan de studie Geneeskunde aan de Universiteit Utrecht. Zij had bijzondere interesse in de neurologie en de kindergeneeskunde en sloot haar studie in 2005 af met wetenschappelijk stages op de afdeling kinderfysiotherapie onder begeleiding van dr. R.H. Engelbert en prof. dr. P.J.M. Helders en op de afdeling kinderneurologie onder begeleiding van dr. P.C. van Rijen en prof. dr. O. van Nieuwenhuizen. Beide stages resulteerden in een artikel in een internationaal medisch-wetenschappelijk tijdschrift. In 2005 behaalde zij haar artsexamen en werkte zij enige maanden als arts-assistent neurologie in het Erasmus MC te Rotterdam. In 2006 werd zij aangenomen voor de opleiding neurologie in het UMC Utrecht (opleiders: prof. dr. J. van Gijn, prof. dr. J.H.J. Wokke). In 2007 begon zij met haar promotieonderzoek naar de immunologie, genetica en behandeling van multifocale motorische neuropathie onder supervisie van prof. dr. L.H. van den Berg en dr. W.L. van der Pol. Tijdens haar opleiding en promotieonderzoek was zij bestuurslid van de Vereniging Arts-Assistenten in opleiding tot Neuroloog (VAAN) en was zij adviserend lid vanuit de VAAN van het bestuur van de Nederlandse Vereniging voor Neurologie (NVN).

LIST OF PUBLICATIONS

1. Vlam L, van der Pol WL, Cats EA, Straver DCG, Piepers S, Franssen H, van den Berg LH. Multifocal motor neuropathy: clinical features, pathophysiology and treatment. Nat Rev Neurol, in press.
2. Cats EA, Vlam L, Seelen M, van Vught PWJ, van den Berg LH, van der Pol WL. Multifocal motor neuropathy is not associated with genetic variation in *PTPN22*, *BANK1*, *Blk*, *FCGR2B*, *CD1A/E* and *TAG-1* genes. J Peripher Nerv Syst, in press.
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10. Vrancken AF, Gathier CS, Cats EA, Notermans NC, Collins MP. The additional yield of combined nerve/muscle biopsy in vasculitic neuropathy. Eur J Neurol 2011;18(1):49-58.
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12. Sutedja NA, Otten HG, Cats EA, Piepers S, Veldink JH, van der Pol WL, van den Berg LH. Increased frequency of HLA-DRB1*15 in patients with multifocal motor neuropathy. Neurology 2010;74(10):828-832.

13. Cats EA, van der Pol WL, Piepers S, Notermans NC, van den Berg-Vos RM, van den Berg LH. New liquid intravenous immunoglobulin (10 % IVIg) for treatment of multifocal motor neuropathy: a prospective study of efficacy, safety and tolerability. J Neurol 2008;255(10):1598-1599.

14. Cats EA, van der Pol WL, van den Berg LH. Case 40-2007: a man with weakness in the hands. N Engl J Med 2008;358(17):1869.

15. Cats EA, Kho KH, Van Nieuwenhuizen O, Van Veelen CW, Gosselaar PH, Van Rijen PC. Seizure freedom after functional hemispherectomy and a possible role for the insular cortex: the Dutch experience. J Neurosurg 2007;107(4 Suppl):275-280.

16. Tolboom N, Cats EA, Helders PJ, Pruijs JE, Engelbert RH. Osteogenesis imperfecta in childhood: effects of spondylodesis on functional ability, ambulation and perceived competence. Eur Spine J 2004;13(2):108-113.