creased. Throat swab and stool culture results were not remarkable. Her serum had IgG antibodies to GT1a (titer, 500) and GQ1b (4,000).

FS had been diagnosed by the primary physician. From day 6, she received 1 g methylprednisolone intravenously daily for 3 days, and on days 11, 14, and 17 underwent three sessions of immunoadsorption therapy with a tryptophan-conjugated column [20]. She began to recover from blepharoptosis on day 10 and from limb weakness on day 12. The CSF protein concentration was 39 mg/dl with 2 cells/μl on day 19. Consciousness was regained, and bulbar palsy disappeared. Ophthalmoplegia lessened from day 27. She could walk without support but could not remain standing on one foot owing to residual truncal ataxia. Because she had consciousness disturbance, ophthalmoparesis, and ataxia together with hemisensory disturbance, a long tract sign, BBE could have been diagnosed, but because she also had weakness of the oropharynx, neck, and arms, the diagnosis could have been PCB. The final diagnosis therefore was PCB/BBE.

3.5. IgG antibodies to other gangliosides

Most of the patients who had the anti-GT1a IgG antibody also had IgG antibodies against other gangliosides, in particular against GQ1b (Table 3). Anti-GQ1b IgG was present in all the patients with FS, BBE, AO, FS/GBS, BBE/GBS, PCB/FS, or PCB/BBE, whereas 83% of the patients with PCB had that antibody. Patients with PCB had anti-GM1b and anti-GD1a IgG antibodies as did those with PCB/FS and PCB/BBE. Anti-GM1, anti-GM2, and anti-GalNAc-GD1a IgG antibodies were detected only in those patients with GBS, FS/GBS, or BBE/GBS.

3.6. Neurological features and diagnoses for patients with anti-GT1a but not anti-GQ1b IgG

A statistical analysis was performed to investigate possible associations between clinical features and the coexistence of anti-GQ1b IgG antibody. Of the anti-GT1a IgG-positive patients, nine (6%) were negative for anti-GQ1b IgG. Their clinical features were compared with those of the other 131 patients with anti-GQ1b IgG (Table 4). These nine more often had a history of diarrhea, bulbar palsy, and limb weakness than had the others. In contrast, ophthalmoparesis, ataxia, and superficial sense impairment were rare. The frequency of hypo- or areflexia did not differ significantly between those patients with and without anti-GQ1b IgG.

For six of the nine patients without anti-GQ1b IgG the diagnosis was GBS, for one PCB, and for two unclassified. Of these nine patients, three patients with GBS had anti-GM1 IgG, two with GBS and one with PCB had anti-GD1a IgG, and one with PCB had anti-GM1b IgG.

Table 4
Anti-GT1a positive-patients with and without anti-GQ1b IgG activity

	Anti-GQ1b IgG antibody :		*p* Value	Odds ratio	95% CI
	Negative	Positive			
Number	9	131			
Gender (men/women)	7/2	70/61	0.14		
Median age (range)	39 (18–71)	41 (4–86)	0.80		
Antecedent illness:	(%)	(%)			
Upper respiratory infection	44	83	0.01	0.2	0.05 to 0.6
Diarrhea	67	21	0.007	7.4	2.1 to 26.4
Neurological signs during the illness:					
Limb weakness	100	51	0.0006	26.2	3.9 to 174.0
Hypo- or areflexia	78	90	0.25		
Bulbar palsy	78	40	0.03	5.3	1.2 to 23.0
Facial weakness	67	34	0.053		
Dysarthria	56	44	0.36		
Autonomic nervous disturbance	33	15	0.96		
External ophthalmoparesis	22	89	0.00002	0.03	0.01 to 0.1
Cerebellar-like ataxia	11	76	0.0002	0.04	0.009 to 0.2
Superficial sense impairment	11	57	0.008	0.09	0.02 to 0.5
Deep sense impairment	11	24	0.33		
Internal ophthalmoparesis	0	40	0.01	0.08	0.010 to 0.6
Blepharoptosis	0	38	0.02	0.08	0.01 to 0.7
Consciousness disturbance	0	10	0.40		
Pathological reflex	0	8	0.47		
CSF:					
Median cell (range) (/μl)	2 (1–4)	4 (0–176)	0.22		
Median protein (range) (mg/dl)	66 (51–107)	44 (11–225)	0.16		

CI = confidence interval; CSF = cerebrospinal fluid.

3.7. Case report of an unclassified patient who had the anti-GT1a but not the anti-GQ1b IgG

During pregnancy, a 33-year-old woman had flu-like symptoms. Four days later, she had difficulty in swallowing and suffered rhinolalia aperta (day 1). The next day she could neither eat nor drink. On day 4, she developed neck and arm weakness and was admitted to a hospital. Facial and bulbar palsies were found. Muscle power was decreased in her neck and arms (4 on the MRC scale). All tendon reflexes were

normal. Her coordination, sensory, and autonomic functions were intact. *C. jejuni* and *H. influenzae* were negative in the stool and throat swab cultures. Her serum showed isolated, elevated anti-GT1a IgG titer (16,000). GBS was diagnosed by the primary physician. She underwent plasma exchange on days 11, 12, and 32. Her rhinolalia and limb weakness began to lessen after the first session. Mild bulbar palsy and neck weakness remained at discharge (Day 40). Although, except for normal tendon reflexes, PCB might have been diagnosed, she was grouped as unclassified.

4. Discussion

The 140 patients with anti-GT1a IgG antibodies had a variety of clinical features. The triad of FS, ophthalmoplegia, ataxia, and areflexia were frequent because half the population sample consisted of FS patients. We elsewhere reported the frequencies of neurological signs in patients with anti-GQ1b IgG [15]. They were similar to those in the patients with anti-GT1a IgG in the current study. The GBS population, however, was larger in the anti-GT1a IgG-positive (16%) than in the anti-GQ1b IgG-positive group (4%). This is due in part to the presence of patients with anti-GT1a IgG without GQ1b reactivity, for most of whom GBS was diagnosed. We found that the nine patients who had anti-GT1a without GQ1b reactivity frequently had had preceding diarrhea, bulbar palsy, and muscle weakness in the neck and four limbs, whereas those patients with anti-GQ1b/GT1a IgG had had ophthalmoplegia and ataxia. The coexistence of anti-GQ1b IgG was one cause of the variety of clinical features seen in the anti-GT1a-positive patients.

Anti-GT1a IgG has been detected in patients with acute oropharyngeal palsy without limb weakness [5,6], in a patient with polyneuritis cranialis who developed neither limb weakness nor ataxia and had preserved deep tendon reflexes [21], and in one with GBS who, during the recovery phase, had the clinical features of PCB [22]. These findings are further proof that patients with anti-GT1a IgG have a variety of clinical features.

Our study showed that typical PCB patients were relatively rare in the anti-GT1a-positive population. Notably, for several patients there was an overlapping diagnosis of PCB/FS or PCB/BBE. Bulbar palsy, the cardinal sign of PCB, also was the initial sign in patients with FS or BBE. In contrast, PCB patients did not always have as the initial symptom, regional weakness of the oropharynx, neck, and arms; some initially experienced unsteady gait or ataxic speech. Moreover, patients with PCB/FS or PCB/BBE had IgG antibodies against GQ1b, GM1b, and GD1a, as did patients with typical PCB, FS, or BBE. These findings support the view that PCB is a condition continuous with FS and BBE.

There were unclassified diagnoses for some of our patients. One had bulbar palsy and weakness of the neck and arms, but normal deep tendon reflexes. The clinical features of that patient were similar to those in the first reported case of PCB with anti-GT1a IgG [7]. Plasma exchange seemed to mitigate the neurological deficits. These patients did not fulfill our criteria for PCB. Interestingly, four patients with axonal neuropathy associated with *C. jejuni* infection, in whom deep tendon reflexes were preserved, have been reported [23]. Although none fulfilled the criteria for GBS, three of them benefited from plasmapheresis. Specific treatment therefore can be recommended even if neurological findings do not fulfill the criteria for GBS or PCB. Some patients who suffered progressive weakness in the oropharynx, neck, and arms did not satisfy the criteria for PCB. An anti-GT1a antibody assay, in addition to an electrophysiological study and CSF investigation, may prove as a useful supplementary test for making differential diagnoses and choosing therapies.

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Effect of methylprednisolone in patients with Guillain-Barré syndrome

Sir—The results of the randomised controlled trial by R van Koningsveld and colleagues (Jan 17, p 192)[1] show a possible beneficial effect of methylprednisolone when given with intravenous immunoglobulin to patients with Guillain-Barré syndrome (GBS), after adjustment for various factors known to affect the prognosis of disease. However, van Koningsveld and colleagues did not discuss the presence of autoantibodies to GM1 ganglioside, which is also an important prognostic factor in the disease.

GBS is divided into primary axonal and demyelinating forms.[2] The presence of IgG antibody against GM1 gives rise to axonal GBS. A Dutch group previously showed that the presence of antibodies against GM1 defines a distinct subgroup of patients in whom plasma exchange is less effective than intravenous immunoglobulin.[3] In patients without antibodies against GM1, there was no difference between the treatments. Although intravenous immunoglobulin combined with methylprednisolone seemed to be more effective than intravenous immunoglobulin alone in anti-GM1-positive patients, further studies were needed to confirm the findings because the analysis was retrospective and numbers were small.[3] The pathogenesis of axonal GBS could therefore be different from that of demyelinating GBS, and the efficacy of van Koningsveld and colleagues' combined treatment could vary correspondingly.

In September, 2001, when van Koningsveld and colleagues first presented their results at the Peripheral Nerve Society meeting in Austria, we switched the first-line treatment for GBS from intravenous immunoglobulin alone to the combined treatment. Axonal GBS was electrophysiologically diagnosed in ten of 16 consecutive GBS patients admitted to our hospital between September, 2001, and December, 2003. All ten patients with axonal GBS had IgG antibodies to several gangliosides including GM1, and high anti-GM1 IgG antibody titres were detected in five. None of the six patients with demyelinating GBS had the autoantibodies. We noted an improvement of one or more grades on the GBS disability score in all five patients with high anti-GM1 IgG antibody titres 1 month after the combined therapy.

The frequency of positivity for anti-GM1 IgG in GBS patients is higher in Asia than in Europe: 40% in Japan and 42% in northern China,[4] compared with 14% in the Netherlands.[3] If the Dutch groups previous observation[3] were confirmed by their larger, prospective study,[1] the combined therapy could benefit many patients with axonal GBS associated with anti-GM1 IgG antibody in Asia. Whether subgrouping of GBS patients by the presence or absence of anti-ganglioside antibodies helps to optimise treatment for individual patients should be elucidated.

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Sir—R van Koningsveld and colleagues[1] show that there is no benefit in treating patients with Guillain-Barré syndrome with corticosteroids. In view of these results, and considering that, even today, about 15% of patients with Guillain-Barré syndrome die or are left disabled, Richard Hughes, in his accompanying Commentary,[2] points to the need for new immunological treatments specific for the underlying pathological processes.

Current treatments target the pathogenetic antibodies directed against peripheral nerve tissues, either eliminating them from the circulation (plasma exchange), or competing with them for the Fc receptor of the macrophages involved in the stripping of myelin sheaths (intravenous immunoglobulins). Furthermore, intravenous immunoglobulins are implicated in idiotype-anti-idiotype interactions, competitive inhibition of complement activation, and enhancement of pathogenic autoantibody clearance. However, none of these treatments specifically acts against the source of the autoantibody: the pathogenetic B-cell clone.

Rituximab, a chimeric, human, IgG1 monoclonal antibody specific for the CD20 antigen expressed on the surface of B lymphocytes, has produced encouraging results in the treatment of some types of autoimmune disease (including autoimmune haemolytic anaemia, rheumatoid arthritis, and idiopathic membranous nephropathy).[3–5]

After only two or three doses, this chimeric monoclonal antibody induces rapid depletion of B lymphocytes, and impairment of antibody production lasting 4–6 months. The mechanism by which treatment with rituximab seems to be effective in autoimmune diseases is still not completely defined. The simplest explanation is that the source of pathogenic antibodies is removed, but some studies have also shown that rituximab might interfere with and modulate the immune system by influencing the mechanisms involved in the antigen presentation and help to T lymphocytes.

Compared with other immunosuppressive agents for the treatment of antibody-mediated autoimmune disorder, this drug has the advantage of inducing selective B-cell immunosuppression, thus sparing cellular immunity mediated by T cells and natural killer cells. Moreover, rituximab has shown limited toxic effects—mainly sporadic, mild, infusion-related events.

On the basis of these arguments, do van Koningsveld and colleagues think that rituximab could be used in a clinical trial involving patients with Guillain-Barré syndrome (ie, intravenous immunoglobulins plus rituximab *vs* intravenous immunoglobulins plus placebo)?

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Authors' reply

Sir—The results of our trial showed no significant difference between treatment with methylprednisolone plus intravenous immunoglobulin and intravenous immunoglobulin alone when no adjustment was made for prognostic factors (p=0·06). After adjustment for age and f-score, there was an effect in favour of the combined treatment, which increased after additional adjustment for a low number of days between onset of weakness and randomisation (≤4 days or >4 days), a low compound muscle action potential (≤4 mV), and the presence of cytomegalovirus infection (p=0·01). In all these subgroups, methylprednisolone was better than placebo.

Keiichiro Susuki and Nobuhiro Yuki suggest that the combined treatment is especially effective in axonal GBS with high-titre anti-GM1 antibodies. We will certainly address the possible relation between the presence of antibodies against GM1 and functional outcome in our trial. We fully agree with their suggestion that an appropriate therapeutic approach should be established for each GBS subgroup.

In his Commentary, Richard Hughes[1] concluded that steroids have no benefit in GBS. We do not agree with this statement because the primary outcome criterion should also be assessed after adjustment for known prognostic factors such as age. Additionally, the conclusion is partly based on pooling the data from this trial with those of other steroid trials in GBS. However, our trial was the only one that aimed to test a possible synergistic effect between steroids and intravenous immunoglobulin.[2] A comparison has been made with multifocal motor neuropathy, in which steroids can increase weakness. On the basis of the results of our trial, there is no evidence that steroids harm GBS patients. Moreover, there are indications that the combination could hasten recovery in GBS.

Gabriele Rossi and Franco Locatelli suggest the use of rituximab for treatment of GBS. This is an interesting suggestion because, despite intravenous immunoglobulin (with or without methylprednisolone), about 20% of patients are still unable to walk unaided after 6 months; any potential useful additional treatment to intravenous immunoglobulin should be considered.

However, whether this additional treatment would be rituximab is questionable. First, although autoantibody production and molecular mimicry seems to be an important mechanism in GBS, it is reasonable to assume that T-cell help is also of importance.

CME Acute motor axonal neuropathy after *Mycoplasma* infection

Evidence of molecular mimicry

K. Susuki, MD, PhD; M. Odaka, MD, PhD; M. Mori, MD, PhD; K. Hirata, MD, PhD; and N. Yuki, MD, PhD

Abstract—*Background:* Patients with Guillain-Barré syndrome (GBS) after *Mycoplasma pneumoniae* infection often have antibodies to galactocerebroside (GalC). Electrodiagnosis may show acute inflammatory demyelinating polyneuropathy (AIDP). *Methods:* The authors report a patient with acute motor axonal neuropathy (AMAN) after *Mycoplasma* infection and review seven cases of *Mycoplasma*-associated GBS. They investigated anti-GalC serology under various conditions associated with *Mycoplasma* infection. *Results:* The patient had immunoglobulin (Ig)G and IgM antibodies against GM1 and GalC, which cross-reacted. During the acute phase, IgM selectively immunostained axons. The cholera toxin B-subunit and rabbit anti-GM1 IgG stained a band in the lipid extract from *M pneumoniae*, indicative of the presence of a GM1 epitope. Six *Mycoplasma*-associated GBS patients with anti-GalC antibodies had non-AIDP electrodiagnoses, whereas one with *Mycoplasma*-associated AIDP had no anti-GalC antibodies. Anti-GalC antibodies were positive in two of five patients who had neurologic diseases other than GBS after *Mycoplasma* infection and in one of 12 who had acute respiratory disease caused by *M pneumoniae* not followed by a neurologic disease. *Conclusions:* Anti-GalC antibodies in *Mycoplasma*-associated GBS may be an epiphenomenon. In certain cases, anti-GM1 antibodies induced by molecular mimicry with *M pneumoniae* may cause acute motor axonal neuropathy.

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Guillain-Barré syndrome (GBS) is the postinfectious autoimmune disease prototype. Molecular mimicry has been shown between certain antecedent infectious agents and peripheral nerve molecules. Lipo-oligosaccharides of *Campylobacter jejuni* have structures identical to the terminal tetrasaccharide of GM1 expressed on peripheral nerve axolemma.[1,2] Patients with *C jejuni*-associated GBS often have anti-GM1 immunoglobulin (Ig)G antibody and the electrodiagnosis of acute motor axonal neuropathy (AMAN).[3] *Haemophilus influenzae*, which also has the GM1 epitope, appears to be another antecedent agent in AMAN.[4] GBS patients recently infected with cytomegalovirus (CMV) frequently have anti-GM2 IgM antibody,[5] which may be induced by GM2 epitope expression in host cells infected with CMV.[6]

Galactocerebroside (GalC) epitope is present in *Mycoplasma pneumoniae*,[7] the infection that precedes 5% or 6% of GBS cases.[3,8] GalC is a major glycolipid in peripheral nerve myelin, and sensitization of rabbits with it induces demyelinating polyneuropathy.[9] Patients with GBS after *Mycoplasma* infection often have anti-GalC antibodies.[10,11] Eleven of 13 anti-GalC-positive GBS patients had predominantly demyelinative features, but no detailed electrophysiologic data were provided.[11] Features of GBS after *Mycoplasma* infection and the pathophysiologic role of anti-GalC antibodies have yet to be determined. A patient who developed AMAN associated with anti-GM1 antibody after *Mycoplasma* infection, with a pathogenesis speculated to be based on molecular mimicry, is reported, and the pathophysiologic role of anti-GalC antibodies discussed.

Methods. *Patient 1.* A previously healthy, 29-year-old man developed fever and dry cough. Twelve days later he was admitted to a district hospital and administered antibiotics. Two days after admission, he experienced bilateral weakness in the upper and lower right limbs (day 1) which gradually worsened. On day 3 he developed tetraparesis. On referral to our hospital on day 5, he was alert, and his cranial nerve functions were normal. Medical Research Council grades were 3 for the bilateral upper limbs, 0/4 for the right/left tibial anterior muscle, and 3/5 for the right/left gastrocnemius muscle. Deep tendon reflexes were decreased. The plantar response was flexor. Neither sensory nor autonomic nerve dysfunction was detected. Serum anti-*M pneumoniae* antibody (particle agglutination test; 640) and cold agglutinin (2,048) titers were increased. Antibodies to *C jejuni* and *H influenzae* were negative. CSF on day 6 showed raised protein (69 mg/dL) and a slightly elevated cell count (12/μL). Nerve conduction study (NCS) results on day 11 (table 1, figure 1) were compatible with the electrophysiologic criteria for AMAN.[12] Compound muscle action potential (CMAP) amplitudes were decreased, motor nerve conduction velocities (MCV) and distal latencies were preserved, there were no excessive temporal dispersions or nerve conduction blocks, and sensory NCS results were within normal limits. He was given IVIg therapy (400 mg/kg/day, 5 days). His muscle power first increased then decreased, and the same therapy and protocol was given from day 31. After treatment, he recovered gradually

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Table 1 Nerve conduction study results on Patient 1 (day 11)

		Motor				Sensory	
Side	Nerve	DL (ms)	CV (m/s)	Distal CMAP Amp (mV)	F latency (ms)	CV (m/s)	SNAP Amp (μV)
Right	Median	4.0	52	10.5	28.8	60	60.5
	Ulnar	3.9	53	2.2		58	62.7
	Tibial	5.5	39	1.3	NE		
	Sural					45	17.5
Left	Median	4.2	50	4.7	NE	56	77.1
	Ulnar	3.0	51	1.8		55	50.0
	Tibial	5.0	39	1.2	NE		
	Sural					48	32.6

Amplitude measured between the positive and negative peaks of the evoked potentials.
DL = distal latency; CV = conduction velocity; CMAP = compound muscle action potential; Amp = amplitude; SNAP = sensory nerve action potential; NE = not elicited.

and on day 55 was discharged from the hospital. Four months after the onset of neurologic symptoms, his muscle power had returned to normal. At that time, his anti-*M pneumoniae* antibody (80) and cold agglutinin (64) titers had decreased. CMAP amplitudes gradually returned to normal but without the slow components produced by remyelination (see figure 1).

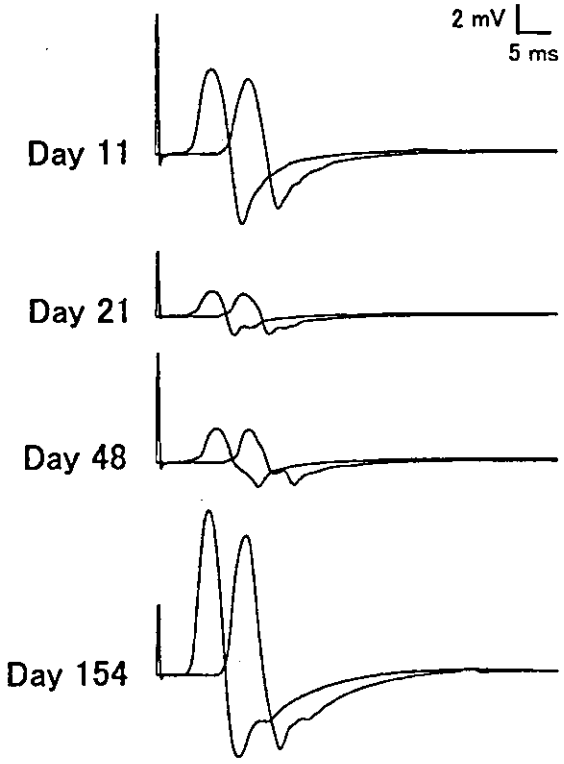

Figure 1. Right median nerve serial nerve conduction study results. Compound muscle action potentials were recorded by supramaximal stimulation to the wrist and elbow. Waves are superimposed. On day 21, compound muscle action potential amplitudes markedly decreased. Demyelination findings such as nerve conduction block, temporal dispersion, prolonged distal latency, and delayed motor conduction velocity are absent. Amplitudes have returned to normal on day 154 with none of the slow components caused by remyelination.

ELISA. An ELISA was done as reported elsewhere[13] with minor modifications. IgG and IgM antibody activities against glycolipids GalC, asialo-GM1, GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GT1a, GD1b, GT1b, GQ1b, and sialosyl lactosaminyl paragloboside (SLPG) were investigated. Asialo-GM1 was purchased from Alexis Biochemicals (San Diego, CA), GalC and GM1 from Sigma (St. Louis, MO), and GM2 from Funakoshi (Tokyo, Japan). Synthetic GM1b was a gift from Dr. M. Kiso (Department of Applied Bioorganic Chemistry, Gifu University, Gifu, Japan).[14] GalNAc-GD1a was prepared from bovine brain as described elsewhere.[15] GD1a, GD1b, and GT1b were gifts from Dr. Y. Hirabayashi (RIKEN, Saitama, Japan). GT1a was purchased from IsoSep (Tullinge, Sweden), and GQ1b from Dia-Iatron (Tokyo, Japan). SLPG was prepared from bovine cauda equina.[16] One hundred picomoles of GalC and five picomoles of one of the other glycolipids were placed in separate microtiter plate wells. Serum was diluted serially starting at 1:500. Anti-glycolipid antibody titer was the highest dilution at which the absorbance at 492 nm was 0.1 or more. Serum was considered positive for anti-glycolipid antibody when the titer was 500 or more. Serum samples from Patient 1 obtained 2 days before onset, during the acute phase (day 5), and after recovery from neurologic symptoms (day 144) were tested.

Absorption study. To determine antibody cross-reactivities, an absorption study was done as described elsewhere[17] on the serum obtained from Patient 1 on day 5. Anti-GM1 and anti-GalC antibodies were absorbed by the glycolipids GM1, GalC, GQ1b, glucosylceramide (GlcCer), and lactosylceramide (LacCer). GlcCer and LacCer were gifts from Dr. T. Taki (Department of Biochemistry, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan). Absorption rates were expressed as percentages of the optical densities with and without absorption.

Thin-layer chromatography with immunostaining. Serum (day 5) from Patient 1 was examined by thin-layer chromatography (TLC) with immunostaining, as described elsewhere[18] but with minor modifications. Serum obtained from a patient with *C jejuni*-associated AMAN during the acute phase and plasma from a rabbit sensitized with GalC were the positive controls. The rabbit developed quadriparesis, and the ELISA showed plasma obtained within 1 week after onset was positive for anti-GalC IgG antibody. Demyelination findings were confirmed by NCS.[19]

Immunohistochemical study. A Wister rat was anesthetized deeply and perfused transcardially with 1 mL/g of 0.1 *M* phosphate-buffered saline (PBS; pH 7.4), then with 1 mL/g of 2% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). After perfusion, the spinal nerve roots were removed and postfixed on ice for 30 minutes with 2% paraformaldehyde in 0.1 *M* phosphate buffer. Samples were cryoprotected at 4 °C overnight in 20% sucrose solution then frozen in isopentane at −70 °C. Sections 6 μm thick, cut with a cryostat, were dried on MAS-coated slides (Matsunami Glass, Osaka, Japan) and after being blocked with buffer (1.5% normal goat serum in PBS) for 20 minutes incubated at 4 °C

overnight with serially diluted serum. Sera used were obtained from Patient 1 during the acute phase (day 5) and after recovery (10 months after onset), and from a patient with *C jejuni*-associated AMAN during the acute phase (positive control) and a healthy human (negative control). The test sections were incubated at 20 °C for 60 minutes with biotin-conjugated anti-human IgM or IgG antibodies (Vector Laboratories, Burlingame, CA) diluted in PBS (1:200). Samples also were incubated first at 4 °C overnight with diluted plasma from the GalC-sensitized demyelinating neuropathy rabbit or with diluted plasma from an AMAN model rabbit sensitized with a bovine brain ganglioside mixture (Cr-4 in the previous report[20]) then at 20 °C for 60 minutes with biotin-conjugated anti-rabbit IgG antibody (Vector Laboratories). The AMAN model rabbit developed tetraparesis. Anti-GM1 IgG antibody was detected in the ELISA of plasma obtained within a week after onset. Axonal degeneration of the sciatic nerve was confirmed pathologically. The samples then were incubated at 20 °C for 30 minutes with the avidin-biotinylated enzyme complex (Vector Laboratories), after which they were incubated for 2 minutes at 20 °C in a solution of 50 m*M* Tris-hydrochloric acid buffer (pH 7.6) containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide. The reaction was terminated by washing with PBS, then the slides were treated with 0.05% osmium tetroxide in distilled water for 2 minutes to intensify the 3,3'-diaminobenzidine tetrahydrochloride reaction products.

Search for the GM1 epitope in M pneumoniae. A lipid solution extracted from lyophilized *M pneumoniae* complement fixation antigen (Denka Seiken, Tokyo, Japan), as described elsewhere,[7] was layered and separated on TLC plates. To detect whether *M pneumoniae* had the GM1 epitope, the plates were incubated at 20 °C for 2 hours with the peroxidase-conjugated cholera toxin B-subunit (List Biologic Laboratories, Campbell, Canada; 1:2,000 dilution), which specifically recognizes the GM1-oligosaccharide. Antibody activities in plasmas from the AMAN model rabbit and demyelinative polyneuropathy rabbit also were examined as above.

Patients with GBS subsequent to M pneumoniae *infection.* Retrospective observations were made of patients who had developed GBS after *M pneumoniae* infection and been referred to our neuroimmunologic laboratory between 1996 and 2002 for serum anti-ganglioside antibody tests. They had had preceding respiratory infectious symptoms and elevated anti-*M pneumoniae* antibody (particle agglutination test). Their clinical records were reviewed to clarify the neurologic signs, laboratory findings, and electrophysiologic data. The electrodiagnosis of acute inflammatory demyelinating polyneuropathy (AIDP) or AMAN was made as described elsewhere[12] based on NCS findings during the acute phase. When the AMAN pattern and amplitude reduction of the sensory nerve action potentials (<80% of the lower normal limit) were present, acute motor-sensory axonal neuropathy was diagnosed. Serum samples obtained during the acute phase of neurologic illness were assayed for anti-glycolipid antibodies by an ELISA as described above.

Anti-GalC serology. To clarify the pathophysiologic roles of anti-GalC antibodies, IgM and IgG activities were examined by an ELISA, as above, of sera from four groups: consecutive samples from patients with various neurologic diseases who had been referred to our laboratory for serologic tests over a 2-month period; acute phase sera from GBS patients with a well-confirmed electrodiagnosis who were seen at Chiba University Hospital or affiliated hospitals and at Dokkyo University School of Medicine Hospital; *Mycoplasma*-associated neurologic diseases other than GBS were investigated retrospectively among patients who had been referred to us for serologic tests; sera from patients with clinically diagnosed acute respiratory diseases caused by *M pneumoniae* but who had no neurologic symptoms also were used in the anti-GalC and anti-GM1 antibody assays.

Results. *Anti-glycolipid antibody assays.* Patient 1. The ELISA of serum obtained from Patient 1 on day 5 showed high titers; 4,000 for the anti-GM1 IgM and IgG antibodies and 8,000 for the anti-GalC IgM and IgG antibodies (table 2). Both the anti-asialo-GM1 IgM and IgG antibody titers (2,000), as well as the anti-SLPG IgM antibody titer (500), were increased. Serum obtained 2 days before the onset of neurologic symptoms showed similar antibody activities; high titers of 4,000 for the anti-GM1 IgM, IgG, and anti-GalC IgM antibodies and 8,000 for anti-GalC IgG antibody. By day 144, the IgM and IgG antibody titers to GM1 and to GalC (500 each) had decreased.

Anti-GM1 antibodies were absorbed by GalC (figure 2) at rates of 89% (IgM) and 71% (IgG). Anti-GalC antibodies were absorbed by GM1 at rates of 72% (IgM) and 40% (IgG). None of the antibodies against GM1 and GalC were absorbed by GQ1b, a nonreactive antigen. The IgM from Patient 1 reacted slightly with both GlcCer and LacCer, whereas IgG did not react. Anti-GM1 and anti-GalC IgM antibodies were not absorbed by the glycolipids.

The reactivity of IgM and IgG with GM1 was confirmed on TLC plates (figure 3). IgM reacted with GalC. IgG did not. High anti-GalC IgG antibody activity was detected in the plasma from the rabbit sensitized with GalC.

Immunohistochemical study. IgM obtained on day 5 from Patient 1 selectively immunostained axons of rat ventral roots (figure 4), whereas that obtained from his serum 10 months after onset of neurologic symptoms produced no specific staining. Like the IgM from Patient 1, the IgG from the AMAN model rabbit and the patient with *C jejuni*-related AMAN selectively stained axons, whereas IgG from the GalC-sensitized rabbit did not. The IgG obtained on day 5 from Patient 1 did not produce specific staining, nor did the IgM and IgG from a healthy human.

GM1 epitope in M pneumoniae. The cholera toxin B-subunit stained a band in the lipid extract from *M pneumoniae,* as did the IgG from the AMAN model rabbit (figure 5). Anti-GM1 activities of the reagent and the IgG were confirmed. Neither the IgG nor IgM from the GalC-sensitized rabbit reacted with that band, but they did react with another band in the *M pneumoniae* lipid extract.

Clinical, electrophysiologic, and serologic features of patients with GBS after M pneumoniae *infection.* A preceding *M pneumoniae* infection was confirmed in seven GBS patients, including Patient 1. Their clinical, electrophysiologic, and serologic features are shown in table 2. Clinical symptoms of Patients 1 and 6 fluctuated after the initial therapy, another session being required. Patient 1 fulfilled the electrophysiologic criteria for AMAN, and Patient 3 those for AIDP.[12] NCS findings for Patient 2 showed the isolated F wave absence. Patient 4 had mild MCV delay in the lower limbs, which abnormality did not satisfy the criteria for AIDP. CMAP amplitudes were decreased in the right median and ulnar nerves of Patient 5, but MCV and distal latencies were normal. In Patients 2, 4, and 5, which mechanism, axonal damage or demyelination, was responsible for the abnormality could not be determined. Both anti-GalC IgM and IgG antibodies were present in Patients 1, 2, 4, 6, and 7. Patient 5 had low anti-GalC IgM antibody titer. Anti-GalC and anti-GM1 activities coexisted in Patients 1 (IgM and IgG classes), 2 (IgM), and 6 (IgG).

Anti-GalC serology. Anti-GalC serology is illustrated in (table 3). Serum samples from 129 patients with various neurologic diseases were examined: GBS, 57; Fisher syndrome, 6; Bickerstaff's brainstem encephalitis, 3; acute ophthalmoparesis, 2; chronic inflammatory demyelinating polyneuropathy, 14; multifocal motor neuropathy, 4; and other neurologic disorders, 43 (motor neuron disease, 9; polyneuropathy, 6; acute sensory neuropathy, 4; mononeuritis multiplex, ophthalmoparesis, and brainstem encepha-

Table 2 Patients with Guillain-Barré syndrome after Mycoplasma *infection*

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age, y/sex	29/M	29/M	32/M	11/F	2/F	47/M	13/M
Neurologic findings							
Ophthalmoplegia	–	–	–	–	–	–	–
Facial palsy	–	Bilateral	Bilateral	–	–	–	–
Bulbar palsy	–	–	+	–	–	–	–
Limb weakness	+	+	+	+	+	+	+
Hypo- or areflexia	+	+	+	+	+	+	+
Sensory disturbances	–	Hyperesthesia	Hypesthesia	–		Impaired position sense	–
Ataxia	–	–	+		–	Sensory ataxia	–
Autonomic nerve dysfunction	–	Anisocoria	–	Urinary retention, hypotension	–	–	–
CSF							
Cells (/μL)	12	45	4	12	23	3	11
Total protein (mg/dL)	69	61	107	33	16	66	63
Anti-*Mycoplasma* antibody titer	640	2,560	1,280	5,120	5,120	1,024	1,280
Cold agglutinin titer	2,048	64	16	1,024		1,024	64
Nerve conduction study	AMAN	Absent F wave	AIDP	Mild MCV delay	Decreased CMAP amplitude	Normal	
Anti-glycolipid antibody titers IgM antibodies to							
GM1	4,000	16,000	–	–	–	–	–
GalC	8,000	4,000	–	2,000	500	4,000	4,000
GM1b	–	4,000	–	1,000	–	–	–
SLPG	500	4,000		–		–	
Asialo-GM1	2,000	16,000		4,000		–	
Others	–	2,000: GM2, GD1a, GalNAc-GD1a, GT1b, and GQ1b 4,000: GD1b	–	–	–	–	–
IgG antibodies to							
GM1	4,000	–	–	–	–	1,000	–
GalC	8,000	32,000	–	1,000	–	16,000	8,000
GM1b	–	2,000	–	–	–	–	–
SLPG	–	–		–		–	
Asialo-GM1	2,000	4,000		–		2,000	
Others	–	2,000: GD1b	–	–	–	–	–

Blanks: information not available.
AMAN = acute motor axonal neuropathy; AIDP = acute inflammatory demyelinating polyneuropathy; MCV = motor nerve conduction velocity; CMAP = compound muscle action potential; Ig = immunoglobulin; GalC = galactocerebroside; SLPG = sialosyl lactosaminyl paragloboside.

litis, 3 each; polyneuritis cranialis and transverse myelitis, 2 each; cerebral infarction, epilepsy, cervical spondylosis, meningoencephalitis, radiculomyelitis, acute disseminated encephalomyelitis, Creutzfeldt-Jakob disease, acute autonomic and sensory ataxic neuropathy, neuralgic amyotrophy, myopathy, and tetanus, 1 each). Low IgM titer (500) for anti-GalC antibody was found only in the sample from a patient who developed GBS subsequent to *Mycoplasma* infection (Patient 5). Anti-GM1 IgG antibody was positive in nine patients with GBS alone. In two of the nine, *C jejuni* was isolated from stool samples and their sera did not have anti-GalC antibodies. Anti-GM1 IgM antibody was positive in four patients: three with GBS and one with multifocal motor neuropathy.

Serum anti-GalC antibodies of 136 GBS patients were examined: 49 with AIDP, 62 with AMAN, 2 with acute motor-sensory axonal neuropathy, and 23 with unclassified electrophysiology. Twelve of the 23 with unclassified NCS findings had isolated F wave absence. Three of 136 patients had had a preceding *Mycoplasma* infection (Patients 1, 2, and 3). Although anti-GalC antibodies were detected in the sera from Patients 1 (AMAN) and 2 (F wave absence), none of the other samples, including those from all 49 AIDP patients, showed anti-GalC antibody activity.

Five patients had various neurologic manifestations associated with *Mycoplasma* infection. Sera were obtained during the acute phase of neurologic illness: 6 days after the onset of respiratory disease from a patient with encephalitis, 10 days after from one with transverse myelitis, 14 days after from one with meningitis and polyneurop-

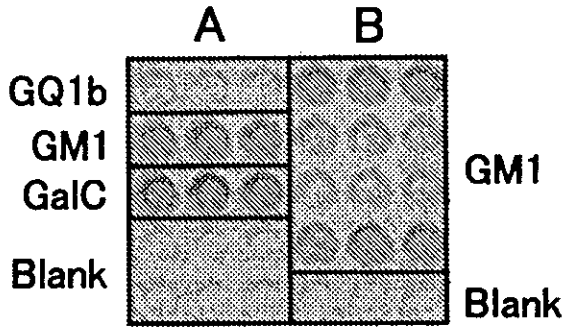

Figure 2. Microtiter plate from the anti-GM1 immunoglobulin M (IgM) antibody absorption study. (A) Serum samples diluted 1:500 were added to the wells, and the plate incubated at 4 °C overnight. Each sample was tested once in triplicate. (B) After incubation, the samples were used as the primary antibodies in the standard ELISA. They show decreased anti-GM1 IgM activity after incubation in wells coated with galactocerebroside, indicative of the cross-reactivity of the anti-GM1 IgM antibody with the glycolipid.

athy, 17 days after from one with acute cerebellar ataxia, and 18 days after from one with meningoencephalitis and polyneuropathy. Two had elevated anti-GalC antibody titers during the acute phase: an IgM titer of 1,000 in one patient with acute cerebellar ataxia and 16,000 in one with meningoencephalitis and polyneuropathy. None of the five had anti-GM1 IgM or IgG antibody activity.

Twelve serum samples were examined from patients who had respiratory diseases caused by *M pneumoniae* but were without neurologic symptoms. Samples were obtained within several days of the onset of respiratory symptoms. An anti-GalC IgM antibody titer of 8,000 was detected in one sample, but no sample showed anti-GalC IgG activity. Anti-GM1 IgM and IgG antibodies were negative in all the sera.

Discussion. Patient 1 had tetraparesis with hyporeflexia 2 weeks after developing fever and a dry cough. There was neither cranial nerve nor sensory nerve involvement. NCS findings showed markedly

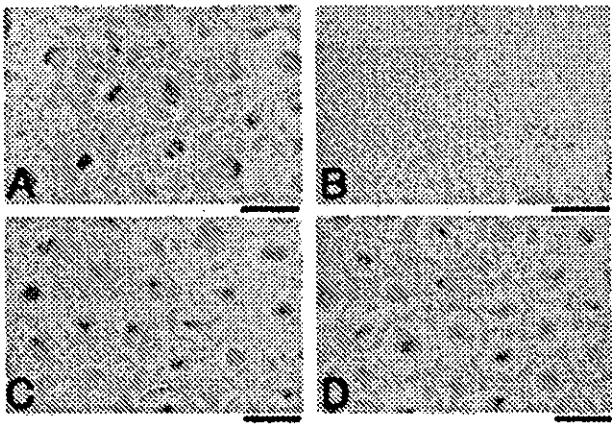

Figure 4. Immunohistochemical study results. Rat ventral roots have reacted with (A) serum obtained on day 5 from Patient 1 (dilution 1:250), and (B) 10 months after onset (dilution 1:100); (C) serum from a patient with acute motor axonal neuropathy (AMAN) subsequent to Campylobacter jejuni *infection (dilution 1:200); and (D) plasma obtained from an AMAN model rabbit sensitized with bovine brain ganglioside mixture within 1 week after onset of limb weakness (dilution 1:500). (A) Axons are selectively immunostained by the immunoglobulin (Ig)M from Patient 1 during the acute phase. (B) There is no selective immunostaining at the axons by the IgM from Patient 1 after recovery. The staining pattern is similar to that of the IgG for (C) the AMAN patient and (D) the AMAN model rabbit. Scale bars = 10 μm.*

decreased CMAP amplitudes without demyelination and preserved sensory nerve functions: neurologic and electrophysiologic findings compatible with AMAN. The serologic evaluation detected antecedent *M pneumoniae* infection. An ELISA showed both anti-GM1 and anti-GalC antibody present in the acute phase serum. Antibody activities 2 days before onset of neurologic illness were almost the same as those on day 5, indicative that the antibodies were the primary cause of GBS, not secondary phenomena produced by nerve damage, as discussed elsewhere.[21]

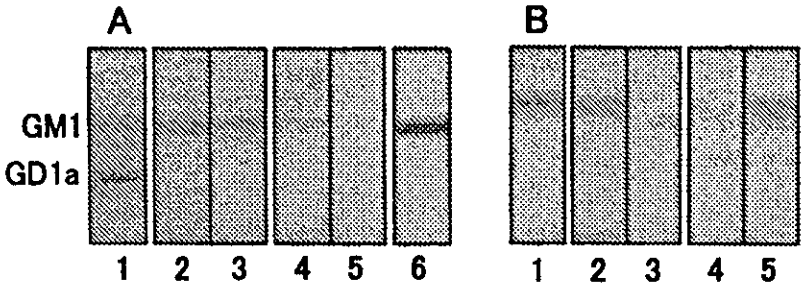

Figure 3. Thin-layer chromatogram with immunostaining. (A) Bovine brain ganglioside mixtures (the fraction enriched with GM1 and GD1a) and (B) galactocerebroside (GalC) were layered on thin-layer chromatography plates, which were developed with chloroform-methanol-0.2% calcium chloride in water (5:4:1). The plates next were stained with orcinol/sulfuric acid for hexose (lane 1), immunoglobulin (Ig)M (lane 2), and IgG (lane 3) from Patient 1 on day 5; IgM (lane 4) and IgG (lane 5) from a GalC-sensitized rabbit within 1 week after the onset of limb weakness; IgG from a patient who developed an acute motor axonal neuropathy (AMAN) after Campylobacter jejuni *enteritis (lane 6). Anti-GM1 IgM and IgG antibodies clearly are present in serum from Patient 1, as well as from the patient with* C jejuni*-related AMAN. Anti-GalC IgM antibody reactivity is present, but no IgG antibody reactivity in the serum from Patient 1. This pattern differs markedly from that of the plasma from the GalC-sensitized rabbit, which shows high anti-GalC IgG antibody activity but no anti-GM1 IgM and IgG antibody activities.*

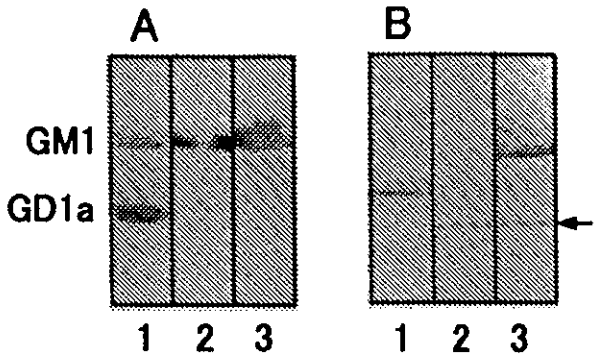

Figure 5. GM1 epitope in Mycoplasma pneumoniae *shown in an immunostained thin-layer chromatogram. (A) Bovine brain ganglioside mixtures (the fraction enriched with GM1 and GD1a) and (B) lipid extract from the lyophilized* Mycoplasma pneumoniae *complement fixation antigen were layered on thin-layer chromatography plates. The plates were developed with chloroform-methanol-0.2% calcium chloride in water (5:4:1), then stained with orcinol/sulfuric acid for hexose (lane 1), the peroxidase-conjugated cholera toxin B-subunit (lane 2), and the immunoglobulin G (IgG) from an acute motor axonal neuropathy model rabbit obtained within 1 week after onset of limb weakness (lane 3). (A) Both the cholera toxin B-subunit and rabbit IgG are bound to GM1. (B) The cholera toxin B-subunit is bound to a band in the lipid extract from* M pneumoniae, *which also reacted with rabbit IgG (arrow).*

Anti-glycolipid antibodies other than anti-GalC and anti-GM1 antibodies have been reported in *Mycoplasma*-associated GBS: anti-SLPG IgM antibody in a patient who showed markedly delayed MCV and temporal dispersion,[22] and in one with anti-GM1b IgG antibody (NCS was not performed).[23] Although anti-SLPG IgM antibody was present in Patient 1, its titer was not as high as the anti-GM1 and anti-GalC antibody titers. TLC with immunostaining confirmed both IgM and IgG activities against GM1. An immunohistochemical study showed that during the acute phase the patient's IgM selectively stained axons, whereas his IgG did not. Moreover, IgM from serum obtained after his recovery did not cause specific staining. The IgG from a GalC-sensitized rabbit did not stain axons. These findings indicate that anti-GM1 IgM antibody is necessary to the development of axonal dysfunction.

In a similar case,[24] the patient developed severe tetraparesis and areflexia without sensory disturbance after *Mycoplasma* infection. NCS results were compatible with AMAN. Anti-GM1 IgM antibody titer was high during the acute phase, whereas anti-GM1 IgG antibody titer was negative. Anti-GalC antibody was not tested in that case report.

Both the cholera toxin B-subunit and IgG antibodies from the AMAN model rabbit stained a band in the *M pneumoniae* lipid extract. Anti-GM1 IgG activity in a patient who developed GBS after a *Mycoplasma* infection was inhibited by preincubation with the *M pneumoniae* reagent.[7] These findings are evidence that the GM1 epitope is present in *M pneumoniae* and indicate that molecular mimicry exists between the bacterium and peripheral nerve tissue. Anti-GM1 IgM antibody may have been induced by the GM1 epitope of *M pneumoniae*, the preceding infectious pathogen, and caused axonal dysfunction in our Patient 1. The chemical structure of the *M pneumoniae* isolated from AMAN patients needs to be determined, as has been done for *C jejuni*.[1]

Anti-GM1 antibodies also have been reported in patients with neurologic manifestations other than GBS associated with *Mycoplasma* infection: in one with chronic polyneuropathy,[25] and in another with meningoencephalitis and cerebellitis.[26] In our series, anti-GM1 antibodies were negative in all five patients who had neurologic illnesses other than GBS preceded by *Mycoplasma* infection. In those patients, the periods between the respiratory disease and serologic examination were similar to those between the prior illness and onset of GBS. Although samples were obtained earlier than those from GBS patients, anti-GM1 antibodies also were negative in all 12 patients who had an acute respiratory disease caused by *M pneumoniae* but no neurologic symptoms.

We confirmed the previous finding that a GalC epitope is present in the *M pneumoniae* lipid ex-

Table 3 *Anti-galactocerebroside antibody assay results*

Patients	IgM	IgG
Consecutive patients with various neurologic conditions (n = 129)	1 (Patient 5)	None
Patients with electrophysiologically verified GBS (n = 136)		
AIDP (n = 49)	None	None
AMAN (n = 62)	1 (Patient 1)	1 (Patient 1)
AMSAN (n = 2)	None	None
Unclassified (n = 23)	1 (Patient 2)	1 (Patient 2)
Patients with neurologic diseases other than GBS (n = 5)	2	None
Respiratory diseases caused by *Mycoplasma* but no neurologic symptoms (n = 12)	1	None

Ig = immunoglobulin; GBS = Guillain-Barré syndrome; AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; AMSAN = acute motor-sensory axonal neuropathy.

tract[7]; therefore, *M pneumoniae* carries both the GM1 and GalC epitopes. Although GalC is a major myelin component, antibodies from Patient 1 reacted with axons rather than myelin. In that patient, antibody reactivity to GM1 may have been more pathogenic than that to GalC.

As in Patient 1, anti-GalC and anti-GM1 antibodies coexist in some patients with *Mycoplasma*-associated GBS. Electrophysiologic data, however, vary. In our series, Patient 2 had high anti-GM1 as well as anti-GalC IgM antibody titer, but the NCS showed isolated F wave absence, which has been interpreted as demyelinative conduction block in proximal nerve segments when distal CMAP are preserved. During the early phase of GBS associated with anti-ganglioside antibodies, however, the block of F wave generation may be caused by axonal conduction abnormalities in the nerve roots, the mechanism of which may be physiologic conduction block or axonal degeneration, as in AMAN.[27] Which mechanism, demyelination or axonal dysfunction, was responsible for the abnormality could not be determined from NCS data. The latter interpretation, however, seems likely for Patient 2, who had anti-glycolipid antibodies. NCS findings showed no abnormalities in Patient 6 who had anti-GM1 as well as anti-GalC IgG antibodies. Interestingly, positive anti-GM1 antibody activities have been reported in three of four anti-GalC positive GBS patients who had had a *Mycoplasma* infection.[10] No NCS findings were mentioned in that report, but two of the four patients were confirmed to have AIDP electrophysiologically (S. Kusunoki, personal communication). NCS was not performed for the others. Of the 11 anti-GalC-positive patients with *Mycoplasma*-related GBS,[11] three had coexisting anti-GM1 and anti-GalC antibodies (IgM class in two, IgG in one) but there were no NCS findings for those three (C.W. Ang, personal communication).

Similar anti-glycolipid antibody patterns of coexisting anti-GalC and anti-GM1 activities may be the cause of demyelination in some patients and axonal dysfunction in others. This may be because of differences in the fine specificities of the antibodies, as reported for anti-LM1 IgG antibodies.[17] Cross-reactivity of anti-GalC antibody with GM1 has been reported.[28] Similarly, in Patient 1, anti-GalC antibody cross-reacted with GM1 and anti-GM1 antibody with GalC. The reacting epitopes of these antibodies may be the terminal β1-linked galactosyl groups common to GM1 (Gal β1–3 GalNAc β1–4 [NeuAc α2–3] Gal β1–4 Glc β1–1 Cer), and GalC (Gal β1–1 Cer), but GD1b with the same terminal groups was not reactive. These antibodies did not cross-react with GlcCer (Glc β1–1 Cer), LacCer (Gal β1–4 Glc β1–1 Cer), or GM2 (GalNAc β1–4 [NeuAc α2–3] Gal β1–4 Glc β1–1 Cer), and their reactive epitopes remain unknown. In contrast, in Patient 2 no anti-GM1 antibodies were absorbed by GalC, and no anti-GalC antibodies were absorbed by GM1 (data not shown). The cross-reactivity of those autoantibodies was not investigated in an earlier study.[10]

Although anti-GalC antibody is considered a cause of AIDP, we could not confirm it. One patient with anti-GalC activity (Patient 4) showed mild MCV delay but did not satisfy the electrophysiologic criteria for AIDP. Patient 3, for whom the electrodiagnosis was AIDP, did not have anti-GalC antibodies. High anti-GalC antibody titers were detected in Patient 1 with AMAN and in Patient 2 who had F wave absence. None of the 49 patients with electrophysiologically verified AIDP had anti-GalC antibodies.

Our findings confirmed only the association of anti-GalC antibodies with *Mycoplasma* infection. Out of 129 consecutive cases of various neurologic disorders, only one patient with *M pneumoniae*-associated GBS had the anti-GalC IgM antibody (Patient 5). Anti-GalC antibody was negative in all the others, including two who developed anti-GM1 IgG-positive GBS after *C jejuni* enteritis. Six of seven patients who developed GBS after *Mycoplasma* infection had anti-GalC antibodies. Anti-GalC antibodies also were positive in patients who had acute cerebellar ataxia, meningoencephalitis and polyneuropathy (present study), encephalitis,[29] and encephalomyelitis[30] associated with *Mycoplasma* infection. Furthermore, anti-GalC antibodies were detected in 1 of 12 (present study), 6 of 33,[31] 8 of 32,[29] and 11 of 19[11] serum samples from patients who had respiratory diseases caused by *M pneumoniae* infection but no neurologic signs. The number of anti-GalC antibody-positive cases may have been underestimated in our study because the 12 serum samples were obtained within several days of the onset of respiratory disease, whereas the times when samples were obtained were not mentioned in previous reports.[11,29,31]

Our findings suggest that the presence of anti-GalC antibodies is an epiphenomenon related to preceding *M pneumoniae* infection and that these antibodies do not have a pathophysiologic role in the development of GBS and other neurologic illnesses. The pathophysiologic function of anti-GM2 IgM antibody in CMV-related GBS is still controversial because it frequently has been found in GBS patients who did not have a preceding CMV infection and in non-GBS patients who had acute CMV infection.[32] In contrast, patients who had had *C jejuni* enteritis but did not develop a neurologic disorder did not have the anti-GM1 IgG antibody,[33,34] indicative that that antibody is important to the pathophysiologic function in *C jejuni*-associated AMAN.

Patients who develop GBS after *Mycoplasma* infection have diverse clinical, serologic, and electrophysiologic findings. Careful, detailed studies are required to clarify the features of *Mycoplasma*-associated GBS. Our study showed that in certain cases AMAN associated with anti-GM1 antibody may develop after *Mycoplasma* infection. The GM1 epitope was found to have been present in the *M pneumoniae* lipid extract, additional evidence that

the pathogenesis of GBS is based on molecular mimicry.

Acknowledgment

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Acute facial diplegia and hyperreflexia

A Guillain–Barré syndrome variant

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Abstract—Two patients with acute facial diplegia and hyperreflexia are described. Both patients had serologic evidence of preceding *Campylobacter jejuni* infection and antiganglioside IgG antibodies as well as other laboratory and electrophysiologic findings suggesting Guillain–Barré syndrome (GBS). IV immunoglobulin produced recovery. Hyperreflexia does not necessarily exclude the diagnosis of a GBS variant. Antiganglioside antibodies can help with diagnosis in difficult cases.

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Bilateral simultaneous facial palsy, an uncommon neurologic manifestation, can result from a number of etiologies.[1] A frequent cause is Guillain–Barré syndrome (GBS), and the loss of deep tendon reflexes helps establish this diagnosis.[1] When bifacial weakness is accompanied by hyperreflexia, cerebral diseases such as brainstem encephalitis, pontine glioma, and stroke must be considered. We describe two patients with a misleading neurologic presentation of acute facial diplegia with brisk tendon jerks that was ultimately determined to represent a GBS variant.

Case reports. *Patient 1.* A 34-year-old woman had watery diarrhea for 3 days. Two weeks later, she experienced numbness in both hands (day 1), followed by horizontal diplopia and right facial weakness on day 7. She was admitted on day 9, at which time neurologic examination showed moderate facial diplegia. She could not close both eyes tightly or purse her lips, predominantly on the right side. Taste, lacrimation, and hearing were normal. Abduction was minimally restricted in the right eye. Biceps and patellar reflexes were brisk, and the plantar response was flexor. She reported numbness in both hands, but sensory testing was normal. Brain MRI was unremarkable. Nerve conduction studies of the limbs were normal. Blink reflex testing on day 13 revealed facial nerve involvements (table). Administration of IV immunoglobulin (IVIg; 0.4 g/kg/day) and methylprednisolone (0.5 g/day) for 5 days rapidly ameliorated her distal limb paresthesias. Facial diplegia gradually lessened and resolved by day 58. On day 135, she was free of neurologic symptoms, and deep tendon reflexes were normal.

Patient 2. A 32-year-old woman experienced difficulty in swallowing (day 1), mild weakness of the right hand (day 3), neck weakness (day 4), and facial weakness (day 6). There were no symptoms of a preceding infection. On day 14, examination showed facial diplegia. Tear flow was increased, but taste and hearing were normal. The patient reported difficulty swallowing, but neurologic evaluation of the lower cranial nerves was unremarkable. Strength in the neck muscles was moderately decreased. There was mild weakness in both upper limbs. Grip was 11 kg on the right side and 12 kg on the left side. Deep tendon reflexes were brisk throughout, with flexor plantar responses. CSF protein was elevated with a normal cell count (see the table). Brain MRI was unremarkable. Nerve conduction studies of the limbs were normal on day 15. A motor conduction study of the left facial nerve showed a low amplitude (0.052 mV) with an essentially normal latency (3.6 milliseconds). Blink reflex testing on day 29 showed delayed R1 and R2 latencies (see the table). She received IVIg (0.4 g/kg/day, 5 days) and methylprednisolone pulse therapy (1 g/day, 3 days). Symptoms reached a nadir on day 23, after which the patient began improving. She was almost fully recovered by day 72. One year after onset, there were no neurologic symptoms or findings.

Serologic findings. Serologic assay, as reported elsewhere,[2] confirmed evidence of recent *Campylobacter jejuni* infection in both patients (see the table). Antibodies to cytomegalovirus *Haemophilus influenzae* and *Borrelia Burgdorferi* were negative in both paitents. Serum IgG and IgM antibodies to gangliosides GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, and GQ1b were investigated by ELISA. During the acute phase, high IgG antibody titers to gangliosides, including GT1a, were detected (see the table). Thin-layer chromatography with immunostaining[3] confirmed IgG activity to GT1a in Patient 1 and to GT1a and GD1a in Patient 2 (figure). An absorption test was performed.[4] In Patient 1, anti-GT1a IgG antibody was absorbed by GQ1b and anti-GQ1b IgG was absorbed by GT1a at rates of 45 and 38%, respectively. This indicated cross-reactivity of these antibodies. None of the antibodies against GT1a and GQ1b was absorbed by GM1 or GD1a, which were nonreactive antigens. In contrast, in Patient 2, no anti-GT1a IgG was absorbed by GQ1b or GD1a, and no anti-GD1a IgG was absorbed by GT1a.

Discussion. Both our patients presented with an acute onset of facial diplegia accompanied by other mild symptoms such as diplopia, dysphagia, and neck and limb weakness. The presence of hyperreflexia confused us, and in both patients, cerebral disease was initially suspected. Brain MRI was unremarkable, but electrophysiologic studies indicated peripheral involvement of the facial nerves. Both patients had features suggestive of GBS: watery diarrhea in Patient 1 and CSF albuminocytologic dissociation in Patient 2. Patients with bifacial but no (or minimal) limb weakness, acral paresthesias, and diminished tendon jerks are considered to have a regional variant of GBS called facial diplegia and paresthesias.[5] Hyperreflexia, however, was inconsistent with the diagnosis of GBS.

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Table Features of two patients with acute facial diplegia and hyperreflexia

Features	Patient 1	Patient 2
Age, y/sex	34/F	32/F
Ophthalmoplegia	Minimal	No
Facial weakness	Bilateral	Bilateral
Bulbar palsy	No	Minimal
Cervical weakness	MRC 4	MRC 3
Limb weakness	No	Mild in both upper limbs
Deep tendon reflexes	Brisk	Brisk
Sensory disturbance	Paresthesias in both hands	No
Ataxia	No	No
CSF		
Cells, /μL	1	3
Total protein, mg/dL	29	67
Blink reflex, ms, right/left		
R1 (mean ± SD = 10.5 ± 0.8)*	Not evoked/12.4	15.2/14.8
Ipsilateral R2 (30.5 ± 3.4)*	Not evoked/35.2	36.8/41.4
Contralateral R2 (30.5 ± 4.4)*	Not evoked/35.8	40.2/36.0
Serum antibody titers to *C. jejuni*		
IgG (<2,000)*	4,000/1,000	32,000/4,000
IgA (<500)*	2,000/<250	1,000/<250
IgM (<500)*	500/500	<500/<500
Antiganglioside serology		
IgG antibody titers to (<500)*	GT1a: 4,000/<500	GT1a: 16,000/1,000
	GQ1b: 4,000/<500	GD1a: 16,000/2,000

Serologic findings were evaluated with sera obtained during the acute phase/after recovery: day 9/day 58 in Patient 1 and day 14/day 72 in Patient 2. CSF examination was done on day 11 in Patient 1 and on day 25 in Patient 2.

* Normal range.

MRC = Medical Research Council grade; Ipsilateral R2 = R2 components elicited by ipsilateral stimulation; Contralateral R2 = R2 elicited by contralateral stimulation.

Some patients who develop a motor form of GBS after *C. jejuni* enteritis may manifest brisk deep tendon reflexes.[6,7] The mechanism that causes hyperreflexia in these cases is unknown. Hyperexcitability of the motor neurons and dysfunction of the spinal inhibitory interneurons have been proposed.[7]

Antiganglioside serology also supported the diagnosis of a GBS variant. Patient 1 had anti-GT1a and anti-GQ1b IgG antibodies that cross-reacted with each other. This antibody pattern is characteristic of Fisher syndrome[4] and may explain the minimal ophthalmoparesis in this patient. Patient 2 had anti-GT1a and anti-GD1a antibodies without cross-reactivity, minimal bulbar palsy, and neck and mild upper limb weakness. These features are similar to those observed in a previously reported patient.[8] GD1a and GQ1b are both present in human facial nerve.[9] Whether GT1a is present in human facial nerves as well is not known. GBS patients with anti-GT1a antibodies do, however, often develop facial palsy.[3] It is not clear why facial palsy was more prominent than other cranial nerve palsies in our patients. It may represent individual

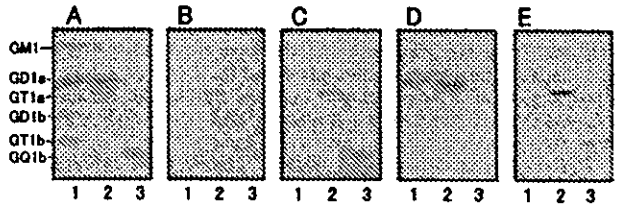

Figure. Thin-layer chromatogram with immunostaining. Lane 1, bovine brain ganglioside mixtures; lane 2, fraction containing GD1a and GT1a, separated from bovine brain ganglioside mixtures by Q-Sepharose column chromatography; lane 3, authentic GQ1b ganglioside. (A) Plate stained with orcinol/sulfuric acid for hexose. (B) Anti-GT1a IgG antibody is detectable in serum obtained from Patient 1 on day 9, whereas anti-GQ1b IgG reactivity is questionable. (C) Both anti-GQ1b and anti-GT1a IgG antibodies are present in serum from a patient with acute ophthalmoparesis. (D) Both anti-GT1a and anti-GD1a IgG antibodies clearly are present in serum obtained from Patient 2 on day 14. (E) Serum from a patient with typical pharyngeal-cervical-brachial weakness showing monospecific IgG antibody to GT1a.

variations in ganglioside distribution. Some *C. jejuni* strains isolated from patients with GBS have lipo-oligosaccharides that bear a GT1a-like structure,[10] and molecular mimicry may have been involved in the development of the facial palsy in our patients.

When acute bifacial weakness occurs, identifying GBS is important because these patients may benefit from IVIg therapy. Hyperreflexia does not exclude GBS or one of its variants. Features such as limb paresthesias, CSF albuminocytologic dissociation, electrophysiologic blink studies, and anti-ganglioside IgG antibodies can be helpful in diagnosing GBS in such cases. Finding evidence of a preceding *C. jejuni* infection can also be very useful.

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Usefulness of anti-GQ1b IgG antibody testing in Fisher syndrome compared with cerebrospinal fluid examination

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Abstract

Fisher syndrome (FS), a variant of Guillain–Barré syndrome (GBS), is a rare disorder, and there are few reported studies of a large number of patients with FS. Cerebrospinal fluid (CSF) albuminocytological dissociation was found in 59% of 123 FS patients during the first 3 weeks of illness, while serum anti-GQ1b IgG antibody was positive in 85%. Whereas the incidence of CSF albuminocytological dissociation increased from the first to second weeks in FS, anti-GQ1b IgG antibody peaked in the first week, but there was no CSF albuminocytological dissociation. Statistically, anti-GQ1b antibody testing was superior to a CSF examination in supporting a diagnosis of FS during the first 3 weeks of illness, especially in the first week.

Keywords: Fisher syndrome; Anti-GQ1b IgG antibody; Cerebrospinal fluid; Albuminocytological dissociation; Guillain–Barré syndrome

1. Introduction

The presence of cerebrospinal fluid (CSF) albuminocytological dissociation is evidence of Guillain–Barré syndrome (GBS), but "*After the first week of symptoms*, CSF protein concentration is elevated or has been shown to rise on serial lumbar punctures", as stated in the criteria established for GBS (Asbury and Cornblath, 1990). Fisher syndrome (FS) is considered a variant of GBS, strong evidence being that both conditions share a common CSF feature, albuminocytological dissociation (Fisher, 1956). FS, however, is a rare disorder, the estimated annual incidence rate being 0.09 per 100,000 population (Emilia-Romagna Study Group on Clinical and Epidemiological Problems in Neurology, 1998). Few studies have been made of large numbers of patients with FS. Clinical features of 50 consecutive patients with FS recently were reported, but no CSF findings were given (Mori et al., 2001). We therefore investigated the frequency of CSF albuminocytological dissociation and its temporal profile in a large number of FS patients.

Chiba et al. (1992) first reported that all six patients with FS that they studied had anti-GQ1b IgG antibody during the acute phase, which strong association was confirmed by other studies (Yuki et al., 1993; Willison et al., 1993). Most of the FS patients tested had this autoantibody. Equally significant was the absence of anti-GQ1b IgG antibody in the normal and other-disease control groups, indicative of the antibody's high specificity for FS. Our previous studies showed diagnostic specificities of anti-GQ1b IgG antibody in FS patients and also anti-GM1 and anti-GD1a IgG antibodies in GBS patients (Yuki et al., 1993; Tagawa et al., 2002). Because antibody titers peak at the time of clinical presentation then decay rapidly during clinical recovery, our main aim was to clarify whether anti-GQ1b antibody testing is more useful than a CSF examination for supporting the diagnosis of FS during the early phase of illness. In contrast, the IgG class of autoantibody to GM1 or GD1a specifically is present during the acute phase in patients with axonal GBS (Ho et al., 1999; Kornberg et al., 1994; Ogawara et al., 2000; Tagawa et al., 2002). Moreover, anti-GQ1b IgG antibody has been detected in GBS with ophthalmoplegia (Chiba et al., 1993). We therefore also investigated whether combined anti-GM1, anti-GD1a and anti-GQ1b antibody testing would be helpful for supporting a diagnosis of GBS.

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2. Patients and methods

2.1. Patients

Serum anti-ganglioside antibody testing was done between September 1995 and December 2000. Serum samples from 289 FS and 785 GBS patients were referred to us from university and district general hospitals throughout Japan. Because tentative diagnoses were made by the primary physicians, one of us (Y.N.) reviewed the medical records of each patient and made a definite diagnosis based on our proposed criteria for FS (Odaka et al., 2001) and the criteria for GBS (Asbury and Cornblath, 1990). The patients with overlapping of GBS and FS were excluded in this study. One hundred twenty-eight patients fulfilled the criteria for FS and 324 for GBS. Of these, 123 FS and 295 GBS patients were selected from May 1989 to December 2000 because serum had been taken from each one before treatment with plasmapheresis or intravenous immunoglobulin. CSF samples from the FS patients were obtained 1–3 times (median: 1) and from the GBS patients 1–4 times (median: 1), all within 3 weeks of the onset of neurological symptoms. Sera from 72 patients with other neurological diseases (49 patients with diabetic neuropathy, 12 with transverse myelitis and 11 with amyotrophic lateral sclerosis), which were taken during the same period, were used as a control group to calculate sensitivity and specificity for anti-ganglioside IgG antibodies.

2.2. Anti-ganglioside antibody assay and CSF examination

Serum IgG antibodies to GM1, GD1a and GQ1b were measured by the enzyme-linked immunosorbent assay as described elsewhere (Yuki et al., 1997). In brief, serum samples diluted 1:500 were added to wells of microtiter plates and the plates incubated at 4 °C overnight. Peroxidase-conjugated goat anti-human γ-chain-specific antibody (Dako, Glostrup, Denmark; diluted 1:1000) was added and the plates kept at 20 °C for 2 h. Color was developed with *o*-phenylenediamine. After 15 min, the reaction was stopped by an addition of 2 *N* hydrochloride. To correct for assay variations, well-characterized serum with high anti-GM1 IgG antibody titer was the internal standard. The mean value for triplicate reference wells without antigen was subtracted from the mean value for triplicate wells with the sample. One of our colleagues, Ms. Tsuchiya, who was blinded to the diagnoses, calculated the optical densities. Serum was judged positive when the optical density at 492 nm was 0.1 or more. The controls in our anti-ganglioside assay were 69 healthy Japanese. When the cutoff value was set at 0.1, the frequencies of positivity were 0% for every anti-ganglioside IgG antibody.

CSF samples were tested at the various hospitals. Albuminocytological dissociation was defined as a raised protein concentration (more than 45 mg/dl) associated with a count of 10 or fewer mononuclear leukocytes per microliter.

2.3. Statistical analysis

Differences in baseline data between FS and GBS were analyzed by the Mann–Whitney *U* test (two-tailed) and chi-squared test. Before doing a two-way analysis of variance (ANOVA) and the two-tailed Student's *t* test, the natural logarithmic transformation of the CSF protein concentrations was performed. A group mean comparison of the natural logarithm of the CSF protein concentration for each week was made by a two-way ANOVA. The two-tailed Student's *t* test was used to identify which group comparison accounted for a significant *p* value. The McNemar test was used when data for each patient were paired for serum anti-ganglioside IgG antibodies and CSF albuminocytological dissociation. Statistical analyses were conducted with Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS 11.0J (SPSS, Chicago, IL, USA) software. A *p* value of less than 0.05 was considered significant. Ninety-five percent confidence intervals (CI) were calculated from the odds ratios (OR).

3. Results

A statistical difference between FS and GBS was found for age (Mann–Whitney *U*-test, $p=0.003$) but not for sex or days when blood and CSF were taken (Table 1).

3.1. CSF protein concentrations during the first 3 weeks

Fig. 1 shows that CSF protein concentrations increased during the first 3 weeks of FS and GBS. There was no significant interaction between syndromes and the each week of CSF collection (two-way ANOVA, $p=0.25$). In each

Table 1
Baseline characteristics of patients with Fisher and Guillain–Barré syndromes

Variable	Fisher syndrome ($n=123$)	Guillain–Barré syndrome ($n=295$)
Sex (male/female)	77/46	184/111
Median age, years (range)	50 (2–92)	36 (0–87)
Blood taken	$n=123$	$n=295$
Median of days from onset (range)	7 (1–21)	6 (1–21)
1st week, *n* (%)	73 (59)	170 (58)
2nd week, *n* (%)	44 (36)	97 (33)
3rd week, *n* (%)	6 (5)	28 (10)
CSF taken	$n=184$	$n=440$
Median of days from onset (range)	8 (1–21)	8 (1–21)
1st week, *n* (%)	91 (50)	195 (44)
2nd week, *n* (%)	55 (30)	152 (35)
3rd week, *n* (%)	38 (20)	93 (21)

CSF data include the repeated samples in one patient.

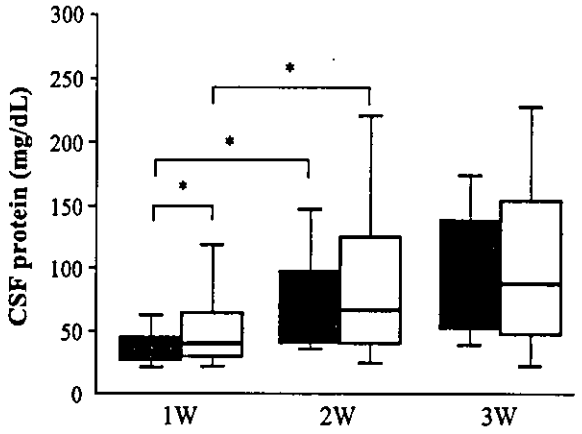

Fig. 1. Median CSF protein concentration in Fisher syndrome (FS: gray bar) and Guillain–Barré syndrome (GBS: white bar) for 3 weeks after onset of neurological symptoms. FS: first week (1W), $n=91$, median 34 mg/dl; second week (2W), $n=55$, median 55 mg/dl; third week (3W), $n=38$, median 84 mg/dl. GBS: 1W, $n=195$, median 40 mg/dl; 2W, $n=152$, median 68 mg/dl; 3W, $n=93$, median 83 mg/dl. Median lines shown within bars represent the 25th to 75th percentile. Error bars indicate the 10th and 90th percentiles. *$p<0.001$: after natural logarithmic transformation and the two-tailed Student's t-test followed a two-way ANOVA.

syndrome, the mean CSF protein concentration during the first week was lower than during the second one (two-tailed Student's t test, for each $p<0.01$: FS, 95% CI: 0.42–0.78; GBS, 95% CI: 0.29–0.61). The difference in mean CSF protein concentrations for FS and GBS was statistically in the first week (two-tailed Student's t test, $p<0.01$, 95% CI: 0.11–0.42) but not in the second and third weeks.

3.2. Comparison with the frequencies of serum anti-ganglioside antibodies and raised CSF protein concentration between GBS and FS

Table 2 shows the frequencies of serum anti-ganglioside IgG antibodies tested and raised CSF protein concentration during the first, second and third weeks. In the patients with FS, anti-GQ1b IgG antibody was positive in more than 80% from the first to third weeks, whereas anti-GM1 and anti-GD1a IgG antibodies were positive in 30% or less for each week. The frequency of anti-GQ1b IgG antibody was significantly higher than that of the anti-GM1 and anti-GD1a IgG antibodies ($p<0.01$ in each week). In the patients with GBS, anti-GM1 and anti-GD1a IgG antibodies were positive in 49% and 44% for the first week ($p=0.39$), 32% and 20% for the second ($p=0.70$), and 18% and 11% for the third ($p=0.71$). The frequencies of anti-GQ1b IgG antibody were lower than those of anti-GM1 IgG antibody during the first and second weeks ($p<0.01$), but, during the third week, there was no difference in frequencies of the three anti-ganglioside antibodies. The frequencies of anti-GQ1b IgG antibody were higher in the patients with FS than in the patients with GBS from the first to third weeks ($p<0.01$ in each week), and the frequencies of anti-GM1 IgG antibody were higher in the GBS patients than in the FS patients during the first and second weeks ($p<0.01$). Frequencies of raised CSF protein concentration in both FS and GBS increased from the first to third weeks. These frequencies were much higher than those of anti-GQ1b, anti-GM1, or anti-GD1a IgG antibodies in GBS patients during the second ($p<0.01$, OR: 0.34, 95% CI: 0.22–0.63) and third weeks ($p<0.01$, OR: 0.11, 95% CI: 0.04–0.29), but lower than those of anti-GQ1b IgG antibody in FS during the first ($p<0.01$, OR: 0.06, 95% CI: 0.03–0.14) and second weeks ($p=0.047$, OR: 0.31, 95% CI: 0.10–0.94).

3.3. Frequencies of serum anti-ganglioside IgG antibodies and CSF albuminocytological dissociation during the first 3 weeks

Anti-GM1 (15%), anti-GD1a (28%) or anti-GQ1b (85%) IgG antibody was positive in 86% of those with FS during the first 3 weeks, whereas anti-GM1 (41%), anti-GD1a (33%) or anti-GQ1b (19%) IgG antibody was positive in 53% of those with GBS. IgG antibodies against GQ1b, GM1 and GD1a during the first 3 weeks were negative in all the 72 patients with other neurological diseases. Compared with the control group, the sensitivity and specificity of anti-GQ1b IgG antibody in FS were 85% and 100%, respectively. In contrast, anti-GQ1b IgG antibody test in GBS was found to be the low sensitivity (19%) but the high specificity (100%). The sensitivity of both anti-GM1 and anti-GD1a IgG antibodies were

Table 2
Frequency of serum anti-ganglioside IgG antibodies and raised CSF proteins during the first, second and third weeks

	Fisher syndrome ($n=123$)			Guillain–Barré syndrome ($n=295$)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Blood taken	$n=73$	$n=44$	$n=6$	$n=170$	$n=97$	$n=28$
IgG anitibody to						
GQ1b, n (%)	59 (81)	39 (89)	6 (100)	41 (24)	13 (13)	2 (7)
GM1, n (%)	12 (16)	7 (16)	0	84 (49)	31 (32)	5 (18)
GD1a, n (%)	21 (29)	13 (30)	1 (17)	75 (44)	19 (20)	3 (11)
GQ1b, GM1 or GD1a, n (%)	61 (84)	39 (89)	6 (100)	107 (63)	42 (43)	7 (25)
CSF taken	$n=91$	$n=55$	$n=38$	$n=195$	$n=152$	$n=93$
Raised protein concentration, n (%)	23 (25)	39 (71)	32 (84)	85 (44)	102 (67)	70 (75)

CSF data include the repeated samples in one patient.

higher in GBS (41% and 33%, respectively) than in FS (15% and 28%, respectively), but anti-GM1 and anti-GD1a IgG antibody tests were the high specificity (100%) in both FS and GBS. CSF albuminocytological dissociation was detected in 59% of the FS patients and in 62% of the GBS patients. As shown in Fig. 2, 33% (black bar) of the FS patients had anti-GQ1b IgG antibody but no CSF albuminocytological dissociation; 7% (white bar) had albuminocytological dissociation but no anti-GQ1b antibody. In the McNemar test, the frequency of the former was significantly higher than that of the latter ($p<0.01$, OR: 4.56, 95% CI: 2.18–10.66). In contrast, 20% (black bar) of the GBS patients had anti-GM1, anti-GD1a or anti-GQ1b antibody but no CSF albuminocytological dissociation; 29% (white bar) had albuminocytological dissociation but no anti-ganglioside antibodies. The frequency of albuminocytological dissociation alone was higher than that of antibody alone ($p=0.02$, OR: 0.67, 95% CI: 0.46–0.95). These findings indicate that during the first 3 weeks of illness, testing of antibodies to GM1, GD1a or GQ1b is inferior to a CSF examination for supporting a diagnosis of GBS, whereas anti-GQ1b antibody testing is superior to a CSF examination for that of FS.

3.4. Frequencies of serum anti-ganglioside IgG antibodies and CSF albuminocytological dissociation during the first, second and third weeks

There were few differences in the frequencies of anti-ganglioside antibodies between the patient proportion of

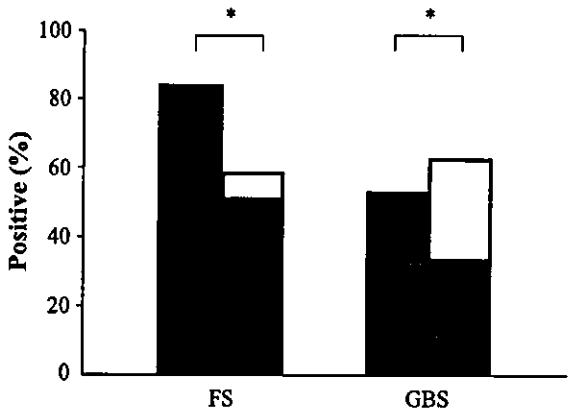

Fig. 2. Frequencies of serum anti-ganglioside IgG antibodies and CSF albuminocytological dissociation in FS and GBS during the first 3 weeks after onset of neurological symptoms. FS: gray bar (51%), serum anti-GQ1b IgG antibody and CSF albuminocytological dissociation; black bar (33%), anti-GQ1b IgG antibody but no albuminocytological dissociation; white bar (7%), albuminocytological dissociation but no anti-GQ1b antibody. Statistical analysis was done by the McNemer test. Significant differences (*) were detected for GBS and FS, on comparison of anti-ganglioside IgG antibodies with albuminocytological dissociation. GBS: gray bar (33%), serum anti-GM1, anti-GD1a or anti-GQ1b IgG antibody and CSF albuminocytological dissociation; black bar (20%), anti-GM1, anti-GD1a or anti-GQ1b IgG antibody but no albuminocytological dissociation; white bar (29%), albuminocytological dissociation but no anti-ganglioside antibodies.

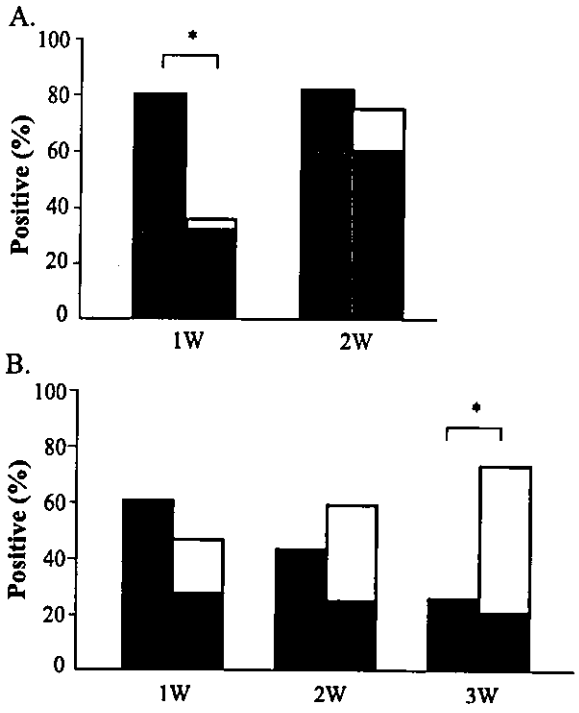

Fig. 3. Frequencies of serum anti-ganglioside IgG antibodies and CSF albuminocytological dissociation in FS and GBS during the first (1W), second (2W) and third (3W) weeks after onset of neurological symptoms. (A) Fisher syndrome: gray bar, serum anti-GQ1b IgG antibody and CSF albuminocytological dissociation; black bar, anti-GQ1b IgG antibody but no albuminocytological dissociation; white bar, albuminocytological dissociation but no anti-GQ1b antibody. 1W ($n=54$): gray=32%, black=48%, white=4%; 2W ($n=28$): gray=61%, black=21%, white=14%. There was a significant difference (*) for 1W in the comparison of anti-GQ1b IgG antibody with albuminocytological dissociation. (B) Guillain–Barré syndrome: gray bar, serum anti-GM1, anti-GD1a or anti-GQ1b IgG antibody and CSF albuminocytological dissociation; black bar, anti-GM1, anti-GD1a or anti-GQ1b IgG antibody but no albuminocytological dissociation; white bar, albuminocytological dissociation but no anti-ganglioside antibodies. 1W ($n=120$): gray=28%, black=33%, white=19%; 2W ($n=72$): gray=25%, black=18%, white=35%; 3W ($n=19$): gray=21%, black=5%, white=53%. Statistical analysis was done by the McNemer test. Significant difference (*) was detected for 3W in the comparison of anti-ganglioside IgG antibodies with albuminocytological dissociation.

Table 2 and the patient proportion of pair-matched study for each week. Fig. 3A shows the results by week of a pair-matched FS study. In the first week, 48% (black bar) of 54 FS patients had anti-GQ1b IgG antibody but no CSF albuminocytological dissociation, and 4% (white bar) had albuminocytological dissociation but no anti-GQ1b antibody. The former frequency was significantly higher than the latter ($p<0.01$, OR: 13.00, 95% CI: 3.26–113.02). In the second week, this frequency difference disappeared. This means that in the first week of FS, anti-GQ1b antibody testing is superior to a CSF examination as a marker that supports the diagnosis of FS, but after the second week antibody testing is no longer useful. During the first week of GBS, 33% (black bar) of 120 patients had anti-GM1, anti-GD1a or anti-GQ1b IgG antibody but no CSF albuminocy-