Table 1 Clinical features and antiganglioside complex antibodies in anti-GD1b-positive Guillain-Barré syndrome

Patient no.	Age, y/sex	Antecedent infection	Loss of deep sensation	Cerebellar signs	Ophthalmoplegia	Ptosis	Deficits of other cranial nerves	Antiganglioside complex antibodies
Ataxia: n = 9, age 58 (25–70)*								
1	70/F	R	(+)			(-)	9, 10, 11	(-)
2	58/F	R	(+)			(-)	7	(-)
3	56/M	R*		(+)	(+)	(+)	7, 9, 10, 11, 12	(-)
4	58/F	(-)	(+)			(+)	(-)	(-)
5	25/M	GI		(+)		(-)	9, 10	(-)
6	63/M	R	(+)			(+)	7, 9, 10	GM1/GD1a, GM1/GT1b
7	51/M	R	(+)				(-)	GM1/GT1b
8†	36/M	R				(+)	5	(-)
9	67/F	R	(+)				(-)	(-)
Nonataxia: n = 8, age: 35 (19–48)*								
10	48/F	R					7	GM1/GD1a, GM1/GT1b
11	36/M	R					(-)	GM1/GD1a, GM1/GT1b
12	26/F	R				(+)	7	GM1/GT1b, GM1/GT1a, GQ1b/GM1
13	36/F	GI					7, 9, 10	GM1/GD1a, GM1/GT1b
14	39/M	R				(+)	7, 9, 10, 11, 12	GM1/GT1b
15	33/F	R				(+)	(-)	GM1/GT1b
16	22/F	R			(+)	(+)	9, 10	GM1/GD1a, GM1/GT1b, GQ1b/GD1a
17	19/M	(-)				(+)	(-)	GQ1b/GD1b, GT1a/GD1b

*Age = median (range) years. Patients with ataxia were older than those without ataxia ($p = 0.003$, unpaired t test).
†Mycoplasma infection diagnosed serologically (complement-fixation reaction: 1:128).
‡His case history did not distinguish between sensory ataxia and cerebellar ataxia. Patient 1 (ataxia group) is patient A.
R = respiratory; GI = gastrointestinal.

(0.2 μg) with ELISA, which was conducted in duplicate. Antibody activities to the mixture antigens were compared with those to single GD1b antigen in each serum sample. Moreover, these sera were investigated for antibodies to GSCs consisting of two of the above gangliosides except for GD1b.

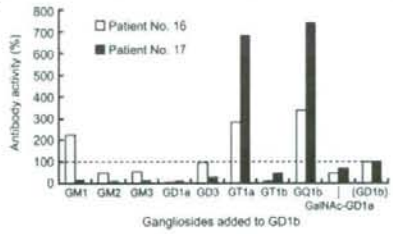
Association between the affinity of anti-GD1b antibodies and the development of ataxia. We analyzed the clinical features of patients with GD1b-mono IgG and subdivided them into two groups based on the presence or absence of ataxia; i.e., the ataxia and nonataxia groups. Patients who exhibited sensory or cerebellar ataxia irrespective of limb weakness during the course of the disease were categorized into the ataxia group. Between the two groups, we analyzed the association of the clinical features and antibody activities to GSCs containing GD1b. In statistical comparison with antibody activities to each GSC between the two groups, unpaired t test was used. Two-tailed p values of < 0.05 were considered significant. These analyses were performed with SPSS software (12.0, SPSS Inc., Chicago).

RESULTS Specificity of the anti-GD1b antibody in the representative serum. The antibody activity of patient A's serum was markedly decreased when an antigen mixture of GD1b and each of the gangliosides, such as GD1a, GD3, GT1a, GT1b, GQ1b, and GalNAc-GD1a, was used instead of GD1b alone (figure 1A). The addition of GM1 had no effect on anti-GD1b activity even if GM1 was added up to 0.6 μg (figure 1B). In combination with GD1a, on the other hand, anti-GD1b activity was strongly inhibited.

Clinical features and antiganglioside complex antibodies in anti-GD1b-positive GBS. The subjects were 17 GD1b-mono IgG-positive GBS patients whose corrected ODs of IgG anti-GD1b antibody were more than 0.3 and whose functional grading scales were 2 or more. The clinical features and anti-GSC antibodies of the subjects are shown in table 1. The ataxia group patients were significantly older than those in the nonataxia group. Six of the nine patients in the ataxia group had deep sensory disturbance and two patients exhibited cerebellar dysfunction. For the remaining one patient, neither the sensory type nor cerebellar type could be determined. Antecedent respiratory infection, ptosis without external ophthalmoplegia, and cranial nerve deficits were frequent in both patient groups. It is noteworthy that all of the nonataxia group patients had anti-GSC antibodies and that, among the anti-GSC antibodies, the target GSCs did not contain GD1b.

Immunoabsorption test on serum from Patient 11 was performed using GD1b, GM1/GD1a, and GM1/GT1b antigens, as described previously.[26] The percentage absorption of anti-GD1b antibody activ-

Figure 2 ELISA on sera from patients 16 and 17



IgG antibody activities to GD1b combined with one of several gangliosides are expressed relative to the response to single GD1b antigen (100%). Their antibody activities increased markedly when GQ1b or GT1a was added to GD1b in a well.

ity was 88.0% by the GD1b antigen, 38.0% by GM1/GD1a, and 62.4% by GM1/GT1b. The percentage absorption of anti-GM1/GT1b antibody activity was 88.6% by GD1b, 51.7% by GM1/GD1a, and 75.1% by GM1/GT1b. The percentage absorption of anti-GM1/GD1a antibody activity was not evaluated because its activity was not sufficient to perform immunoabsorption test (corrected OD; less than 0.2).

Antibody activities to mixture antigens containing GD1b in anti-GD1b-positive GBS. Sera of the 17 GD1b-mono IgG-positive patients were investigated in antibody activities to mixture antigens containing GD1b. In the nonataxia group, sera of patients 16 and 17 exhibited a remarkably strong reaction to the antigen mixture of GD1b in combination with GQ1b or GT1a (figure 2). Those two patients were excluded from analyses of antibody activity because their extreme increase of antibody activity had a significant effect on the results of the analyses and may lead to incorrect conclusion. As a result, sera from nine patients in the ataxia group and six patients in the nonataxia group were used to compare the antibody activities to the mixture of antigens containing GD1b between both groups. Antibody activities to each mixture antigens are shown in table 2 and figure 3. Corrected OD of IgG antibody to single GD1b was used as a standard (100%) and each antibody activity to mixture antigens containing GD1b was compared between ataxia and nonataxia groups (table 2 and figure 3). The reactivities of the anti-GD1b IgG antibodies in both the ataxia and nonataxia groups were reduced when a mixture antigen was used, except for with a mixture of GD1b and GM1. In particular, the reactivities to such antigens as GD1b combined with GD1a, GT1b, GQ1b, and GalNAc-GD1a were significantly further attenuated in the ataxia group than in the nonataxia group. Analysis on data of all nonataxia patients including 16 and 17 did not change this conclusion (mean percentage of antibody activities to each mixture antigens in nonataxia group: GD1b/GM1 118%, GD1b/GM2 60.5%, GD1b/GM3 61.6%, GD1b/GD1a 43.9%, GD1b/GD3 73.2%, GD1b/GT1a 140%, GD1b/GT1b 53.4%, GD1b/GQ1b 231%, GD1b/GalNAc-GD1a 59.7%).

DISCUSSION Occasionally patients with GBS have severe ataxia at the onset but not ophthalmoplegia.[21] Most of these patients experience some weakness, and the condition may progress to typical GBS, whereupon the ataxia is obscured. A previous review showed several cases of ataxic GBS, two of which the reviewer himself experienced.[22] The first patient had staggering gait, arm ataxia, less leg ataxia, areflexia, right abducens palsy, and moderate reduction of vibratory sensation in the hands, without Romberg sign. The second had severe gait and limb ataxia and areflexia without ophthalmoplegia. Sensory loss appeared later.

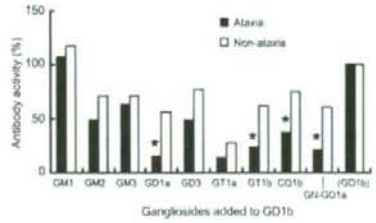
Since we first demonstrated anti-GSC antibodies in GBS sera,[15] anti-GSC antibodies have been rou-

Table 2 Comparison of IgG antibody activities to mixture antigens containing GD1b between ataxia and nonataxia groups

	GD1b	GD1b/GM1	GD1b/GM2	GD1b/GM3	GD1b/GD1a	GD1b/GD3	GD1b/GT1a	GD1b/GT1b	GD1b/GQ1b	GD1b/GalNAc-GD1a
Ataxia (n = 9)										
Mean (%)	100	108	48.9	63.6	15.8	49.1	14.5	23.6	37.3	21.3
SD (%)		47.4	34.2	41	14.6	38.4	14.3	23.2	31.3	18.3
95% CI (%)		71.6-144	22.6-75.2	32.1-95.1	3.6-28	19.6-78.6	3.5-25.5	5.37-41.4	13.2-61.4	7.23-35.4
Nonataxia (n = 6)										
Mean (%)	100	117	71.2	71.2	56	76.8	28.1	62.3	75.2	60.7
SD (%)		26.7	18.8	22	23.8	18.3	19.4	16.1	18.6	16
95% CI (%)		89-145	43.2-90.9	48.1-94.3	31-81	57.6-96	7.74-48.5	45.4-79.2	55.7-94.7	43.9-77.5
p Value*		0.53	0.049	0.51	<0.0001	0.014	0.035	<0.0001	0.0008	<0.0001

Antibody activity to a single GD1b is used as a standard (100%).
*Unpaired t test was used for comparison of antibody activities between the two groups. Two-tailed p values were shown.

Figure 3 Comparison of IgG antibody activities between ataxia and nonataxia groups



IgG antibody activities to GD1b combined with one of several gangliosides are expressed relative to the response to single GD1b antigen (100%). Black bars express antibody activities in the ataxia group, and white bars in the nonataxia group. Each asterisk indicates remarkable difference ($p < 0.0001$) between the ataxia and nonataxia groups.

tinely investigated on GBS sera in our laboratories and we found the representative serum obtained from patient A, who had profound ataxia as described in the above articles.[21,22] We were intrigued by the ELISA results showing that the anti-GD1b activity of the representative serum disappeared in wells to which GD1a, GT1a, GT1b, GQ1b, or GalNAc-GD1a was added to GD1b. This prompted us to investigate a series of GD1b-mono IgG-positive sera in the same manner.

The results on sera from a larger number of GD1b-mono IgG-positive patients showed that the addition of gangliosides with two or more sialic acids to GD1b inhibits binding of the anti-GD1b antibody to GD1b antigen in sera from patients with GBS. On the other hand, the addition of a ganglioside with one sialic acid, such as GM1, had little effect on binding of the anti-GD1b antibody to GD1b. Our previous results indicate that GD1b interacts with gangliosides, such as GD1a and GT1b, to form a novel epitope.[15,23] In such GSCs, GD1b may undergo conformational changes to such an extent that antibodies specifically recognizing GD1b cannot bind to it any more. Our present results are compatible with this hypothesis.

The binding activities of anti-GD1b antibodies in the ataxia group of patients are affected to a greater degree by such conformational changes. It can be considered that the more specific the antibody is, the more affected its reactivity should be. Therefore, anti-GD1b antibodies associated with ataxia should be highly specific to GD1b. The anti-GD1b antibodies reported in previous cases may be of this type.[5,6,7]

In the nonataxia group, all patients had antibodies to GSCs, such as GM1/GD1a and GM1/GT1b, even if conventional surveillance using single ganglioside antigens showed elevation of only anti-GD1b antibodies. Recently, we have pointed out that anti-GSC-positive GBS sera often have IgG anti-GD1b activity and that 74% of patients whose sera reacted to GM1/GD1a and GM1/GT1b also had IgG anti-GD1b antibodies.[23] The absorption study showed that the anti-GD1b antibody in a nonataxia group patient was absorbed on GSC antigen-coated wells. This finding suggested that anti-GD1b antibodies of the patients in the nonataxia group might react with a part of the terminal residues of GD1b, similar to the conformation of clustered epitopes in the GM1/GT1b complex. It is likely that terminal residues of GD1b conformationally resemble glycoepitopes formed in GM1/GD1a or GM1/GT1b.

It was not predictable that sera from patients16 and 17 would show intense activity to GD1b/GQ1b and GD1b/GT1a. The target antigens for serum antibodies from these patients are virtually complex epitopes of GD1b/GQ1b or GD1b/GT1a, rather than GD1b itself. This specificity is similar to that reported by ourselves in some patients with Fisher syndrome.[16] Both of those patients exhibited ptosis and one exhibited ophthalmoplegia. Interestingly, anti-GD1b-positive patients often present with ptosis without external ophthalmoplegia, with or without ataxia, the reason for which remains to be resolved.

As stated above, the binding ability of anti-GD1b antibodies was inhibited by the addition of gangliosides, such as GD1a, to GD1b antigen, strongly supporting that GD1b undergoes conformational change in GSCs. The number and configuration of sialic acids in the added ganglioside may determine whether the conformation of GD1b is significantly affected. The binding activities of anti-GD1b IgG antibodies associated with ataxia were significantly further attenuated by such a conformational change of GD1b, suggesting that they are highly specific for GD1b. Thus, GD1b-specific IgG antibodies that can bind to GD1b tightly are required to cause ataxia in GBS. Moreover, it is inferred that GD1b does not coexist with gangliosides, such as GD1a, GT1a, GT1b, or GQ1b, in the membrane of dorsal root ganglia cells. Anti-GD1b antibody has been considered to play an important role in the pathogenesis of ataxia in autoimmune neuropathies.[1,2,10,11] The present results provide further evidence to support the hypothesis that GD1b is a real target molecule for ataxia in autoimmune neuropathy.

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SHORT REPORT

ABSTRACT: In this report we describe a 72-year-old woman who had cytomegalovirus infection–related Guillain–Barré syndrome (GBS) associated with multiple immunoglobulin M (IgM) anti-ganglioside antibodies. She became tetraplegic with respiratory failure, but recovered completely after intravenous immunoglobulin therapy and plasmapheresis. The serum contained high-titer IgM antibody activities to several gangliosides with disialosyl residues (GD1b, GD3, GT1b, GQ1b, and GT1a) and GD1a. These antibodies are often found in sera from patients with chronic sensory ataxic neuropathy, but they occur rarely in GBS.

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COMPLETE RECOVERY OF AN AGED PATIENT WITH GUILLAIN–BARRÉ SYNDROME ASSOCIATED WITH MULTIPLE IgM ANTI-GANGLIOSIDE ANTIBODIES

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Guillain–Barré syndrome (GBS) is an acute immune-mediated peripheral neuropathy that is usually preceded by various types of infection, especially cytomegalovirus (CMV; 20–30% of all cases) and *Campylobacter jejuni* (30–40%).[1,2] Anti-ganglioside antibodies, positive in the sera of about 60% of patients with GBS in Japan, may participate in the development of specific neurologic signs by binding to regions that contain predominantly distinct ganglioside antigens. For example, anti-GQ1b IgG is thought to cause external ophthalmoplegia, presumably by binding to the paranodal regions of ocular motor nerves, where GQ1b is immunolocalized.[3] Anti-GT1a IgGs that do not cross-react with GQ1b are associated with bulbar palsy, neck weakness, and absence of sensory disturbance.[1] In contrast to anti-ganglioside antibodies, IgM antibodies are generally non-specific in GBS. However, in chronic sensory ataxic neuropathy (CAN), a pathogenic role has been suggested for serum IgM antibodies against NeuAc(alpha2–8)-NeuAc(alpha2–3)Gal-configured disialosyl epitopes, which are shared among many types of gangliosides, including GD1b, GD3, GT1b, and GQ1b.[13] We now report a rare case of GBS that occurred after CMV infection and was associated with high titers of IgM antibodies against multiple gangliosides.

Abbreviations: CAN, chronic sensory ataxic neuropathy; CMV, cytomegalovirus; CSF, cerebrospinal fluid; GBS, Guillain–Barré syndrome; IgM, immunoglobulin M; IVIg, intravenous immunoglobulin; NCS, nerve conduction studies; SNAP, sensory nerve action potential

Key words: anti-GD1a IgM antibody; anti-disialosylganglioside IgM antibody; cytomegalovirus; Guillain–Barré syndrome

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CASE REPORT

A 72-year-old woman had rhinorrhea, malaise, a non-productive cough, and a low-grade fever. One week later, she had bilateral dysesthesia in the distal parts of her fingers and soles, which extended to the arms and proximal thighs within 3 days and caused difficulty in walking due to sensory ataxia. The next day the patient was admitted. She had severe loss of deep sensation in the trunk and extremities, mild proximal dominant weakness, and decreased deep tendon reflexes. Loss of superficial sensation (light touch and pin prick) was not apparent. Laboratory tests revealed mild elevation of cerebrospinal fluid (CSF) protein (58 mg/dl) and cell count (8 cells/mm^3, all mononuclear cells). CMV infection was confirmed by a positive serum anti-CMV IgM titer and a 5-fold increase in the anti-CMV IgG titer in serum samples obtained at an interval of at least 2 weeks. Antibodies were quantitatively measured by enzyme immunoassay. Polymerase chain reaction for CMV was not performed. Anti-ganglioside antibodies were examined as described previously, using GM1, GM2, GM3, GD1a, GD1b, GalNAc-GD1a, GD3, GT1a, and GQ1b

as antigens.[5] Our patient had various anti-ganglioside IgM antibodies against GD1a (titer 1:1280, normal <1:40), GD1b (1:640), GD3 (1:320), GT1b (1:640), GQ1b (1:640), and GT1a (1:640), but no other antibodies, including various IgG antibodies. Results of nerve conduction studies (NCS) and sural nerve biopsy are shown in Figure 1. The initial and second NCS showed prolonged F-wave latencies, prolonged distal latencies, reduced conduction velocities, and proximal conduction blocks in the right median and ulnar nerves, suggesting the presence of demyelination. Sensory nerve action potentials (SNAPs) were evoked only in the initial study with reduced velocities and were not be obtained after 10 days. Short-latency somatosensory evoked potentials were not evoked in response to bilateral stimulation of the median or tibial nerves. A teased-fiber preparation of a biopsy specimen of the sural nerve showed segmental demyelination, although abnormalities were mild; several fibers had thin myelin sheaths without axonal degeneration. A brain magnetic resonance imaging (MRI) scan obtained on the first hospital day was almost normal except for marginal ischemic changes. GBS was diagnosed according to the criteria of Asbury et al.[1]

Despite high-dose intravenous immunoglobulin (IVIg) therapy (0.4 g/kg/day for 5 days) from the third day after admission, the patient became tetraplegic with bilateral facial nerve palsy, complete external and internal ophthalmoplegia, and bulbar palsy. Respiratory failure also developed, and she was intubated and received mechanical ventilation from days 19–35. Plasmapheresis was given eight times between days 16 and 28, as this treatment had been effective after a poor response to IVIg therapy.[2] She then recovered gradually after receiving the fifth session of plasmapheresis on day 20 and regained movement in her limbs, face, and eyes over the course of the next 2 weeks. After plasmapheresis, all anti-ganglioside IgM antibodies became almost undetectable. Although CSF protein remained high with a normal cell count 4 weeks after onset (180 mg/dl), she refused a further lumbar tap. Seven months after onset, she began to walk with a cane. After 18 months, she recovered full function and walked without aid. However, her tendon reflexes remained decreased, and NCS showed that the amplitude of M-waves for the ulnar nerve recovered to only 66%. SNAPs in the ulnar and median nerve remained absent despite almost complete recovery of M-waves for the median nerve 5 years after onset.

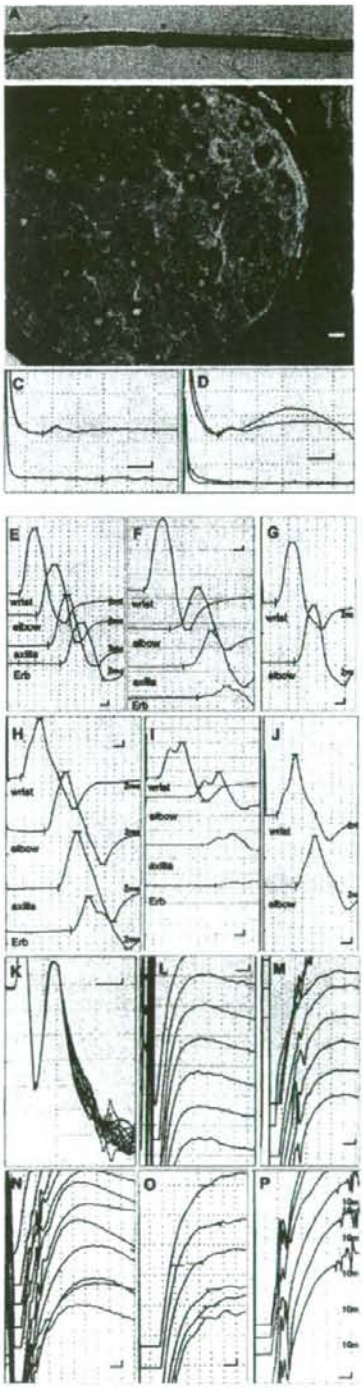
The patient's serum showed high-titer IgM antibody activity not only to gangliosides with disialosyl residues (GD1b, GD3, GT1b, GQ1b, and GT1a), but also to GD1a, which has no disialosyl residue. We therefore performed absorption tests, as described previously,[5] using GD1a and GD1b antigens. The results showed that either GD1a or GD1b antigens absorbed antibodies reactive to both GD1a and GD1b (absorption rate >80%).

DISCUSSION

Our patient had GBS associated with acute sensory ataxia. Severe muscle weakness, including ophthalmoplegia, dysphagia, facial nerve palsies, and respiratory failure, subsequently developed, with high-titer IgM antibodies against gangliosides such as GD1a, GD1b, GD3, GT1b, GQ1b, and GT1a. The presence of IgM antibodies against gangliosides with disialosyl residues, including GD1b, GD3, GT1b, and GQ1b, has been reported in sera from patients with CAN,[13] but it is rare in GBS. Monoclonal anti-GD1b antibodies selectively immunostain large-diameter sensory neurons. Sensitization with GD1b ganglioside induces sensory ataxic neuropathy in rabbits.[7] Deposition of IgM on the myelin sheath of peripheral nerve specimens from patients with IgM antibodies against gangliosides has been reported. This finding indicates that IgM antibodies can access the peripheral nervous system despite their high molecular weight. Thus, the detected IgM anti-ganglioside antibodies, possibly reactive to GD1b as a primary target, were presumably associated with sensory ataxia in our patient.

Our patient also had ophthalmoplegia and respiratory failure. These symptoms are known to occur in CAN; ophthalmoplegia is frequent but respiratory failure is uncommon.[15] Anti-GQ1b IgG antibodies are associated with ophthalmoplegia in GBS and Miller Fisher syndrome (MFS). Kaida et al.[6] reported that antibodies to GQ1b are associated with a risk of mechanical ventilation in GBS but not in MFS, suggesting that factors besides anti-GQ1b antibodies are involved. Thus, the anti-GQ1b IgM antibodies in our patient might have been associated with both ophthalmoplegia and respiratory failure.

Our patient had high-antibody titers against GD1a, as well as gangliosides with disialosyl residues. Because anti-GD1a IgM antibodies have been associated with motor-dominant demyelinating neuropathy, they may have caused the motor involvement in our patient.[10] Absorption studies showed that the same antibodies that react with gangliosides with disialosyl residues also bind to GD1a. The presence of reactivity against GD1a has also been reported in several patients who had sensory ataxic neuropathy with anti-disialosyl IgM antibodies.[13] GD1a may have



a three-dimensional structure similar to that of disialosyl residues. Further study is needed to clarify this issue.

Another characteristic feature of our patient was complete functional recovery from tetraplegia and respiratory failure, although age >50 years and ventilatory support are generally associated with poor outcomes.[8] Axonal damage is usually absent in CMV-related GBS,[5] but it is often present in *Campylobacter*-related GBS.[11] To exclude other types of sensory neuropathy, we performed sural nerve biopsy, which showed the presence of demyelination without axonal damage. To our knowledge, this is the first report of this finding in IgM anti-ganglioside-antibody–related GBS. The lack of axonal damage may at least partly explain the complete recovery in our patient.

FIGURE 1. Findings of sural nerve biopsy and nerve conduction studies in our patient **(A, B)**. The sural nerve biopsy specimen was obtained on the second hospital day. A teased-fiber preparation revealed segmental demyelination **(A)**. Abnormalities of light-microscopic findings were mild, however, and the result showed that several fibers had thin myelin sheaths without axonal degeneration. Scale bars: 10 μm **(B)**. Nerve conduction studies (NCS) were performed on the first hospital day **(C–E, H, K, N)**, 3 weeks later **(F, I, L, O)**, and 5 years later **(G, J, M, P)**. Sensory nerve action potentials (SNAPs) of the right median nerve are shown in **(C)** and those of the right ulnar nerve are shown in **(D)** (scale bars: 2 ms and 2 μV). M-waves of the right median nerve are shown in **(E)–(G)** and those of the right ulnar nerve are shown in **(H)–(J)** (scale bars: 2 ms and 1 mV). F-waves of the right median nerve are shown in **(K)–(M)**, and those of the right ulnar nerve are shown in **(N)–(P)** (scale bars: 10 ms and 40 μV). Motor conduction velocities (m/s), distal M-wave amplitude (mV), and distal latency (ms) were respectively as follows: **(E)** 57.2, 19.0, and 4.0; **(H)** 45.2, 15.2, and 3.16; **(F)** 31.3, 9.12, and 3.54; **(I)** 32.6, 6.58, and 4.02; **(G)** 54.0, 18.7, and 3.22; **(J)** 56.2, 10.6, and 2.82. Normal ranges were: median nerve, 56.2–62.8, 13.1–19.7, and 2.6–3.9; and ulnar nerve, 51.0–65.4, 9.6–23.8, and 3.2–3.8. For sensory conduction studies, SNAPs were evoked only at the onset, with reduced velocities [**(C)** median nerve 45.3 m/s (normal 51.5–66.6 m/s); **(D)** ulnar nerve, 43.1 m/s (normal 49.9–69.0 m/s)], but normal amplitudes [**(C)** median nerve, 2.87 μV (normal range 1.0–25.1 μV); and **(D)** ulnar nerve, 2.81 μV (normal 0.2–18.5)]. The initial NCS showed delayed F-wave latencies [**(K)** median nerve, 29.4 ms (normal 20.5–28.0 ms); and **(N)** ulnar nerve, 33.1 ms (20.0–28.3 ms)], delayed distal latencies, reduced conduction velocities, and proximal temporal dispersion in the ulnar nerve. On the second studies, F-waves became absent **(L, O)**, and conduction blocks occurred in both nerves **(F, I)**; that is, proximal/distal CMAP amplitude ratios of the ulnar and median nerves were 0.2 and 0.35, respectively, indicating demyelination. Five years after onset, when our patient had complete functional recovery, F-waves were evoked with normal latencies (median nerve 28.0 ms, ulnar nerve 28.2 ms). However, the amplitude of M-waves in response to stimulation of the median nerve recovered almost completely, whereas that of M-waves in response to stimulation of the ulnar nerve was only 66%, despite complete functional recovery.

In conclusion, we ascribe the functionally complete recovery in this elderly patient with GBS to demyelination caused by CMV-related GBS. Despite observations in only one patient, some IgM antiganglioside antibodies may be specifically related to certain clinical features of GBS and thus may be as useful as IgG antibodies for early diagnosis.

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GM1/GalNAc-GD1a complex

A target for pure motor Guillain-Barré syndrome

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ABSTRACT

Background: GM1 and GalNAc-GD1a are located on the axolemma of the motor nerves and are believed to be the antigens associated with pure motor Guillain-Barré syndrome (GBS). Furthermore, GM1 and GalNAc-GD1a may exist nearby and colocalize on the axolemma. Ganglioside complex (GSC) antigens associated with GM1 or GalNAc-GD1a can be target antigens in pure motor GBS. We investigated GBS sera for antibodies to a GSC consisting of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a) and analyzed the clinical and electrophysiologic findings of patients with antibodies to GM1/GalNAc-GD1a.

Methods: Sera from 224 patients with GBS were surveyed for antibodies to GSCs consisting of two of nine gangliosides (GM1, GM2, GM3, GD1a, GD3, GT1a, GT1b, GQ1b, and GalNAc-GD1a). We analyzed the clinical and electrophysiologic features of patients with IgG antibodies to the GM1/GalNAc-GD1a complex.

Results: Ten patients with GBS had IgG antibodies to the GM1/GalNAc-GD1a complex. The clinical findings of the 10 patients with GBS were characterized by preserved sensory system and infrequent cranial nerve deficits. According to the criteria established by Hadden et al., electrodiagnostic studies showed a demyelinating pattern in four patients and axonal neuropathy pattern in two. Early motor conduction block at intermediate nerve segments was found in five patients.

Conclusions: GM1 and GalNAc-GD1a may form a complex in the axolemma at nodes of Ranvier or paranodes of the motor nerves, and may be a target antigen in pure motor Guillain-Barré syndrome, especially in the form of acute motor conduction block neuropathy. ***Neurology*® 2008;71: 1683–1690**

GLOSSARY

AMAN = acute motor axonal neuropathy; **AMCBN** = acute motor conduction block neuropathy; **FS** = Fisher syndrome; **GBS** = Guillain-Barré syndrome; **GSC** = ganglioside complex; **LLN** = lower limit of normal; **NCS** = nerve conduction study; **OD** = optical density; **SNAP** = sensory nerve action potential; **TD** = temporal dispersion; **TLC** = thin-layer chromatogram; **ULN** = upper limit of normal.

Gangliosides, glycosphingolipids containing sialic acids, penetrate the outer leaflet of the plasma membrane and are thought to form a cluster with other glycolipids, especially in the glycosphingolipid-enriched membranes and lipid rafts.[1,2] Serum antibodies to gangliosides are found in approximately 60% of patients with Guillain-Barré syndrome (GBS),[3] and recently, we reported that a ganglioside complex (GSC) consisting of two different gangliosides can be a target antigen in GBS and Fisher syndrome (FS).[4-6] The anti-GSC antibody does not specifically react with each constituent ganglioside of the GSC, but with de novo epitopes formed in the GSC, indicating that gangliosides assemble in the plasma membrane and that the clustered glycoepitopes on the membrane are actually involved in immune-mediated events.

Supplemental data at www.neurology.org

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Antiganglioside antibodies may determine the clinical features based on distribution of target gangliosides in peripheral nerves.[7] Anti-GM1 or anti-GalNAc-GD1a antibodies closely correlate with the development of acute motor axonal neuropathy (AMAN),[8-13] which is supported by some immunohistologic studies.[14-17] It is possible that they exist nearby and colocalize on the motor axolemma. Antibodies to GSC antigens associated with GM1 or GalNAc-GD1a may exist and could be associated with pure motor GBS. Based on this hypothesis, we investigated GBS sera for antibodies to GSC, including GM1 or GalNAc-GD1a, and found an antibody to a GSC consisting of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a). In the present study, we described a representative patient with IgG anti-GM1/GalNAc-GD1a antibody, investigated anti-GM1/GalNAc-GD1a antibodies in a large population of patients with GBS, and analyzed the clinical and electrophysiologic findings of anti-GM1/GalNAc-GD1a-positive patients.

METHODS

Representative serum and the antiganglioside antibody assay. A 72-year-old man (patient A) developed weakness in his hands without obvious antecedent infection and, on the next day, he could not walk due to weakness in his leg. At admission (the second day), he presented with decreased tendon reflexes and tetraparesis (grade 2 or 3, Medical Research Council scale), but with no sensory signs and no cranial nerve involvement. He became bedridden on day 6. GBS was diagnosed based on standard criteria.[14] After completion of IV immunoglobulin treatment (400 mg/kg/day) from day 3 to day 7 and IV methylprednisolone (1,000 mg/day) from day 5 to day 7, his limb weakness improved rapidly. On day 14, he was able to stand independently and was transferred to another hospital for rehabilitation. The disease was considered to be a pure motor variant of GBS. The acute phase serum was examined for antiganglioside antibodies using an ELISA, as described elsewhere.[14] Ganglioside antigens used in the ELISA were 200 ng each of GM1, GM2, GD1a, GD1b, GD3, GalNAc-GD1a, GT1a, GT1b, and GQ1b.

The ELISA was also performed for antibodies to GSCs containing two (100 ng each) of the above nine ganglioside antigens, as described elsewhere.[4] Optical density (OD) values were corrected by subtracting the OD of a control well (uncoated well) that had been similarly processed. The criterion of anti-GSC antibodies was determined according to a previous report[4]: i.e., when the corrected OD was more than 0.1, the serum was considered positive. Anti-GM1- or anti-GalNAc-GD1a-positive serum for which the corrected OD of the anti-GM1/GalNAc-GD1a antibody was 0.2 higher than each corrected OD of the anti-GM1 or anti-GalNAc-GD1a antibody were considered anti-GM1/GalNAc-GD1a antibody-positive. If a serum exhibited antibody activities to both GM1 and GalNAc-GD1a, the serum was judged to be anti-GM1/GalNAc-GD1a antibody-positive when the OD value of anti-GM1/GalNAc-GD1a antibody was greater than the sum of the anti-GM1 and anti-GalNAc-GD1a antibodies. The same criteria were applied for estimation of antibodies to other GSCs. ELISAs were performed in duplicate and mean ODs were calculated. Thin-layer chromatogram (TLC) immunostaining was performed for GM1, GalNAc-GD1a, and a mixture of GM1 and GalNAc-GD1a, as described elsewhere.[4,5]

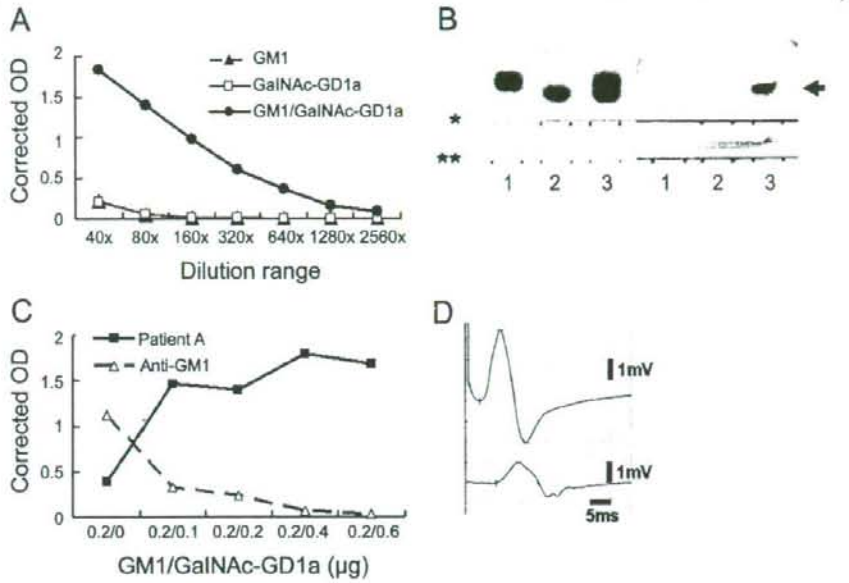
Antibody activity of the serum from the patient against GM1/GalNAc-GD1a was examined with ELISA using a mixture of 200 ng of GM1 and various amounts of GalNAc-GD1a (0, 100, 200, 400, 600 ng). Mouse monoclonal anti-GM1 IgM antibody (Seikagaku-kogyo, Tokyo, Japan) was also examined in the same way for comparison.

Immunoabsorption study on serum from patient A. Immunoabsorption test and ELISA were performed in duplicate using GM1, GalNAc-GD1a, and GM1/GalNAc-GD1a antigens, as described previously.[4,19] Each microtiter plate well was coated with a single ganglioside (500 ng) or a mixture of 250 ng of each ganglioside. Uncoated wells served as controls. Serum from patient A (50 μL) was diluted 1:40 with 1% BSA in PBS and added to both antigen-coated and uncoated wells. The percentage absorption was calculated as described previously.[19]

Serial electrophysiologic investigation of the representative case (patient A). Nerve conduction studies (NCSs) were performed serially in the upper and lower extremities of patient A, i.e., on days 2, 4, 6, 8, 10, 12, and 14, and on weeks 15 and 26, by standard methods[20] in our hospital. The skin temperature was maintained above 32°C. The motor nerves (right median, ulnar, and tibial) were stimulated at conventional sites, and surface electrodes recorded CMAPs. The amplitude of the CMAPs was the negative-peak value. To evaluate CB and temporal dispersion (TD), the amplitude and duration of the negative phase of the CMAP were measured. According to an electrodiagnostic criterion of GBS,[18] we defined partial motor conduction block; i.e., proximal CMAP/distal CMAP amplitude ratio of less than 0.5, but considered this valid only if the distal CMAP amplitude was greater than or equal to 20% of the lower limit of normal (LLN) without excessive TD. According to the criteria described by Oh et al.,[21] we defined TD as an increased duration of negative peak of more than 20% in the median and ulnar nerves and 30% in tibial nerves. The upper limit of normal (ULN) of distal latency and LLN of CMAP and NCV in Japanese were used.

Antiganglioside antibodies, clinical features, and electrophysiologic findings of anti-GM1/GalNAc-GD1a-positive patients. Acute phase sera from patients with GBS between 1994 and 2005 at the National Defense Medical College hospital were investigated for antibodies to GSCs and single ganglioside antigens, as described above. All the patients met the standard diagnostic criteria.[18] Moreover, the same investigation was performed in acute phase sera, which were collected also from patients with clinically defined GBS between October 2005 and April 2006, at various general and teaching hospitals throughout Japan. We asked the attending physicians to send us detailed clinical and electrophysiologic data on each patient with anti-GM1/GalNAc-GD1a antibody. Antecedent gastrointestinal infection was diagnosed when patients had gastrointestinal symptoms, such as nausea, vomiting, abdominal pain, and diarrhea without respiratory symptoms. NCSs in anti-GM1/GalNAc-GD1a-positive patients were evaluated as described

Figure ELISA and thin-layer chromatogram (TLC) immunostaining studies using serum from patient A



(A) Serum from patient A was diluted serially from 1:40 to 1:2,560. The antibody titer was 1:40, 1:40, and 1:1,280 for anti-GM1, anti-GalNAc-GD1a, and anti-GM1/GalNAc-GD1a antibodies, respectively. (B) TLC immunostaining using serum from the patient, diluted 1:100. The developing solvent consisted of chloroform, methanol, and 0.2%$CaCl_2$-$2H_2O$ (50:45:10, vol/vol). GM1 (3 μg) was applied to lane 1, GalNAc-GD1a (3 μg) to lane 2, and both GM1 and GalNAc-GD1a (3 μg each) to lane 3. Immunostaining is shown in the overlapping portions of GM1 and GalNAc-GD1a (arrow). GalNAc-GD1a was applied on the upper line (*) and GM1 on the lower line (**). (C) ELISA results using serum from the patient and mouse monoclonal anti-GM1 IgM antibody. The mouse monoclonal IgM antibody was diluted 1:500 with 1% bovine serum albumin in phosphate-buffered saline. Serum from patient A showed stronger reactivity with GM1/GalNAc-GD1a, whereas the activity of the monoclonal anti-GM1 antibody was definitely inhibited by the addition of GalNAc-GD1a. (D) This figure shows compound muscle action potentials recorded from the abductor pollicis brevis muscle after right median nerve stimulation on day 8. Conduction block was found in the intermediate site of the median nerve.

above, the results of which were categorized as primary demyelinating, primary axonal, unexcitable, equivocal, or normal according to two electrodiagnostic criteria of GBS.[9,10]

RESULTS Anti-GM1/GalNAc-GD1a antibody in the serum of patient A. The corrected OD was 0.22 for IgG anti-GM1 antibody, 0.21 for anti-GalNAc-GD1a, and 1.83 for anti-GM1/GalNAc-GD1a (diluted 1:40) (figure, A). TLC immunostaining showed that the serum antibodies reacted specifically with a GM1/GalNAc-GD1a complex (figure, B). No antibodies to other single gangliosides or GSCs were found in the serum. When GalNAc-GD1a was added to GM1 antigen in the well of an ELISA plate, binding of monoclonal anti-GM1 antibody to GM1 antigen was inhibited, whereas his serum antibody activity to the mixture of GM1 and GalNAc-GD1a increased remarkably (figure, C).

Immunoabsorption study using serum from patient A. An immunoabsorption study using serum from patient A showed that the serum antibodies reacted specifically with GM1/GalNAc-GD1a. The mean percentage absorption of anti-GM1/GalNAc-GD1a antibody activity was 27.3% by the GM1 antigen, 15.4% by GalNAc-GD1a, and 98.8% by GM1/GalNAc-GD1a.

Serial nerve conduction study results of patient A. Representative NCS results in patient A are shown in figure D and figures e-1 and e-2 on the *Neurology*® Web site at www.neurology.org. In the early phase of the disease, CBs were found in intermediate segments of the upper and lower extremities, with TD in his tibial nerve (figure e-1). Serial recordings showed that distal CMAP amplitude and proximal/distal CMAP ratio gradually improved after treatment, without excessive TD of the proximal CMAPs in the upper extremities (figure e-2). CB and TD in the lower extremities were prolonged, but improved to disappear in the 15th week. Motor conduction velocity, distal motor latency, and sensory conduction velocity were almost normal during the disease course. Sensory nerve action potential (SNAP) amplitudes of the

Table 1 Antiganglioside antibodies in patients with Guillain-Barré syndrome with IgG anti-GM1/GalNAc-GD1a antibody

No.	IgG anti-GM1/GalNAc-GD1a	IgG anti-GM1	IgG anti-GalNAc-GD1a	Other antibodies to single ganglioside antigens*	Other anti-GSC IgG antibodies*
1†	1.83	0.22	0.21	(−)	(−)
2	0.51	(−)	0.13	IgG: GD1b (2+)	GM1/GD1a (3+), GM1/GT1b (3+)
3	1.07	(−)	0.21	IgG: GD1b (−)	(−)
4	0.89	0.47	0.24	(−)	(−)
5	0.88	0.31	0.22	IgG: GD1b (+)	(−)
6	1.03	(−)	(−)	IgG: GD1a (+), GT1b (+)	(−)
7	0.8	(−)	(−)	(−)	(−)
8	1.28	(−)	0.19	(−)	(−)
9	0.33	(−)	(−)	IgG: GD1b (+)	GM1/GD1a (3+), GD1a/GD1b (2+),
				IgM: GM1 (3+), GD1b (2+), GalNAc−GD1a (2+), GQ1b (+)	GM1/GT1b (2+), GD1b/GT1b (2+)
10	1.55	0.76	0.35	IgG: GD1b (+)	(−)

*Criteria for antibody activities: +: 0.1 to 0.3 (corrected optical density), 2+: 0.3 to 0.8, 3+: 0.8 to 1.3, 4+: more than 1.3.
†Patient A.

median and ulnar nerves decreased transiently, although they were within normal limits.

Antiganglioside antibodies and clinical features of anti-GM1/GalNAc-GD1a-positive GBS patients. Four of 35 patients with GBS (including patient A) in the National Defense Medical College hospital and 6 of 189 patients with GBS at other hospitals had IgG anti-GM1/GalNAc-GD1a antibodies. According to clinical data sent from the other hospitals, the six patients also met the standard GBS criteria.[18] Antiganglioside antibodies in anti-GM1/GalNAc-GD1a-positive patients are shown in table 1. One patient had no antiganglioside antibodies except for IgG anti-GM1/GalNAc-GD1a antibody. Patients 2 and 9 had not only anti-GM1/GalNAc-GD1a antibodies but also had those against other GSCs with higher titers. Clinical features of the anti-GM1/GalNAc-GD1a antibody-positive patients are shown in table 2. The mean age of the patients was 49.6 years. Pathogens of antecedent infections were not identified. Two patients had cranial nerve involvement, one of whom had only bilateral ptosis without diplopia (patient 4). Of two patients with sensory symptoms, patient 5 presented with dysesthesia without objective sensory loss and had had diabetes mellitus. His hemoglobin A1c level at admission was 9.6% (normal level; <6.0%). Patient 7 was considered to have mild loss of the sense of vibration in his feet because he felt vibration during 6 seconds of vibration testing with a tuning fork. In patient 10, deep tendon reflexes decreased only in the lower extremities, and became normalized at day 16. Dysautonomia such as bladder-bowel dysfunction, orthostatic hypotension, instability of blood pressure, and arrhythmia was not observed in any patients. Muscle atrophy and ataxia were also not evident.

Electrophysiologic findings of anti-GM1/GalNAc-GD1a-positive GBS patients. The results of NCS in anti-GM1/GalNAc-GD1a-positive patients are shown in table 3. Four patients were classified as AIDP and two as AMAN based on the criteria described by Hadden et al.[10] Acute conduction block was found in intermediate sites of the forearm in patients 1, 2, and 8 and in the peroneal nerves of patient 6. When the criteria of Ho et al.[8] were applied, four patients were classified as AIDP and three as AMAN. Sensory nerve conduction studies showed mild decrease of SNAPs in the median nerves of patient 1 and sural nerves of patient 7, and mild delay of sensory conduction velocity in the median nerves of patient 7. SNAPs of the median and ulnar nerves in patient 8 were not evoked. Abnormality of the sensory NCS in patients 7 and 8 might have been associated with advanced age.

DISCUSSION The present study shows that GM1/GalNAc-GD1a may be a target antigen in pure motor GBS. Clinical analyses showed that anti-GM1/GalNAc-GD1a-positive patients resembled IgG anti-GM1-positive or anti-GalNAc-GD1a-positive patients with GBS in view of selective involvement of the motor fibers, infrequent disturbance of the cranial nerves, and distal-dominant limb weakness, which are consistent with the clinical features of a pure motor variant or AMAN variant,[9,12] although the sample size in this study was small. Moreover,

Table 2 Clinical features of anti-GM1/GalNAc-GD1a antibody-positive patients with Guillain-Barré syndrome

No.	Age, y/sex	Antecedent infection	Onset to nadir	F score at peak*	Involved cranial nerves	Sensory signs	DTR†	Predominant weakness	Therapy (response)‡
1	72/M	No	6	4	(−)	(−)	(−)	Distal	IVIg/pulse (effective)
2	17/F	Resp	4	4	(−)	(−)	(−)	Global	DFPP (effective)
3	28/M	Resp	N/A	4	(−)	(−)	(−)	Distal	IVIg (N/A)
4	72/F	GI	7	2	3	(−)	(−)	Global	IVIg (N/A)
5	42/M	Resp	5	2	(−)	(+)§	(+)	Distal	IVIg (effective)
6	49/M	Resp	9	2	(−)	(−)	(+)	Distal	(−)
7	69/F	Resp	2	4	(−)	(+)¶	(−)	Global	(−)
8	70/F	No	N/A	5	3, 6	(−)	(−)	Distal	IVIg (ineffective)
9	26/M	GI	12	3	(−)	(−)	(+)	Distal	DFPP/IA (effective)
10	51/M	Resp	6	2	(−)	(−)	(−)	Distal	DFPP (effective)

*Hughes grading scale is used as Functional score[40]: 1, having minor symptoms and signs but fully capable of manual work; 2, able to walk 5 m or morewithout assistance; 3, able to walk 5 m or more with a walker or support; 4, bedridden or chairbound; 5, requiring assisted ventilation for at least part ofthe day; 6, dead.

†Deep tendon reflexes (DTR) during the course: (−) = absent or decreased, (+) = preserved.

‡Response to therapy was classified into effective or ineffective, based on clinical evaluation by attendant physicians. Pulse = methylprednisolone pulse therapy (1,000 mg/day × 3 days).

§Numbness associated with diabetes mellitus.

¶Mild impairment of vibration sensation.

IVIg = intravenous immunoglobulin; Resp = respiratory infection; DFPP = double filtration plasmapheresis; N/A = not available (indicates in this column that we could not obtain enough information about response to therapy from attendant physicians); GI = gastrointestinal infection; IA = immunoadsorption therapy.

our results indicate that GM1/GalNAc-GD1a provides new clustered epitopes with different conformation and configuration from the terminal carbohydrate structure of GM1 or GalNAc-GD1a. GM1 and GalNAc-GD1a may aggregate and be in close formation at specific loci in the motor axonal membrane, since GM1-like epitopes locate on the axolemma at nodes of the motor nerves and GalNAc-GD1a also locates on axolemma at the nodes and paranodes of the motor nerves.[14,15]

The illness of patient A is analogous to acute motor conduction block neuropathy (AMCBN), which was recently reported as a distinct GBS variant,[22] in view of preserved sensory function, early CB at intermediate nerve segments, and good recovery. The definition and the pathophysiology of AMCBN are under debate and it is uncertain whether AMCBN should be categorized into AIDP, AMAN, or as an acute variant of multifocal motor neuropathy with conduction block. Several similar cases of acute motor neuropathy with multiple CB have been reported.[23-31] These reports describe frequent elevation of antiganglioside antibodies, preserved DTR, and frequent antecedent gastrointestinal infection such as *Campylobacter jejuni* enteritis. Some cases appear to be followed by axonal degeneration.[28] In patients with acute motor neuropathy with multiple CB, IgG anti-GM1 antibody was most common,[23-28] and IgG anti-GalNAc-GD1a was also reported,[25] although CB is not common in the pure motor type of GBS with these antibodies. Considering GM1 and GalNAc-GD1a are localized nearby in the axolemma of the motor nerves, some GM1/GalNAc-GD1a-positive GBS patients may take the form of the AMCBN variant similar to patient A. Serial conduction studies of patient 1 (patient A) showed no findings indicative of remyelination such as prolonged DML, slowing of MCV, and TD, in the later or recovery phase of the disease, except for mild dispersion in the tibial nerve (figure e-2). Hence, demyelination appears not to be the primary cause of the CB and limb weakness in this patient, although slight paranodal demyelination may be associated with such dispersion. Axonal degeneration is also unlikely because of the rapid improvement of limb weakness and NCS findings. The rapid resolution of CB might result from early reversible changes on the axolemma.[11] The anti-GM1/GalNAc-GD1a antibody is likely to cause such early reversible block and may be more closely associated with AMCBN than is the anti-GM1 or anti-GalNAc-GD1a antibody.

The mechanism of CB in the AMCBN or MMN has not yet been elucidated. A recent electrophysiologic study demonstrated that Na^+-K^+ pump activity may be impaired at the site of CB and cause depolarization with intracellular accumulation of Na^+.[32] The high intracellular Na^+ could eventually raise intracellular Ca^{++} levels through the Na^+-

Table 3 Nerve conduction study (NCS) results of anti-GM1/GalNAc-GD1a antibody-positive patients with Guillain-Barré syndrome

Patient no.	Days to NCS	No. of tested motor nerves	NCS					Classification of NCS*	
			CB	TD†	Prolonged DML	Decreased MCV	CMAP <20% LLN	Hadden	Ho
1§	4	3	3	1	0	0	0	(Dem)	(Axon)
	14	3	2	1	0	1	0	Dem‡	Equivocal‡
2	4	2	1	1	1	1	1	(Axon)	(Dem)
	53	2	0	0	2	1	1	Dem‡	Dem‡
3	3	2	0	0	1	1	0	(Dem)	(Dem)
	8	4	0	0	2	0	0	Equivocal‡	Equivocal‡
5	6	5	0	0	1	1	0	Equivocal‡	Axon‡
6	11	4	1	1	1	1	0	Equivocal‡	Dem‡
7	6	3	0	0	0	0	2	(Axon)	(Axon)
	20	3	0	0	0	0	2	Axon‡	Axon‡
8	4	3	2	0	2	1	1	(Dem)	(Dem)
	30	2	1	0	2	0	1	Dem‡	Dem‡
9	6	4	1	0	0	1	1	(Dem)	(Equivocal)
	13	4	0	0	0	0	2	(Axon)	(Axon)
	33	4	0	0	1	0	2	Axon‡	Axon‡
10	12	3	0	1	2	0	0	(Dem)	(Dem)
	23	3	0	1	2	0	1	Dem‡	Dem‡

*Numbers in columns of NCS indicated numbers of affected motor nerves. The NCS results of patient 4 were not described because they were unavailable. The NCS results were judged according to the criteria of Hadden et al.[10] and Ho et al.[8]
†TD: negative CMAP duration prolongation >20% normal limits (U/E), 30% (L/E).[21]
‡Final diagnosis.
§Patient A.
Days to NCS = days from disease onset to NCS; CB = conduction block; TD = temporal dispersion; DML = distal motor latency; MCV = motor conduction velocity; CMAP = complex muscle action potential; LLN = lower limit of normal; Dem = primary demyelinating; Axon = primary axonal.

Ca^{++} exchanger, resulting in axonal degeneration. If such physiologic (functional) block becomes more severe or prolonged, axonal degeneration could be induced. Therefore, CB, slowing of MCV, and low CMAP indicative of axonal loss could coexist to variable extents in the nerve tested.[33] Indeed, only two or three out of 10 patients with anti-GM1/GalNAc-GD1a antibodies were classified into the AMAN pattern based on their electrodiagnostic findings (table 3). Such diversity of electrophysiologic findings might be explained by differences in the intensity of the lesion in axolemma, although the effect of other antiganglioside antibodies should be taken into consideration.

In a recent pathologic study using nerve specimens obtained from patients with MMN, neither overt segmental demyelination nor onion bulb formation were observed at the site of CB, whereas pathologic alteration of axon was more prominent than myelin pathology.[33] It is inferred from such findings that prime target antigens associated with antibody-mediated CB are components of the axolemma of the nodes of Ranvier, rather than those of paranodal myelin.[34] Taken together, the binding of anti-GM1/GalNAc-GD1a antibodies to GM1/GalNAc-GD1a in the axonal membrane at the nodes of Ranvier may induce CB. In view of the dense cluster of voltage-gated Na^+ channels in the axolemma at nodes of Ranvier, the antibody-antigen interaction at the nodes may cause CB through alteration of regulatory function of Na^+ channels.

Conduction abnormalities at intermediate sites of the extremities are uncommon early in the course of GBS,[34,35] and distal nerve terminals and nerve roots are vulnerable to immune attack by autoantibodies because the blood–nerve barrier is anatomically deficient.[36] The high occurrence of CB at intermediate sites, an electrophysiologic feature associated with anti-GM1/GalNAc-GD1a antibodies, raises the possibility that anti-GM1/GalNAc-GD1a antibodies cause breakdown of the blood–nerve barrier.

Classification of the NCS results in some patients did not concur between the electrodiagnostic criteria described by Hadden et al. and by Ho et al. The criteria of Ho et al. do not regard CB as a feature of

demyelination, which may partly explain the difference of the classification. As mentioned above, axonal dysfunction can exhibit CB or abnormal MCV in electrodiagnostic studies, and electrophysiologic classification does not always reflect the actual pathologic and physiologic condition of damaged nerves. We did not discuss the difference and the effectiveness of the two criteria because they were not the main focus of the present study.

Our study showed antecedent gastrointestinal infection less frequently than the above previous reports. The reason for this is not clear. Considering the molecular mimicry hypothesis in GBS,[37,38] GM1/GalNAc-GD1a may be expressed also in the lipooligosaccharides of organisms causing antecedent infection. A recent study has shown that the lipooligosaccharides fraction of *C jejuni* isolated from GBS patients with anti-GSC antibodies expressed GSC-like structures.[39] Identification of pathogens causing antecedent infection is necessary to uncover the origin of anti-GM1/GalNAc-GD1a antibodies.

A monoclonal anti-GM1 antibody could not bind significantly to GM1/GalNAc-GD1a (figure, C). If GM1 and GalNAc-GD1a form a complex with new configuration in the axolemma at the nodes of the motor nerves, the anti-GM1/GalNAc-GD1a antibody would be more accessible to the axolemma at the nodes than GM1-specific antibodies. It will be important to examine antibody activity to GM1/GalNAc-GD1a in sera from patients with pure motor GBS.

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Review

Antibodies against gangliosides and ganglioside complexes in Guillain–Barré syndrome: New aspects of research

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Abstract

Guillain–Barré syndrome (GBS) is an acute autoimmune neuropathy, often preceded by an infection. Serum anti-ganglioside antibodies are frequently elevated in titer. Those antibodies are useful for diagnosis. Some of them also may be directly involved in the pathogenetic mechanisms by binding to the regions where the respective target ganglioside is specifically localized. We have recently found the presence of the antibody that specifically recognizes a new conformational epitope formed by two gangliosides (ganglioside complex) in the acute-phase sera of some GBS patients. In particular, the antibodies against GD1a/GD1b and/or GD1b/GT1b complexes are associated with severe GBS requiring artificial ventilation. Some patients with Miller Fisher syndrome also have antibodies against ganglioside complexes including GQ1b; such as GQ1b/GM1 and GQ1b/GD1a. Gangliosides along with other components as cholesterol are known to form lipid rafts, in which the carbohydrate portions of two different gangliosides may form a new conformational epitope. Within the rafts, gangliosides are considered to interact with important receptors or signal transducers. The antibodies against ganglioside complexes may therefore directly cause nerve conduction failure and severe disability in GBS. More study is needed to elucidate the roles of the antibodies against ganglioside complexes.

Keywords: Ganglioside; Peripheral nerve; Guillain–Barré syndrome; Raft; Glycolipid; Autoantibody

In immune-mediated neuropathies such as Guillain–Barré syndrome (GBS), anti-ganglioside antibodies are frequently present in the patients' sera. There are diversities in the carbohydrate sequences of the gangliosides. Each ganglioside has unique distribution within the peripheral nervous system. Anti-ganglioside antibodies can be used as useful markers for diagnosis. In addition, because the gangliosides are localized in the plasma membrane with their carbohydrate portions extended to the extracellular spaces, the anti-ganglioside antibodies may be involved in the pathogenetic process.

1. Anti-ganglioside antibodies in Guillain–Barré syndrome (GBS) and related disorders

Guillain–Barré syndrome (GBS) is an acute, self-limited immune-mediated motor-dominant polyneuropathy. An infection, involving respiratory or gastrointestinal tract frequently precedes the neurological onset. In GBS, either the myelin or the axon of the peripheral nervous system is the target of the autoimmune mechanism. Demyelinating type is referred to as acute inflammatory demyelinating polyneuropathy (AIDP), whereas the primary target is axonal membrane in acute motor axonal neuropathy (AMAN) [1]. Recent serological investigations using a panel of the gangliosides revealed that approximately 60% of the acute-phase GBS sera had antibodies, predominantly IgG class, against at least one ganglioside [2,3]. The antibody titers are highest in the acute-phase and decrease with clinical improvement. It suggests that elevation of the antiglycolipid antibody titer may not be a result of the damage to

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the peripheral nerve but may be directly associated with the pathogenetic mechanisms. Microorganisms of the antecedent infections of GBS sometimes are shown to have carbohydrate structures similar to those of gangliosides. Molecular mimicry mechanism has therefore been suggested as the mechanism of producing anti-ganglioside antibodies [3]. Anti-ganglioside antibodies are useful for diagnosis and also may be important pathogenetic factors of GBS.

Miller Fisher syndrome (MFS) is a variant of GBS, characterized by the triad of ophthalmoplegia, ataxia, and areflexia. It shares several features with GBS; frequent presence of antecedent infection, acute and self-limited clinical course, and albuminocytological dissociation in cerebrospinal fluid. We reported in 1992 that anti-GQ1b IgG antibody is specifically and frequently (more than 90%) present in the acute-phase MFS sera [4]. The titers of this antibody are also elevated in the acute-phase sera of GBS with ophthalmoplegia [5], "atypical MFS" (ophthalmoplegia without ataxia [5] or ataxia without ophthalmoplegia [6] of acute post-infectious self-limited clinical course), and Bickerstaff's brainstem encephalitis [7], which have clinical characteristics of MFS and central nervous system involvement. On the other hand, this antibody is not detected in sera from patients with other diseases. The elevation of serum anti-GQ1b IgG antibody titers is therefore closely associated with the occurrence of acute ophthalmoplegia and/or ataxia caused by such pathogenetic mechanisms as those underlying GBS. The antibody assay has been used for differentiating this syndrome from other immunological or neurological diseases, such as multiple sclerosis and myasthenia gravis.

Biochemical analysis of ganglioside fractions extracted from cranial nerves and spinal nerve roots (ventral and dorsal roots) showed that GQ1b is present in any of those nerves although the oculomotor, trochlear and abducens nerves had a relatively higher content of GQ1b [8]. In addition, an immunohistochemical study of the human nervous system using a mouse monoclonal anti-GQ1b antibody (7F5) showed that the paranodal myelin of the extramedullary portion of the cranial nerves innervating the extraocular muscles (oculomotor, trochlear and abducens nerves) was specifically immunostained [5]. No significant staining was observed in other cranial or peripheral nerves. The paranodal region is essential to nerve conduction and is the site of the earliest pathological change in GBS. Specific binding of the anti-GQ1b IgG to the paranodal myelin of the cranial nerves innervating extraocular muscles may cause ophthalmoplegia in MFS and GBS. The same monoclonal antibody also immunostained a subset of neurons in the dorsal root ganglia (DRG) of humans [6]. Binding of the anti-GQ1b IgG antibody to such neurons may be associated with the development of ataxia in MFS.

Several other gangliosides are also known as targets for antibodies in the acute-phase sera of GBS patients [9–14] (Table 1). Each antibody is associated with specific clinical features. The association can be explained by specific localization of the target molecule. Sensitization of rabbits with GD1b ganglioside, which is localized in the large sensory neurons in DRG, induces an animal model of immune-mediated sensory ataxic neuropathy [15]. This model confirms the assumption that anti-ganglioside antibodies may determine the clinical phenotype by binding to the respective ganglioside antigens that have a unique distribution in the peripheral nervous system.

Table 1
Anti-ganglioside antibodies in GBS (localization of ganglioside and associted clinical features)

Target antigen	Class	Localization of antigen	Clinical features	Ref.
GQ1b	IgG	Paranodal myelin of cranial nerves III, IV, VI Some neurons in DRG	Miller Fisher syndrome GBS with ophthalmoplegia Bickerstaff's brainstem encephalitis	[4,5,7]
GD1b (monospecific)	IgG	Paranodal myelin Large neurons in DRG	AIDP Ataxic GBS	[9]
GalNAc-GD1a	IgG	Periaxonal membrane	AMAN Pure motor GBS	[10,11]
LM1	IgG	Myelin	AIDP	[12]
GM1	IgG	Not determined	AMAN Pure motor GBS	[13]
GD1a	IgG	Not determined	AMAN	[14]

2. Antibodies to ganglioside complexes in GBS

2.1. Anti-GD1a/GD1b complex antibodies

Gangliosides have characteristics of forming clusters in the plasma membrane [16]. In the clusters, the carbohydrate structure of a ganglioside may interact with each other to form a novel epitope. We recently revealed that some GBS patients had serum antibodies that specifically recognize the novel glycoepitopes formed by two individual ganglioside molecules and named such antibodies as "anti-ganglioside complex antibodies" [17].

How we found the presence of anti-ganglioside complex antibodies is as follows. We investigated a serum from a patient with acute severe flaccid tetraparesis by an enzyme-linked immunosorbent assay (ELISA) and thin-layer chromatogram (TLC)-immunostaining [17]. We first found an unidentified immunoreactive band in the position just below GD1a on TLC of a crude ganglioside fraction from bovine brain. ELISA results were negative for each of the test gangliosides (GalNAc-GD1a, GM1, GM2, GM3, GD1a, GD1b, GD3, GT1b, and GQ1b). But the serum IgG bound strongly to the well coated with the mixture of GD1a and GD1b gangliosides (GD1a/GD1b) (Fig. 1). In TLC-immunostaining using a developing solvent of chloroform/methanol/0.2%$CaCl_2$–$2H_2O$ (50:45:10), positive staining was present in the lane in which both GD1a and GD1b were developed, but not in the lanes in which GD1a or GD1b was developed respectively (Fig. 2). The immunostaining was only present in the overlapping portion of GD1a and GD1b. In another developing solvent (C/M/0.2%$CaCl_2$–$2H_2O$, 30/65/10) that separated the positions of GD1a and GD1b more than the previous one, the immunostaining disappeared. Mixing GD1a and GD1b may produce a new conformational glycoepitope which is different from that of GD1a or GD1b alone and the antibody may specifically recognize such a new glycoepitope.

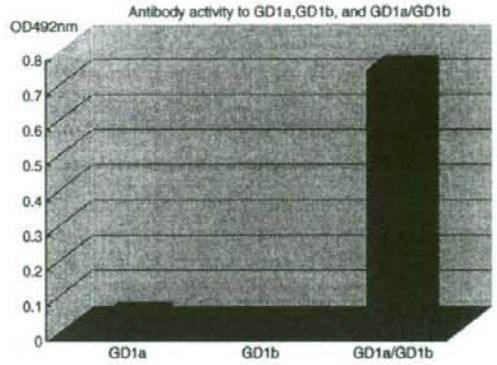

Fig. 1. ELISA result with anti-GD1a/GD1b IgG-positive sera. The antibody activities were expressed as optical density (OD) values. When the OD is more than 0.1, a serum is considered positive [17]. This patient's serum IgG shows strong reaction with a mixture of GD1a and GD1b (GD1a/GD1b) but does not react with GD1a or GD1b alone. The detailed experimental procedures were described elsewhere [17].

We next investigated antibodies in sera from 234 GBS patients using a mixture of two of the four major gangliosides (GM1, GD1a, GD1b and GT1b) as antigens [18]. Some patients with IgG anti-GD1a or anti-GD1b antibody activities have IgG activities against a mixture of GD1a/GD1b with much higher OD values than those of the anti-GD1a or anti-GD1b antibodies. When the corrected anti-GD1a/GD1b antibody OD was higher than the corrected anti-GD1a or anti-GD1b antibody OD by more than 0.2, we considered anti-GD1a/GD1b antibody was positive. We also used the above-mentioned criteria for the presence of the other anti-ganglioside complex antibodies. The results showed that 39 patients (17%) had antibodies against at least one of the mixture antigens. Among them, antibodies against GD1a/GD1b and GD1b/GT1b complexes are significantly associated with severe GBS requiring artificial ventilation [18]. Those antibodies against ganglioside complexes can be useful markers of severe GBS and may provide a clue to solve the pathogenetic mechanism of severe GBS.

Gangliosides are located in the cell membranes with carbohydrate portions on the outer surfaces, and are preferentially packaged with cholesterol, forming lipid rafts. Within rafts, gangliosides are considered to interact with important transmembrane receptors or signal transducers [16,19]. Antibodies to a ganglioside complex therefore may have effects on the function of the axon or Schwann cell through their binding to clustered epitopes of glycosphingolipids in the plasma membrane microdomains. Thus, they may directly induce nerve conduction failure and severe disability in patients with GBS. Further investigation is necessary to clarify the roles of anti-ganglioside complex antibodies in the pathogenetic mechanism of GBS.

2.2. Antibodies to ganglioside complexes including GQ1b

Because MFS is considered to be a variant of GBS, MFS patients may also have anti-ganglioside complexes antibodies. Presence of anti-ganglioside complexes antibodies in MFS therefore was investigated using seven ganglioside antigens; GM1, GM2, GD1a, GD1b, GT1a, GT1b and GQ1b [20].

Acute-phase serum samples were collected from 12 MFS patients, 10 of whom had IgG anti-GQ1b antibodies. ELISA results showed that seven of the 12 patients had serum antibodies to ganglioside complexes such as GQ1b/GM1, GQ1b/GD1b, GQ1b/GD1a, GT1a/GM1, GT1a/GD1b, GT1a/GD1a and GQ1b/GT1b, but not to ganglioside complexes without GQ1b or GT1a. One patient had no anti-GQ1b or anti-GT1a antibodies, but had antibodies to GQ1b/GM1 and GT1a/GM1. TLC-immunostaining also showed specific immunoreactivity against the overlapping portion of the two gangliosides [20]. In contrast to GBS, no patients had antibodies to the complexes consisting of two of the four major gangliosides, GM1, GD1a, GD1b and GT1b.

Based on the results of anti-ganglioside complex antibody assay, the 12 MFS patients could be subdivided into the three groups; antibody negative for ganglioside complexes (5 patients), anti-GQ1b/GM1-positive (5 patients), and anti-GQ1b/GD1a-positive (2 patients).

Because sensory signs were infrequent in MFS patients with antibodies to GQ1b/GM1 but were frequent in patients with other antibodies, such differences in antibody specificity may have some influence on clinical features in MFS. However, the clinical relevance of such anti ganglioside complexes antibodies needs to be investigated in a larger number of MFS patients.

2.3. Future studies

In view of the characteristic formation of clusters of gangliosides in the plasma membrane, anti-ganglioside complexes antibodies may cause nerve dysfunction more efficiently than those specific to a single ganglioside. Future study on the localization of each ganglioside complex is necessary. Animal model of the autoimmune neuropathy with anti-ganglioside complexes antibodies should also be developed. Through such investigations, possible roles of ganglioside complexes in the

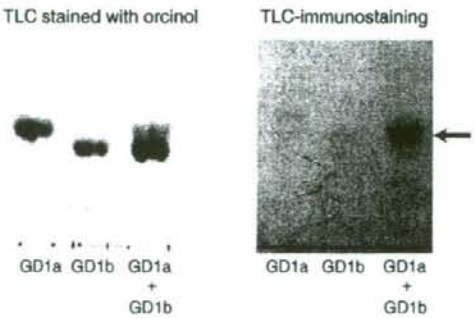

Fig. 2. TLC-immunostaining with anti-GD1a/GD1b IgG-positive sera. Positive staining was present in the lane in which both GD1a and GD1b were developed, but not in the lanes in which GD1a or GD1b was developed respectively. The antibody specifically bound to the overlapping portion of GD1a and GD1b (arrow). The detailed experimental procedures were described elsewhere [17].

plasma membrane and clinical relevance of antibodies against ganglioside complexes will be clarified.

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