1. GUILLAIN-BARRÉ SYNDROME

GBS is an acute, self-limited motor-dominant polyneuropathy. An infection, involving respiratory or gastrointestinal tract commonly precedes the neurological onset. GBS is considered as an autoimmne disease, of which the target is either the myelin or the axon of the peripheral nervous systems. The former is referred to as acute inflammatory demyelinating polyneuropathy (AIDP) and the latter acute axonal motor neuropathy (AMAN)[1]. Diagnosis of GBS is made by the characteristic clinical course, signs, and symptoms. Electrophysiological and cerebrospinal fluid examination, elevation of protein content but not of cell count (alubuminocytological dissociation), have been known to be useful for diagnosis. Recent serological investigations using a panel of the glycolipid antigens revealed that approximately 60% of sera from patients with acute-phase GBS had antibodies, predominantly IgG class, against at least one glycolipid[2,3]. Gangliosides, which are sialic acid-containing glycolipids and are distributed in the nervous system, are frequently the target antigens for serum antibodies in GBS. The antibody titers are highest in the acute phase and decrease with clinical improvement. In contrast, elevation of protein in cerebrospinal fluid usually becomes obvious on 7-10 days of neurological onset. It suggests that elevation of the antiglycolipid antibody titer may not be a result of the damage to the peripheral nervous system but may be associated with the pathogenetic mechanisms. Microorganisms of the antecedent infections of GBS sometimes are shown to have carbohydrate structures similar to those of such glycolipids as gangliosides. Molecular mimicry has therefore been suggested as the mechanism of producing antiglycolipid antibodies. Antiglycolipid antibodies should be useful for diagnosis and also may be important pathogenetic factors of GBS. Several antibodies associated with GBS and related disorders will be described as below.

a) Anti-GQ1b Antibody

Miller Fisher syndrome (MFS) is a variant of GBS, characterized by the triad of ophthalmoplegia, ataxia, and areflexia. It shares such features as frequent presence of antecedent infection, acute and self-limited clinical course, and albuminocytological dissociation in cerebrospinal fluid with GBS. Specific and frequent (more than 90%) elevation of anti-GQ1b IgG antibody titers in MFS was reported in 1992[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 type brainstem encephalitis[7], which has 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 utilized for differentiating this syndrome from other immunological or neurological diseases, such as multiple sclerosis and myasthenia gravis, even when ophthalmoplegia is present.

GQ1b has two disialosyl residues attached to the gangliotetraose structure (Gal-GalNAc-Gal-Glc-). Although both GT1a and GT1b have carbohydrate sequences similar to that of GQ1b, most of the MFS sera have IgG antibody activity against GT1a as well as GQ1b but not against GT1b[5]. An absorption study showed that the same antibody in MFS sera binds to both GQ1b and GT1a[5]. The serum antibodies in MFS sera may therefore recognize the disialosyl residue attached to the outer galactose of the gangliotetraose structure.

Biochemical analysis of ganglioside fractions extracted from cranial nerves and the 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]. However, an immunohistochemical study of the human nervous system using a mouse monoclonal antibody (7F5) provided us with a reason why anti-GQ1b antibody is closely associated with ophthalmoplegia and ataxia. As the monoclonal antibody 7F5 recognizes not only GQ1b but also GT1a, it has the same binding specificity as the IgG antibodies in MFS sera. The results 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 underlie the pathogenesis of ophthalmoplegia in MFS and GBS (figure 2). The monoclonal antibody 7F5 also immunostained a subset of neurons in the dorsal root ganglia and the cerebellar granule cells of humans[6]. Binding of the anti-GQ1b IgG antibody to those sites may be associated with the development of ataxia in MFS. Considering the looseness of the blood-nerve barrier in DRG, DRG neurons may be more accessible for the antibody in serum. A neurophysiological study suggested the dysfunction of the proprioceptive afferent system in MFS[9]. If the afferent fibers from GQ1b-positive DRG neurons connect with the spinocerebellar tract, the anti-GQ1b IgG antibodies may be involved in the development of ataxia by binding to those GQ1b-positive neurons.

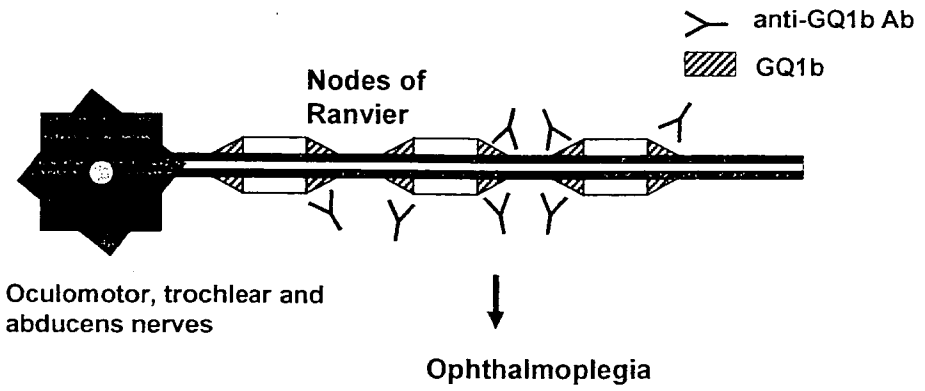

Figure 2. Specific binding of the anti-GQ1b IgG to the paranodal myelin of the cranial nerves innervating extraocular muscles may cause ophthalmoplegia in MFS and GBS.

It was reported that anti-GQ1b antibodies bind to neuromuscular junctions (NMJs), cause massive quantal release of acetylcholine from nerve terminals and eventually block neuromuscular transmission in mouse diaphragm[10]. This resembles the effect of alpha-

latrotoxin. It was also reported that morphological destruction of the motor nerve terminal in mouse hemidiaphragm preparation was induced by anti-GQ1b antibody-positive MFS serum and a mouse monoclonal anti-GQ1b antibody[11]. Those experimental results definitively showed that anti-GQ1b IgG antibodies can potentially cause motor weakness by their action to NMJs. However, the close association between that antibody and ophthalmoplegia may be difficult to explain solely based on those results, because the effect observed in the mouse diaphragma preparation should occur not only in the cranial nerves innervating the ocular muscles but also in the other peripheral nerves. MFS patients sometimes have other cranial nerve palsies. Anti-GQ1b IgG antibodies are also present in patients who have prominent limb weakness as well as ophthalmoplegia (GBS with ophthalmoplegia) as described above. Among GBS patients, it was reported that anti-GQ1b IgG-positive patients more frequently needed assistance with aritificial ventilation[12]. The effect of the anti-GQ1b IgG antibodies on NMJs could be involved in the pathogenesis of general motor weakness that sometimes occur in patients with anti-GQ1b IgG antibodies. Abnormal sudomotor axon reflex in patients with anti-GQ1b IgG antibodies has recently been reported, which suggests that the anti-GQ1b antibody also has similar effects on the neuroglandular junction[13].

Most of the antecedent infections of MFS or GBS with anti-GQ1b IgG antibodies are respiratory tract infections, although approximately 10 % are gastrointestinal infections, in which *Campylobacter jejuni* is the frequent infectious agent. *C jejuni* from stools from anti-GQ1b IgG positive patients has GQ1b-like carbohydrate structure in the lipopolysaccharide (LPS), indicating the antibody produced in response to the GQ1b-like epitope of the bacterial LPS is reactive with GQ1b ("molecular mimicry" mechanism)[14,15].

Antibodies against ganglioside-complexes including GQ1b will be discussed below.

b) Anti-GM1 Antibody

Anti-GM1 antibodies are one of the most frequent antiganglioside antibodies in GBS, detected in approximately 30 % of acute-phase GBS patients' sera[16]. Anti-GM1 IgG antibodies were reported to be associated with severe AMAN[17,18]. In subsequent reports, however, they were shown to be present not only in severe cases but in AMAN with mild clinical course[19]. In addition, other researchers reported that they are detected not only in sera of AMAN but also in those of AIDP[20]. Further investigation by collaboration of several research groups using definite neurophysiological criteria is needed for clarification of the clinical significance of anti-GM1 IgG antibodies in GBS.

It has been reported that GM1 is localized in the nodes of Ranvier and paranodal regions in the peripheral nervous system in studies using cholera toxin and peanut agglutinin lectin[21]. Those probes however bind to several gangliosides as well as GM1. Although GM1 is present in human PNS, specific localization of GM1 in human PNS has not been determined by the use of such probes monospecific to GM1 as the monoclonal antibody GMB16[22].

Sensitization of rabbits with GM1 was shown to induce motor axonal neuropathy. IgG was deposited on the axons of the ventral roots[23]. This experimental model suggests the important role of anti-GM1 antibody in the pathogenesis of motor axonal neuropathy.

Both gastrointestinal and respiratory tract infections can precede GBS with anti-GM1 IgG antibodies. Among the former, *C. jejuni* is the most frequent infectious agent. *C. jejuni* isolated from the stool of the anti-GM1 IgG-positive GBS patients has the carbohydrate structure similar to that of GM1, indicating anti-GM1 antibody is produced by the "molecular mimicry" mechanism[24].

c) Anti-GD1b Antibody

Two gangliosides, GM1 and GD1b, share the terminal galactosyl beta1-3 N-acetylgalactosaminyl (Gal-GalNAc-) residue. Antibodies recognizing the Gal-GalNAc-residue, therefore, bind to both GM1 and GD1b. Antibodies reactive with both GM1 and GD1b are present in approximately 20% of acute-phase GBS sera, anti-GM1 without anti-GD1b reactivity in approximately 10%, and anti-GD1b without anti-GM1 reactivity in approximately 10%[16]. Among the last group, there are monospecific anti-GD1b antibodies, which are reactive with only GD1b but not with other gangliosides or glycolipids. To elucidate the clinical significance of anti-GD1b reactivity, we should investigate the clinical features of GBS patients with monospecific anti-GD1b IgG antibodies.

Among 445 patients with GBS, 70 had anti-GD1b IgG antibodies. Nine of those patients had monospecific anti-GD1b IgG antibodies. Eight of the nine patients had respiratory tract infections before neurological onset. All the nine patients had sensory disturbances. Electrophysiological studies showed that none of the nine patients had AMAN form[25]. In the human PNS, GD1b is localized in paranodal myelin of both motor and sensory nerves as well as primary sensory neurons[22]. Anti-GD1b IgG antibodies may bind to these regions and cause sensory disturbances and demyelination. There are several case reports of GBS with anti-GD1b antibodies in which ataxia is prominent. Rabbits sensitized with GD1b were affected with ataxic neuropathy with high titer anti-GD1b antibodies, as detailed in later[26]. Only 3 of the above 9 patients however had ataxia[25]. Fine specificity of each monospecific anti-GD1b IgG antibodies may be varied and further investigation is necessary to clarify this point.

d) Anti-Galnac-GD1a Antibodies

GalNAc-GD1a is a relatively minor ganglioside which migrates just below GD1a in thin-layer chromatogram (TLC). Anti-GalNAc-GD1a IgG antibodies are reported to be present in about 10% of acute-phase GBS sera[16]. Monospecific anti-GalNAc-GD1a IgG antibodies are closely associated with the pure motor variant of GBS characterized by distal-dominant weakness and infrequent cranial nerve involvement[27]. Subsequent immunohistochemical investigation using a monospecific rabbit antibody showed that the antibody immunostained the periaxonal-axolemma-related region or an inner part of compact myelin of motor nerve fibers[28]. Some sensory nerve fibers however are also immunostained the rabbit anti-GalNAc-GD1a antibody. The reason for the discrepancy between the immunolocalization of GalNAc-GD1a in sensory nerves and the absence of sensory disturbance in patients with

GBS with IgG anti-GalNAc-GD1a antibodies is not known. Anti-GalNAc-GD1a IgM antibodies which are cross-reactive with GM2 are present in sera from GBS with sensory disturbances[29]. The anti-GalNAc-GD1a antibodies with such specificity might bind to GalNAc-GD1a in sensory fibers to cause sensory disturbances, whereas the monospecific anti-GalNAc-GD1a IgG might not. Future investigation is necessary to clarify this point.

e) Antigalactocerebroside Antibodies

Galactocerebroside (Gal-C) is a major glycolipid antigen in the myelin of both the central and peripheral nervous systems. Sensitization of rabbits with Gal-C causes demyelinating neuropathy, and anti-Gal-C antibody was shown to be a demyelinating factor[30]. Although elevation of anti-Gal-C antibody titers is not so common in GBS patients, the antibodies are quite frequently present in GBS subsequent to *Mycoplasma pneumoniae* infection[31]. Anti-Gal-C antibody activities in these sera were inhibited specifically by the *M. pneumoniae* reagent. A rabbit anti-Gal-C antibody recognized several glycolipids in the lipid fraction extracted from *M. pneumoniae*. Although their carbohydrate structure remains to be shown, those glycolipids in M. pneumoniae that are recognized by anti-Gal-C antibody may stimulate the immune system to produce anti-Gal-C antibody ("molecular mimicry mechanism"), which may participate in the pathogenesis of GBS as a demyelinating factor[32].

f) Antibodies to other Gangliosides

Antibodies to such gangliosides as GD1a, GT1b, LM1, GM2, and GM1b have also been reported in the acute-phase sera of GBS[2,3]. Anti-GD1a IgG[33] and anti-GM1b IgG antibodies are reported to be related with AMAN. Patients with anti-LM1 IgG antibodies frequently have anti-GQ1b IgG antibodies as well[34]. Anti-GM2 (IgM and/or IgG) antibodies are known to be associated with preceding cytomegalovirus infection[35](table 1).

Table 1. Clinical features and antiglycolipid antibodies in GBS.

Target antigen	class	localization of antigen	Clinical features
GQ1b	IgG	paranodal myelin of cranial nerves III,IV,VI some neurons in DRG	Miller Fisher syndrome GBS with ophthalmoplegia Bickerstaff brainstem encephalitis
GM1	IgG	not determined	AMAN Pure motor GBS
GD1b (monospecific)	IgG	paranodal myelin large neurons in DRG	AIDP ataxic GBS
GalNAc-GD1a	IgG	periaxonal membrane	AMAN Pure motor GBS
Galactocerebroside	IgG, IgM	myelin	AIDP
GM2	IgG, IgM	not determined	cranial nerve involvement

g) Antibodies to Ganglioside Complexes

i) Anti-GD1a/GD1b Complex Antibodies

Gagliosides have characteristics of forming clusters in the plasma membrane[36]. In the clusters, the carbohydrate structure of a ganglioside may intereact with each other to form a novel epitope. Clustered glycoepitopes of ganglioside complexes in the membrane may be targeted by serum antibodies in GBS patients. We recently revealed that some GBS patients had serum antibodies that specifically recognize the novel epitope formed by two ganglioside molecules and named such antibodies as "anti-ganglioside complex antibodies"[37].

We first investigated a serum from a 31-year-old man who developed acute flaccid tetraparesis after several days of flu-like symptoms. GBS was diagnosed based on Asbury and Cornblath criteria. His acute phase serum was checked for anti-ganglioside antibodies by an enzyme-linked immunosorbent assay (ELISA) and thin-layer chromatogram (TLC) immunostaining. We first found an unidentified immuno-reactive 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). 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 (figure 3). 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. The Image Analyzer assay showed specific immunostaining in the overlapping portion of the GD1a and GD1b antigens (figure).

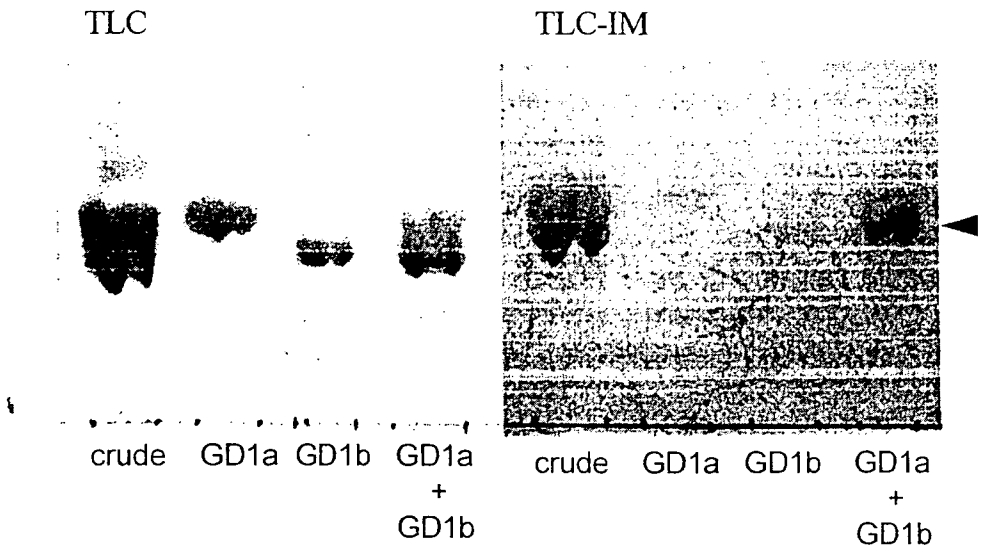

Figure 3. TLC-immunostaining with anti-GD1a/GD1b-positive sera; Positive staining was present in the lane in which both GD1a and GD1b were developed, but not in the lanes in which GD1a or GD1b was developed respectively.

To investigate the frequency of anti-GD1a/GD1b antibody in GBS patients, serum anti-ganglioside antibodies were investigated by ELISAs using 100 consecutive acute-phase GBS sera. Anti-GD1a- or anti-GD1b-positive sera, in which the corrected anti-GD1a/GD1b antibody OD was 0.2 higher than the corrected anti-GD1a or anti-GD1b antibody OD, were considered anti-GD1a/GD1b antibody-positive. The cut-off value (0.2) for the anti-GD1a/GD1b antibody was decided arbitrarily. The results showed that eight out of 100 consecutive patients (8%) with GBS had IgG anti-GD1a/GD1b antibodies. None of the disease or normal control group patients did. Three of the eight GBS patients with IgG anti-GD1a/GD1b antibodies had neither the IgG anti-GD1a nor anti-GD1b antibody. Of 92 GBS patients without IgG anti-GD1a/GD1b antibody, four had IgG anti-GD1a antibody, and eleven had IgG anti-GD1b antibody. TLC immunostaining of the anti-GD1a/GD1b antibody-positive sera gave the same results as for the above patient. Four anti-GD1a/GD1b antibody-positive patients required artificial ventilation (grade 5), and 3 other patients were bed- or chair-bound (grade 4) at peak. Electrophysiological studies showed "primary axonal" in two patients, equivocal" in one patient, which were classified as described elsewhere.[5] Five other patients had no available electrophysiological data.

Anti-GD1a/GD1b antibody-positive sera often showed little or no reactivity to GD1a or GD1b in the ELISAs. And even in the anti-GD1a- or anti-GD1b- positive cases, OD values of the anti-GD1a/GD1b antibodies were much higher than those of the anti-GD1a or anti-GD1b antibodies. It is therefore indicated that mixing GD1a with GD1b produced a ganglioside complex and a new conformational glycoepitope that is different from those of GD1a or GD1b alone.

The anti-GD1a/GD1b antibody-positive sera also had antibody activities to two or more of ganglioside complexes such as GD1a/GM1, GD1b/GT1b, and GM1/GT1b. Future investigation is needed on the presence of antibodies to the various ganglioside complexes using sera from larger number of GBS patients.

Glycosphingolipids are located in the cell membranes with carbohydrate portions on the outer surfaces, and are preferentially packaged with cholesterol, forming lipid rafts. These lipid rafts, protein-linked microdomains in the plasma membrane, are called detergent-insoluble glycolipid-enriched complexes or glycosphingolipid-enriched membranes. Within the plasma membrane microdomains, gangliosides are considered to interact with important transmembrane receptors or signal transducers involved in cell adhesion and signaling[36,38]. 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. Anti-GD1a/GD1b antibody-positive patients with GBS tend to have severe disabilities. Clinical studies of larger numbers of patients with GBS are needed to clarify the significance of anti-GD1a/GD1b antibody.

ii) Antibodies to Ganglioside Complexes Including GQ1b

As described above, the presence in serum of the IgG anti-GQ1b antibody is an excellent diagnostic marker for MFS[4,5]. Because MFS is considered to be a variant of GBS, MFS patients may also have anti-GSC antibodies. Presence of anti-ganglioside complexes

antibodies in MFS therefore was investigated using seven ganglioside antigens; GM1, GM2, GD1a, GD1b, GT1a, GT1b and GQ1b[39].

Acute phase serum samples were collected from 12 MFS patients, 10 (83%) of whom had IgG anti-GQ1b antibodies. ELISA results showed that seven (58%) 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 GSCs without GQ1b or GT1a. Especially, antibodies to GQ1b/GM1, GT1a/GM1, and GT1a/GD1b were frequent. One patient had no anti-GQ1b or anti-GT1a antibodies, but had antibodies to GQ1b/GM1 and GT1a/GM1. In contrast to GBS, no patients had antibodies to the complexes consisting of two of the four major gangliosides, GM1, GD1a, GD1b and GT1b. TLC-immunostaining also showed specific immunoreactivity against the overlapping portion of two gangliosides, including GT1a/GM1, GQ1b/GM1 and GT1a/GD1b.

The ELISA showed that the fine specificity of serum antibodies in MFS patients was heterogeneous. Some serum antibodies had stronger activity with GQ1b/GM1 or GT1a/GM1 than with either GQ1b or GT1a alone, whereas others had little or no activity against GQ1b/GM1 or GT1a/GM1, despite showing intense activity with GQ1b or GT1a. 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).

Antibodies to GSCs containing GQ1b or GT1a, as well as anti-GQ1b and anti-GT1a antibodies, may be critical for development of MFS. Serum antibodies specific to GQ1b/GM1 and GT1a/GM1 may recognize a combination of [Galβ1-3GalNAc] and [NeuAcα2-8NeuAcα2-3Galβ1-3GalNAc] in the terminal residues of gangliotetraose structures. In contrast, serum antibodies specific to GQ1b/GD1a and GQ1b/GT1b may recognize a combination of [NeuAcα2-3Galβ1-3GalNAc] and [NeuAcα2-8NeuAcα2-3Galβ1-3GalNAc] in the terminal residues. On the other hands, some patients had monospecific activities to GQ1b or GT1a. Thus, there are at least three different specificities in MFS-associated antibodies. Because sensory signs were infrequent in MFS patients with antibodies to GQ1b/GM1 and GT1a/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-GSC antibodies needs to be investigated in a larger number of MFS patients before firm conclusions can be drawn.

In view of the characteristic formation of clusters of gangliosides in the plasma membrane, anti-GSC antibodies may cause nerve dysfunction more efficiently than monospecific anti-GQ1b antibody. Future investigations of the localization and possible roles of ganglioside complexes in the plasma membrane are required.

2) IgM Paraproteinemic Neuropathy

IgM paraproteinemia is often associateed with chronic peripheral neuropathies. IgM paraproteins (M-proteins) associated with neuropathies frequently recognize varied carbohydrate epitopes. Each binding specificity of the IgM M-protein is linked with a unique clinical feature[2].

a) IgM M-Protein Specific to Myelin-Associated Glycoprotein, P0-Glycoprotein, and Phosphated Glucuronyl Paragloboside

Approximately half of the IgM M-proteins of such IgM paraproteinemic neuropathies bind to the carbohydrate epitope, sulfated glucuronyl residue, shared by myelin-associated glycoprotein (MAG) and such glycolipids as sulfated glucuronyl paragloboside (SGPG)[40, 41]. MAG and SGPG are localized in myelin. IgM deposits are found in the myelin in sural nerve biopsy specimens. Passive transfer of this IgM M-protein caused demyelination of the peripheral nerve[42]. IgM M-protein of this specificity may play an important role in the pathogenesis of the demyelinating neuropathy.

In addition to MAG and SGPG, P0-glycoprotein and peripheral myelin protein-22 (PMP-22) also have sulfated glucuronyl residue. Some of the IgM M-proteins binding to SGPG strongly immunostained peripheral nerve myelin, whereas the others strongly immunostained the cytoplasm of the Schwann cells surrounding the myelin sheath with only weak staining of the myelin. Western blotting showed that the former IgM M-proteins also reacted strongly with P0 and PMP-22, whereas the latter did not. P0 and PMP-22 are known to be localized in compact myelin. The strong reactivity with P0 and PMP-22 may be associated with the strong immunostaining of the peripheral nerve myelin by the former IgM M-protein[43].

b) IgM M-Protein that Binds to Gangliosides with a Disialosyl Residue

In some patients with IgM paraproteinemic neuropathy, IgM M-protein binds to gangliosides with a disialosyl resudie, such as GD1b, GD3, GT1b and GQ1b. IgM M-protein of this type is specifically associated with sensory ataxic neuropathy[44]. GD1b is localized in the primary sensory neurons with large diameters[22]. The IgM M-protein of this type may therefore bind to GD1b in the primary sensory neurons to cause sensory ataxic neuropathy.

c) Experimental Sensory Ataxic Neuropathy Induced by Sensitization with GD1b

As described above, GD1b ganglioside is a target molecule for serum antibodies in sensory ataxic neuropathy. GD1b is localized in neurons with large diameters in rabbit DRG as well as human DRG. We therefore assumed that sensitization with GD1b may induce sensory ataxic neuropathy in rabbits. As a result, experimental sensory ataxic neuropathy indeed developed in about 50% of the rabbits immunized with GD1b[26]. The affected rabbits lay on the floor with splayed limbs, frequently in a peculiar posture. The limbs moved awkwardly. Muscle power and pain sensation were not affected. Anti-GD1b antibody titers were markedly raised in rabbits immunized with GD1b. Pathological investigation of the affected rabbits showed axonal degeneration in the dorsal root, the dorsal column of the spinal cord, and the sciatic nerve, whereas the ventral root was intact. No demyelination was found. Some nerve cell bodies in DRG had degenerated or disappeared. No lymphocytic infiltration was observed in the affected regions. It is therefore indicated that anti-GD1b antibody may cause specific degeneration of primary sensory neurons mediating deep

sensation. This was supported by the report that degeneration of rabbit sensory neurons could be induced by passive transfer of anti-GD1b antiserum[45]. Monospecific anti-GD1b IgG antibody was shown to be required to induce this sensory ataxic neuropathy[46].

DRG neurons with large diameters depend on neurotrophin-3-mediated trkC signaling. TrkC expression was reported to be reduced in DRG of the affected rabbits in the acute phase[47]. Binding of the anti-GD1b antibody may cause downregulation of trkC expression. It may be involved in one of the pathogenetic mechanisms of this animal model.

This is the first established animal model of autoimmune neuropathy mediated by the antiganglioside antibody. This model confirms the assumption that antiganglioside antibodies may determine the clinical features of autoimmune neuropathies by binding to the respective gangliosides that have a unique distribution in the peripheral nervous system.

CONCLUSION

Antiglycolipid antibodies are useful diagnostic markers in autoimmune neuropathies. Recent investigations showed that they are also involved directly in the pathogenetic mechanisms. Different species of gangliosides may interact with each other to form a novel carbohydrate epitope, which may also be target antigens for serum antibodies in autoimmune neuropathies. Future investigation on antiglycolipid antibodies to clarify the pathogenetic mechanisms of autoimmune neuropathies may also be useful to understand the physiological functions of glycolipids in the nervous system.

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Anti-ganglioside complex antibodies associated with severe disability in GBS☆

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Abstract

Ganglioside complexes (GSCs) are known as target antigens in Guillain–Barré syndrome (GBS). To elucidate the clinical importance of the anti-GSC antibodies in GBS, we investigated serum antibodies to GSCs containing two of the gangliosides, GM1, GD1a, GD1b and GT1b, and analyzed clinical features of anti-GSC-positive GBS patients. Thirty-nine (17%) of 234 GBS patients had IgG anti-GSC antibodies. Anti-GSC-positive GBS had antecedent gastrointestinal infection and lower cranial nerve deficits more frequently than control GBS. The presence of antibody specificity to GD1a/GD1b and/or GD1b/GT1b was significantly associated with severe disability and a requirement for mechanical ventilation.

Keywords: Guillain–Barré syndrome; Ganglioside complex; Antibody; Disability

1. Introduction

Gangliosides concentrate on the surfaces of neurons, with their oligosaccharide portions exposed on the cell surface, and are believed to form clusters extensively in the cell membrane (Hakomori, 2002). Recent studies of plasma membrane molecules have shown that gangliosides appear to be organized in clusters, and to form membrane microdomains together with cholesterol and glycosylphosphatidylinositol (GPI)-anchored proteins (Simons and Toomre, 2000). These microdomains are called lipid rafts or detergent-resistant membranes.

Gangliosides in peripheral nerves are often targeted by serum antibodies in patients with Guillain–Barré syndrome (GBS), acute immune-mediated polyradiculoneuropathy, and interaction between anti-ganglioside antibodies and peripheral nerve gangliosides is believed to cause GBS and its variants (Chiba et al., 1993; Willison and Yuki, 2002; Kaida et al., 2003). We recently demonstrated the presence of antibodies to ganglioside complexes (GSCs) in serum of some GBS patients and suggested that anti-GSC antibodies might function in the development of GBS (Kaida et al., 2004). It has hitherto been inferred that ligands of adhesion molecules such as selectins comprise diverse and complex glycoconjugates, called clustered saccharide patches, in which oligosaccharides are packed closely together to form rigid rodlike structures with multivalency and strict binding-specificity (Norgard et al., 1993; Varki, 1994). The discovery of anti-GSC antibodies in GBS serum suggests that clustered glycoepitopes of GSCs or clustered saccharide patches actually exist on the plasma membrane and are involved in immune-mediated events.

In our previous study (Kaida et al., 2004), eight of 100 GBS patients had IgG antibodies to GD1a–GD1b complexes (GD1a/GD1b) and were predisposed to lower cranial nerve palsy and severe disability. However, statistical significance

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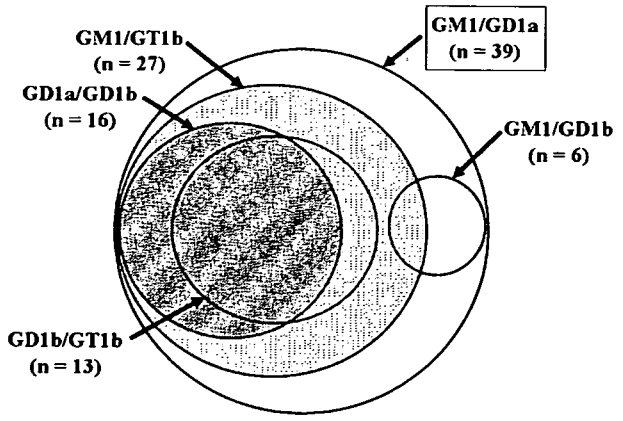

Fig. 1. The Venn diagram shows distribution of anti-GSC(+) group patients based on data in Table 1. All of the anti-GSC(+) group patients had IgG anti-GM1/GD1a antibodies. Anti-GD1a/GD1b-positive patients had also IgG anti-GM1/GD1a and GM1/GT1b antibodies. Ten anti-GSC(+) group patients had IgG anti-GD1a/GD1b and anti-GD1b/GT1b antibodies.

of the association between that antibody and those clinical features has not been demonstrated. In the present study, to clarify the clinical significance of anti-GSC antibodies in GBS, we retrospectively analyzed the clinical features of anti-GSC antibody-positive patients with GBS in a larger population, with a focus on the fine specificity of anti-GSC antibodies and its clinical relevance.

2. Materials and methods

2.1. Patient serum and enzyme-linked immunosorbent assay for anti-ganglioside complex antibodies

Acute phase serum was collected from patients with clinically defined GBS between September 2001 and December 2002 at various general and teaching hospitals throughout Japan. All met the diagnostic criteria of Asbury and Cornblath (Asbury and Cornblath, 1990).

ELISA was done for serum antibodies to gangliosides GM1, GM2, GM3, GD1a, GalNAc–GD1a, GD1b, GD3, GT1b, and GQ1b, as described elsewhere (Kaida et al., 2000). The ELISA for anti-GSC antibodies were performed as described in our previous report (Kaida et al., 2004). GSCs used in the ELISA contained two (0.1 μg each) of four ganglioside antigens, GM1, GD1a, GD1b, and GT1b which

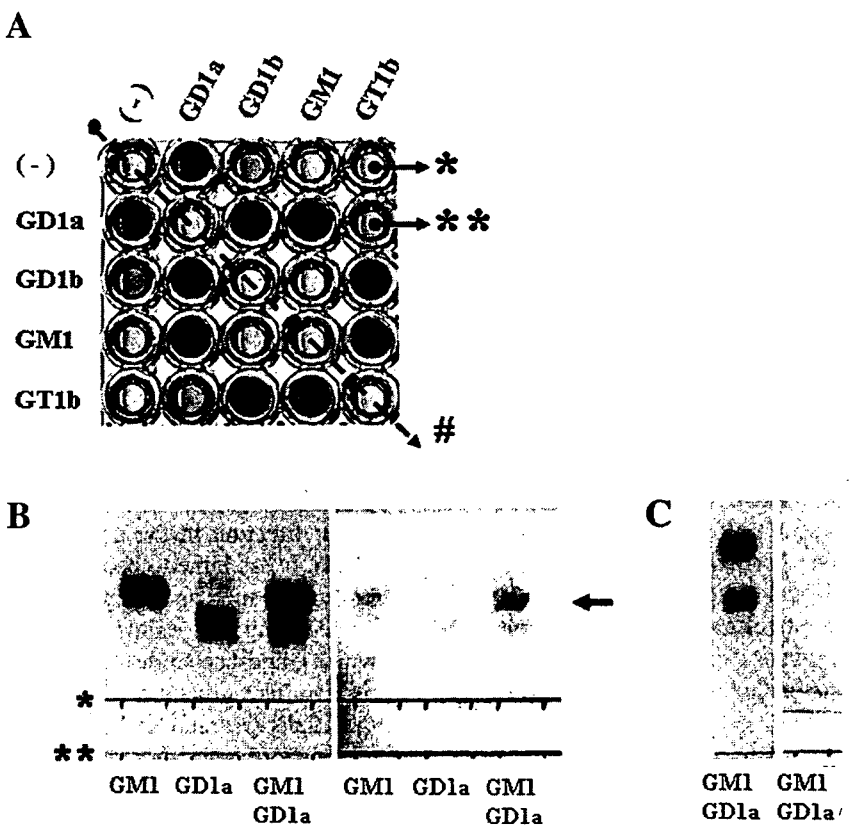

Fig. 2. Representative ELISA results and thin-layer chromatography (TLC) studies for antibodies to GSCs using sera from Anti-GSC(+) group patients. (A) Serum from patient no.16 in the Anti-GSC(+) group was used. A weak reaction is observed in a well containing only GD1a, while strong reactions can be seen in wells containing GD1a/GD1b, GM1/GD1a, GD1b/GT1b, and GM1/GT1b (w/w=0.1/0.1). *=GT1b only (0.2 μg), **=a mixture of GD1a (0.1 μg) and GT1b (0.1 μg). # Five control wells on each plate are indicated by oblique dotted arrows. (B) Serum from patient no.18 was used. TLC bands were visualized with orcinol reagent in the left panels. Immunostaining using anti-GSC antibody-positive sera (diluted 1:100 with 1% BSA in PBS) is shown in the right panels. Immunostaining is shown in the overlapping portions of GM1 and GD1a (arrow). GD1a was applied on the upper line (*) and GM1 on the lower line (**). (C) The immunostaining in the overlapping portions of GM1 and GD1a have disappeared in a solvent system that separated the positions of GM1 and GD1a to a great extent (serum from patient no.18). The developing solvent comprised chloroform, methanol, and 0.2%CaCl2.2H2O as follows; 55/40/10 (v/v, Fig. 2B), 50/45/10 (Fig. 2C).

Table 1
Frequency of antibodies to single gangliosides in anti-GSC(+) and control groups

	Antiganglioside antibody	Antibody specificity in anti-GSC(+) group*					
		GM1/GD1a (Anti-GSC(+))	GM1/GD1a GM1/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b	GM1/GD1a GM1/GT1b GD1b/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b GD1b/GT1b	Control
		(*n*=38)	(*n*=27)	(*n*=16)	(*n*=13)	(*n*=10)	(*n*=170)
IgG class	anti-GM1	6 (16%)	3 (11%)	3 (19%)	2 (15%)	1 (10%)	27 (16%)
	anti-GD1a	4 (11%)	4 (15%)	4 (25%)[#1]	4 (31%)[#2]	4 (40%)[#3]	10 (6.1%)
	anti-GD1b	28 (74%)[#4]	20 (74%)[#4]	8 (50%)[#3]	8 (62%)[#5]	5 (50%)[#1]	9 (5.5%)
	anti-GT1b	3 (7.9%)	3 (11%)[#6]	3 (19%)[#7]	3 (23%)[#8]	3 (30%)[#9]	3 (1.8%)
	anti-GQ1b	6 (16%)	5 (19%)	1 (6.3%)	1 (7.7%)	1 (10%)	15 (9.1%)
	anti-GalNAc-GD1a	7 (18%)	4 (15%)	1 (6.3%)	0	0	26 (16%)**
IgM class	anti-GM1	2 (5.3%)	2 (7.4%)	2 (13%)	2 (15%)	2 (20%)	21 (13%)
	anti-GalNAc–GD1a	1 (2.6%)	1 (3.7%)	0	0	0	15 (9.4%)**

* The Anti-GSC(+) group was divided into four subgroups based on antibody specficity of anti-GSC antibodies. In the GSC(+) group, IgG antibodies to GM2, GM3, and GD3 and IgM to GD1a, GD1b, GT1b, GQ1b, GM2, GM3, and GD3 were negative. # indicates that there are significant differences between an antibody-positive group and remainder. Remainders include control group patients and remaining anti-GSCantibody-positive patients. [#1], $p=0.02$; [#2], $p=0.009$; [#3], $p=0.003$; [#4], $p<0.0001$; [#5], $p=0.0004$; [#6], $p=0.03$; [#7], $p=0.007$; [#8], $p=0.004$; [#9], $p=0.002$ The chi-square test was used to test differences in proportions when the number in a cell was more than 6. If not, Fisher's exact test was used. **$n=166$ patients.

were major gangliosides in human nervous system (Yu and Iqbal, 1979). Gangliosides were mixed for 30 min before application to the ELISA. If a serum has both anti-GD1a and anti-GD1b antibody activities, the serum was judged to be anti-GD1a/GD1b antibody-positive when an OD value of anti-GD1a/GD1b antibody was more than the sum of those of anti-GD1a and anti-GD1b antibodies. As for estimation of antibodies to GM1/GD1a, GM1/GD1b, GM1/GT1b, GD1a/GT1b, and GD1b/GT1b, the same criteria were applied. ELISAs were performed in duplicate and mean ODs were calculated.

2.2. ELISA for anti-Campylobacter jejuni antibodies in serum from anti-ganglioside complex antibody-positive patients

IgG and IgM antibodies to *C. jejuni* were investigated in acute phase serum samples from anti-GSC antibody-positive patients with antecedent gastrointestinal infection using an ELISA kit for C. jejuni (Serion ELISA classic, Campylobacter jejuni IgG/IgM; Institut Virion/Serion, Würzburg, Germany). Serum from patients already diagnosed as having had *C. jejuni* enteritis from stool or serum specimens at other hospitals was excluded from the investigation. In cases where patients had positive or borderline ELISA results for IgM or IgG antibodies to *C. jejuni* and had suffered from diarrhea without respiratory symptoms, they were considered to have had antecedent gastrointestinal infection associated with *C. jejuni*.

2.3. Study population and analyses of clinical and electrophysiological features

Patients with IgG antibodies to at least one combination of two of the four gangliosides, GM1, GD1a, GD1b, and GT1b were defined as anti-GSC-positive patients (anti-GSC(+) group) and grouped according to the specificity of the anti-GSC antibodies. We asked the attending physicians to provide detailed clinical data for those subjects for whom clinical information was insufficient. Patients without data concerning neurological signs and symptoms were excluded from the clinical analysis. Anti-GSC-negative patients with detailed clinical data formed the control group. Patients' disabilities were graded using the Hughes Functional Grading Scale (Hughes et al., 1978). Electrophysiological data were evaluated as described previously and categorized as "primary demyelinating," "primary axonal," "unexcitable," "equivocal," or "normal" (Hadden et al., 1998; Kaida et al., 2001). Clinical features and electrophysiological findings were compared between the anti-GSC(+) and control groups.

2.4. Investigations into the specificity of anti-ganglioside complex antibodies

To investigate the specificities of anti-GSC antibodies in anti-GSC antibody-positive serum samples, immunoabsorption and ELISA were performed using mixtures of more than two gangliosides. Anti-GSC antibodies were absorbed on antigen-coated ELISA wells as described previously (Kaida et al., 2001).

2.5. Statistical analysis

Differences in proportions were tested by the Fisher exact probability test or the χ^2 test. The Mann–Whitney test was used for comparisons of age, onset to nadir (days), and peak disabilities among the groups. Two-tailed p-values <0.05 were considered significant throughout. These analyses were performed with SPSS software (12.0, SPSS Inc., Chicago).

3. Results

3.1. Study population

We collected serum from 234 consecutive GBS patients including 100 patients previously published (Kaida et al., 2004). ELISA showed that 39 (17%) of these patients had IgG antibodies to at least one GSC such as GD1a/GD1b, GM1/GD1a, GD1b/GT1b, GM1/GT1b, or GM1/GD1b. Among the 39 anti-GSC-positive patients, all had IgG anti-GM1/GD1a antibodies and 27 had anti-GM1/GT1b antibodies. Sixteen patients had anti-GD1a/GD1b antibodies, 13 had anti-GD1b/GT1b antibodies, and six had anti-GM1/GD1b antibodies. Ten patients had both anti-GD1a/GD1b and anti-GD1b/GT1b antibodies. Distribution of anti-GSC(+) group patients was described with a Venn diagram (Fig. 1). No patients in either group had anti-GD1a/GT1b antibodies. Eight of the 39 anti-GSC-positive patients were already reported in the previous study (Kaida et al., 2004). There were no serum IgM antibodies to GSCs in any of the 39 patients. Representative ELISA results and TLC immunostaining are shown in Fig. 2. Specific immunostaining was found on the overlapping portions of each ganglioside (Fig. 2B). One in the Anti-GSC(+) group was excluded from the analysis of clinical features because detailed clinical data were unavailable. Twenty-five of the 195 anti-GSC-negative patients also were excluded because insufficient clinical data were available; the remaining 170 patients served as the control group.

3.2. Frequencies of antibodies to single ganglioside antigens

Thirty-four of 39 patients in the anti-GSC(+) group had antibodies to single gangliosides (Table 1). There were no significant differences in the frequency of antibodies to single ganglioside antigens between anti-GSC(+) and the control groups, except for IgG anti-GD1b antibody. One of eight ventilated patients in the anti-GSC(+) group had IgG anti-GQ1b antibody. Although the frequencies of IgG antibodies to GD1a, GD1b, or GT1b were significantly higher in three subgroups (Table 1), their OD values were much lower than those of anti-GSC antibodies (data not

Table 2
Clinical features of GBS patients in anti-GSC(+) and control groups

	Antibody specificity in anti-GSC(+) group*					
	GM1/GD1a (Anti-GSC(+))	GM1/GD1a GM1/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b	GM1/GD1a GM1/GT1b GD1b/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b GD1b/GT1b	Control
Clinical features	(n=38)	(n=27)	(n=16)	(n=13)	(n=10)	(n=170)
Age**	43.2 (37.7–48.8)	45.3 (38.0–52.6)	43.8 (34.4–53.1)	48 (35.8–60.2)	47.1 (33.2–61.0)	48.9 (45.6–52.1) (n=169)
Sex male/female	25/13	18/9	12/4	9/4	7/3	103/67
Antecedent infection						
Respiratory	19 (50%)	12 (44%)	4 (25%)	3 (23%)	2 (20%)	76 (45%)
Gastro-intestinal	17 (45%)[#1] (*C. jejuni*: 12)	14 (52%)[#2] (*C. jejuni*: 9)	12 (75%)[#3] (*C. jejuni*: 8)	10 (77%)[#4] (*C. jejuji*: 6)	8 (80%)[#5] (*C. jejuni*: 5)	38 (22%)
Onset to nadir (days)**	7.8 (6.1–9.6) (n=33)	7.8 (5.4–10.2) (n=24)	9.2 (4.8–13.6) (n=13)	9.7 (5.2–14.1) (n=12)	10.4 (4.3–16.6) (n=9)	7.8 (7.0–8.7) (n=158)
Cranial nerve deficits	(n=35)	(n=25)	(n=15)			(n=156)
positive	22 (63%)	18 (72%)	11 (73%)	10 (77%)	9 (90%)[#6]	80 (51%)
III, IV, VI	12 (34%)[#6]	11 (44%)[#2]	5 (33%)	6 (46%)[#2]	5 (50%)[#7]	25 (16%)
VII	11 (31%)	9 (36%)	7 (47%)	5 (38%)	5 (50%)	57 (37%)
IX, X	17 (49%)	15 (60%)[#8]	10 (67%)[#9]	9 (69%)[#10]	8 (80%)[#11]	48 (31%)
XI, XII	9 (26%)[#12]	7 (28%)[#10]	6 (40%)[#11]	5 (38%)[#9]	4 (40%)[#6]	13 (8.3%)
Sensory loss	9 (26%)	9 (38%)	5 (36%)	5 (42%)	5 (56%)	61 (39%)
	(n=34)	(n=24)	(n=14)	(n=12)	(n=9)	(n=156)
Nerve conduction study	(n=26)	(n=18)	(n=9)	(n=7)	(n=6)	(n=46)
Demyelination	8 (31%)	5 (28%)	3 (33%)	2 (29%)	2 (33%)	18 (39%)
Axonal	6 (23%)	4 (22%)	3 (33%)	3 (43%)	2 (33%)	5 (11%)
Inexcitable	3 (12%)	3 (17%)	1 (11%)	1 (14%)	1 (17%)	4 (8.7%)
Artificial ventilation	8 (22%)	7 (27%)[#13]	6 (38%)[#9]	6 (46%)[#11]	5 (50%)[#14]	19 (11%)
	(n=36)	(n=26)				

* The Anti-GSC(+) group was divided into four subgroups based on antibody specficity of anti-GSC antibodies (see Fig 1). Anti-GM1/GD1b antibody-positive group is omitted in this table due to the small number of patients. ** mean (95% confidence interval) # indicates that there are significant differences between an antibody-positive group and remainder. Remainder include Control group patients and remaining anti-GSCantibody-positive patients. [#1], p=0.008; [#2], p=0.002, [#3], p<0.0001; [#4], p=0.0001; [#5], p=0.0005; [#6], p=0.02; [#7], p=0.03; [#8], p=0.006; [#9], p=0.009; [#10], p=0.01; [#11], p=0.003; [#12], p=0.007; [#13], p=0.03; [#14], p=0.004.

Table 3
Association of antibody specificities with peak disability

	Antibody specificity in anti-GSC(+) group					
	GM1/GD1a (Anti-GSC(+))	GM1/GD1a GM1/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b	GM1/GD1a GM1/GT1b GD1b/GT1b	GM1/GD1a GM1/GT1b GD1a/GD1b GD1b/GT1b	Control
Functional score	(n=36)	(n=26)	(n=16)	(n=13)	(n=10)	(n=170)
F-1	2 (5.6%)	1 (3.8%)	0 (0%)	0 (0%)	0 (0%)	6 (3.5%)
F-2	10 (28%)	7 (27%)	2 (13%)	3 (23%)	2 (20%)	48 (28%)
F-3	7 (19%)	3 (12%)	3 (19%)	1 (7.7%)	0 (0%)	42 (25%)
F-4	9 (25%)	8 (31%)	5 (31%)	3 (23%)	3 (30%)	54 (32%)
F-5	8 (22%)	7 (27%)	6 (38%)	6 (46%)	5 (50%)	19 (11%)
F-6	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.6%)
p value* (two-tailed)	0.63 vs. remainder** (n=170)	0.18 vs. remainder** (n=180)	0.008 vs. remainder** (n=190)	0.024 vs. remainder** (n=193)	0.012 vs. remainder** (n=196)	

*The Mann–Whitney test was used to compare functional score of two groups. **Remainder include Control group patients and remaining anti-GSC antibody-positive patients.

shown). TLC immunostaining using anti-GD1a/GD1b-positive sera showed no or little binding to GD1a or GD1b each, but intense binding to in the overlapping portion of GD1a and GD1b.

3.3. Antecedent Campylobacter jejuni infection in anti-ganglioside complex antibody-positive patients

Four patients in the anti-GSC(+) group had already been diagnosed as having antecedent *C. jejuni* enteritis at other hospitals. Of the remaining anti-GSC(+) group patients with antecedent gastrointestinal infection, eight were IgG anti-*C. jejuni* antibody-positive, one of whom was IgM anti-*C. jejuni* antibody-borderline. Overall, 12 Anti-GSC(+) group patients had antecedent *C. jejuni* enteritis (Table 2).

3.4. Clinical and electrophysiological features of anti-ganglioside complex-positive patients

Clinical features and electrophysiological findings in each group of patients are shown in Table 2. Results of statistical analysis of the clinical features are also shown in Table 2. Anti-GSC(+) group patients more frequently had antecedent gastrointestinal infections, ophthalmoplegia, and lower cranial nerve deficits. Among them, the anti-GD1a/GD1b or anti-GD1b/GT1b-positive patients had significantly higher F-scores at nadir and more frequently needed mechanical ventilation than the remaining subjects including control group patients (Table 3). Of 206 patients who had sufficient clinical data on peak disability, ventilated patients more frequently had IgG anti-GD1a/GD1b antibodies than unventilated ones (6/27, 22%, vs. 10/179, 5.6%) (two-tailed p value=0.009, odds ratio=4.85, χ^2 test). Anti-GD1b/GT1b antibodies also were more frequently found in ventilated patients (6/27, 22%, vs. 7/179, 3.9%) (two-tailed p value=0.003, odds ratio=7.02, χ^2 test). No patients required mechanical ventilation due to any respiratory disorder such as aspiration pneumonia.

In terms of the short-term outcome among patients with a peak F score of more than 2 and adequate follow-up data, improvement by one or more in the F score one month after the onset of GBS was found in 11 (65%) of 17 anti-GSC(+) group and 21 (68%) of 31 control group patients. In the anti-GSC(+) group patients, two (25%) of eight anti-GD1a/GD1b-positive patients and three (43%) of seven anti-GD1b/GT1b-positive patients improved by one or more in the F score one month after the onset of GBS. Anti-GD1a/GD1b-positive patients showed significantly poor recovery compared with the respective remainders (p=0.012, Fisher exact probability). There were no significant differences in treatment between the two groups (Table 4). In this study, because electrophysiological and clinical following-up data were insufficient to statistically compare between anti-GSC(+) and control groups, we could not confirm association of anti-GSC antibodies with electrophysiological results and poor outcome.

Table 4
Therapy in anti-GSC(+) and control groups

Treatment	Anti-GSC(+)	Control
	n=17	n=31
IVIG	10	17
PP	2	3
Combination (IVIG+PP)	3	3
Combination (IVIG+steroid)	2	4
Others	0	4[a]

IVIG = intravenous immunoglobulin.
PP = plasmapheresis, including plasma exchange and immunoadsorption therapy.
[a] Includes prednisolone (1), not done (2), and unknown (1) There were no significant differences in treatment between the two groups.

Table 5
Immunoabsorption study of anti-GSC antibodies with GSCs and single ganglioside antigens using serum from patient No.10

Antigens used for absorption[a]		Absorption rate			
		In anti-GD1a/GD1b antibody activity	In anti-GM1/GD1a antibody activity	In anti-GD1b/GT1b antibody activity	In anti-GM1/GT1b antibody activity
GSCs					
(−)	(Corrected OD)	0.48	0.60	0.67	0.92
GD1a/GD1b		100%	58%	76%	73%
GM1/GD1a		100%	100%	100%	100%
GD1b/GT1b		100%	57%	100%	72%
GM1/GT1b		100%	76%	81%	100%
Single ganglioside antigens					
(−)	(Corrected OD)	0.40	0.68	0.80	0.77
GM1		0%	0%	0%	0%
GD1a		35%	0%	37%	13%
GD1b		58%	5%	20%	11%
GT1b		0%	1%	14%	0%

GSCs = ganglioside complexes.
Uncoated wells served as controls. The serum from the patient No.10 was diluted 1:80 with 1% BSA in PBS.
[a] Each microtiter plate well was coated with a single ganglioside (0.25 μg) or a mixture of 0.2 μg of each ganglioside.

3.5. Immunoabsorption test

Immunoabsorption testing was done with serum from patient no.10 in the anti-GSC(+) group. The serum sample had IgG antibodies to GD1b (a corrected OD value was 0.16 when the serum was diluted 1:40) in addition to anti-GSC antibodies. Results of the immunoabsorption test are shown in Table 5. The serum antibodies reacted more specifically with GSCs than with single ganglioside antigens.

4. Discussion

The present study has shown that 17% of patients with GBS have IgG antibodies to at least one GSC such as GD1a/GD1b, GM1/GD1a, GD1b/GT1b, GM1/GT1b, and GM1/GD1b. The anti-GSC(+) group patients presented more frequently preceding gastrointestinal infection, ophthalmoplegia, and lower cranial nerve deficits than control group patients, although a selection bias in a control group may have influenced the results to some extent. A close association between the presence of anti-GD1a/GD1b and/or anti-GD1b/GT1b antibodies and a need for mechanical ventilation suggest that these antibodies may be useful predictors of a need for mechanical ventilation and a severe course of GBS.

A mixture of GM1 and a phospholipid such as phosphatidic acid, phosphatidylinositol, or phosphatidylserine, intensifies antiganglioside antibody activity in GBS serum (Kusunoki et al., 2003; Hirakawa et al., 2005). Anti-GM1 antibodies, however, appeared not to react with new epitopes generated by mixtures of phospholipids and GM1 because they had no activity toward phospholipids alone, whereas anti-GSC antibodies are likely to react with new epitopes in clustered gangliosides because of the weak activity toward individual ganglioside antigens. Anti-GSC(+) antibodies rarely bound toGM1/GD1b and never to GD1a/GT1b, suggesting that an epitope formed by a combination of [Galβ1–3GalNAc] and [NeuAcα2–3Galβ1–3GalNAc] in the terminal residues of gangliotetraose structures is important for the antibody binding (Fig. 3). When mixtures of three or four gangliosides (GM1, GD1a, GD1b, GT1b) were used as antigens in ELISA, anti-GSC antibody activity was often decreased (data not shown), supporting that epitopes targeted by anti-GSC antibodies are formed in a mixture of two gangliosides and have new specific conformations. We believe that a mixture of two gangliosides is an appropriate antigen for the surveillance of anti-GSC antibodies. The examination of serum antibodies to such complex antigens as GSCs may increase the spectrum of anti-ganglioside antibodies in GBS, enhancing their value as diagnostic markers.

Anti-GD1a/GD1b-and/or anti-GD1b/GT1b-positive patients were more likely to have a poor outcome and be

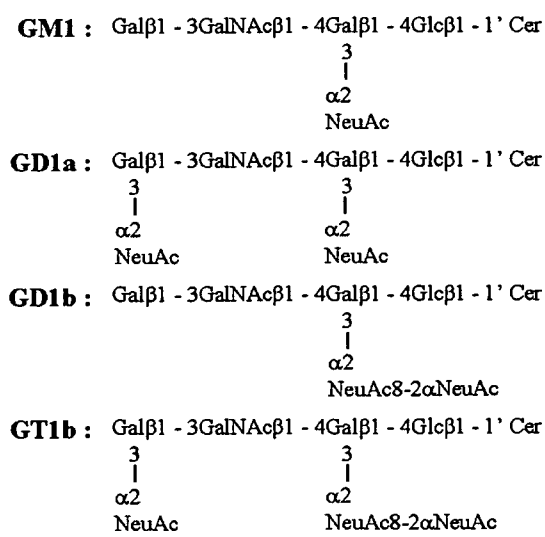

Fig. 3. Carbohydrate structures of GM1, GD1a, GD1b, and GT1b.

refractory to therapy than remaining subjects including control group patients, although more follow-up data are required to confirm the long-term prognosis. Why the anti-GD1a/GD1b-or anti-GD1b/GT1b-positive patients present a severe form of GBS is unclear. The different specificities of the anti-GSC antibodies might influence the peak disability and prognosis in anti-GSC antibody-positive patients. Most of anti-GD1a/GD1b-or anti-GD1b/GT1b-positive sera also react with GM1/GD1a and GM1/GT1b, suggesting that these sera are more multivalent than the antibodies reacting only with GM1/GD1a or GM1/GT1b, or with single ganglioside antigen. Tight binding between such multivalent antibodies and clustered glycoepitopes may correlate with a predisposition to a severe form of the disease.

Antecedent infection plays a critical role in the pathogenesis of GBS. Specific bacterial genes and ganglioside-mimicking structures in LOS of pathogens causing antecedent infection have proven to be essential for the induction of anti-ganglioside antibodies and determine antibody specificities (Ang et al., 2002; Yuki et al., 2004; Godschalk et al., 2004; Koga et al., 2005). Various ganglioside complex-like structures presumably exist in LOS of organisms causing antecedent infection. Cell wall glycoconjugates of *C. jejuni* might share epitopes with the GSCs such as GM1/GD1a or GD1a/GD1b, leading to the induction of anti-GSC antibodies.

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