against ganglioside complex may therefore directly induce nerve conduction failure and severe disability in GBS. More study is needed to elucidate the roles of antiglycolipid antibodies in the pathogenetic mechanisms of autoimmune neuropathies.

INTRODUCTION

In immune-mediated neuropathies such as Guillain-Barré syndrome (GBS) and IgM paraproteinemic neuropathy, antiglycolipid antibodies, which specifically recognize the carbohydrate portion of the glycolipids, are frequently present in the patients' sera. There are diversities in the carbohydrate sequences of the glycolipids (figure 1). Each glycolipid has a unique distribution within the peripheral nervous sysytem. Those antibodies can be used as useful markers for diagnosis. In addition, considering that the glycolipids are localized in the plasma membrane with their carbohydrate portions extended to the extracellular spaces, they may be involved in the pathogenetic process.

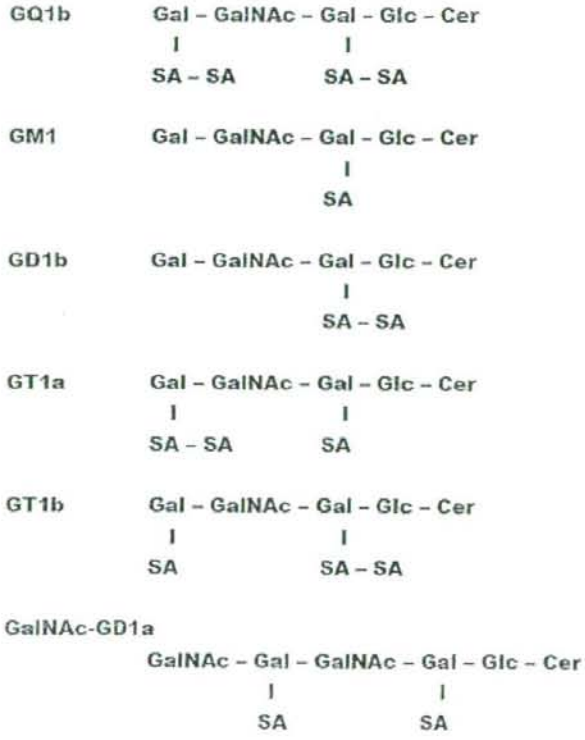

Figure 1. Carbohydrate sequences of gangliosides; Gal: galactose, GalNAc: N-acetylgalactosamine, Glc: glucose; SA: sialic acid, Cer: ceramide, SA-SA: disialosyl residue.

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.

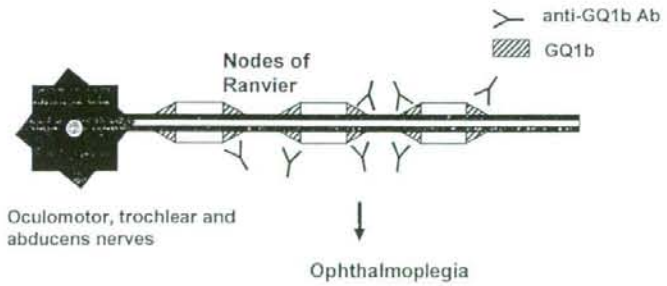

Figure 2. Specific binding of the anti-GQ1b IgG to the paranodal myelin of the cranial nerves innervating extraocular muscles may cause ophthalmoplegia in MFS and GBS.

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).

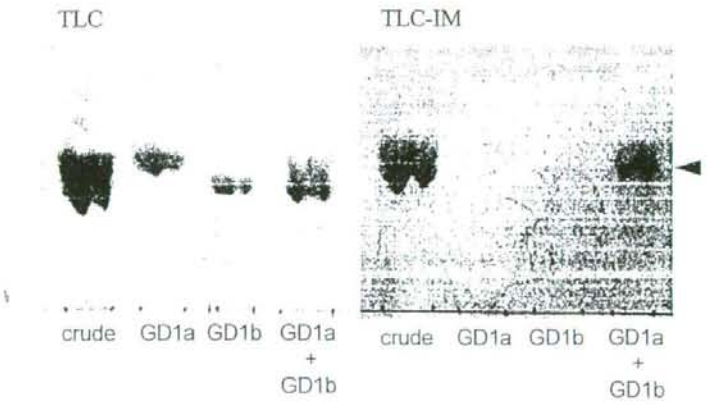

Figure 3. TLC-immunostaining with anti-GD1a/GD1b-positive sera; Positive staining was present in the lane in which both GD1a and GD1b were developed, but not in the lanes in which GD1a or GD1b was developed respectively.

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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LETTER TO THE EDITOR

ABNORMAL SUDOMOTOR AXON REFLEX AND ANTIGANGLIOSIDE ANTIBODIES

Antiganglioside antibodies are frequently present in sera from patients with such autoimmune neuropathies as Guillain–Barré syndrome (GBS) and Miller Fisher syndrome (MFS). Autonomic dysfunction sometimes occurs in autoimmune neuropathies,[6] but its relationship to antiganglioside antibodies is unknown. The quantitative sudomotor axon reflex test (QSART), which can evaluate the postganglionic sudomotor axon quantitatively,[4] can provide a measure of autonomic dysfunction. To investigate the relationship between antiganglioside antibodies and autonomic dysfunction in GBS or MFS, we performed QSART in 15 such patients, and compared the severity of QSART with the results of the antiganglioside antibody assay.

We investigated 6 MFS and 9 GBS patients admitted to our hospital between 1992 and 2004. None of the patients had other diseases affecting sweating function, such as diabetes mellitus. QSART was performed in the left distal leg and foot.[4] The severity of QSART abnormality was rated by the age- and gender-matched sudomotor subscore of the Composite Autonomic Scoring Scale[1] (CASS-sud) in which 0 indicates no deficit and 3 represents maximal deficit. IgM and IgG antibodies against GM1, GM2, GM3, GD1a, GD1b, GD3, GT1b, GQ1b, GA1, and Gal-C in patients' sera were investigated by enzyme-linked immunosorbent assay (ELISA).[3] Fisher's exact test was performed and $P < 0.05$ was considered statistically significant. The patients consented to participate in this study, which had the approval of our institutional review board.

CASS-sud scores were 2 or 3 in 5 of the 15 patients, and 1 or 0 in the remaining 10 patients. Among those 5 patients with high CASS-sud scores, 4 had anti-GQ1b IgG antibodies and 1 had IgM and IgG antibodies against both GM1 and GD1b (Table 1). However, only 1 of the 10 patients with a low CASS-sud demonstrated anti-GQ1b IgG antibody. Occurrence of IgG anti-GQ1b antibody was more frequent in patients with a CASS-sud of 2 or more ($P < 0.05$). High anti-GQ1b titer (++ or +++) was associated with high (3) CASS-sud score, but larger numbers of patients are necessary for statistical analysis.

Table 1. Relationship between CASS-sud and serum antiganglioside antibodies.

Age (years)	Gender	Diagnosis	Antiganglioside antibodies	CASS-sud
52	M	MFS	GQ1b (IgG)++	3
26	M	MFS	GQ1b (IgG)+++	3
22	F	MFS	GQ1b (IgG)+	2
42	F	MFS	GQ1b (IgG)+	2
20	M	GBS	GM1(IgM)+,(IgG)+, GD1b(IgM)+,(IgG)+	2
67	F	GBS	GD1b(IgG)+++	1
28	F	GBS	GM1(IgM)+,(IgG)+	1
13	M	MFS	GQ1(IgG)+	0
57	M	GBS	GM1(IgM)++,(IgG)+	0
21	M	MFS	–	0
26	M	MFS	–	0
29	M	MFS	–	0
22	M	GBS	–	0
17	F	GBS	–	0

Antibody titers are expressed semiquantitatively according to the OD value of the ELISA. OD <0.1 is negative –; + is 0.1–0.3; ++ is 0.3–0.5; and +++ is >0.5. Occurrence of IgG anti-GQ1b antibody was significantly more frequent in patients with a QSART score ≥2 ($P < 0.05$). GBS, Guillain–Barré syndrome; MFS, Miller Fisher syndrome.

Because other autonomic tests such as head-up tilt or the Valsalva maneuver could not be performed in patients with severe weakness, we focused our attention on the QSART in this study. We have shown an association between postganglionic sudomotor dysfunction and anti-GQ1b antibodies. Birklein et al. showed that local injection of botulinum toxin type A reduced focal hyperhidrosis.[1] Botulinum toxin A–G shows a high binding affinity to GQ1b.[2] Anti-GQ1b antibodies therefore might also decrease the activity of postganglionic sudomotor axons by binding to GQ1b as does botulinum toxin. Plomp et al. reported human anti-GQ1b antibodies bind to mouse neuromuscular junctions to induce acetylecholine release from nerve terminals and eventually to block neuromuscular transmission.[5] IgG antibodies against GQ1b therefore might induce a transmission block at the neuroglandular junction by a similar mechanism as at the neuromuscular junction.

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PAPER

Anti-ganglioside complex antibodies in Miller Fisher syndrome

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Background: Some ganglioside complexes (GSCs) are target antigens for serum antibodies in patients with Guillain–Barré syndrome (GBS). Anti-GSC antibodies may be associated with particular clinical features of GBS.
Objective: To investigate antibodies to GSCs in the sera of patients with Miller Fisher syndrome (MFS) characterised by elevation of the IgG anti-GQ1b antibody.
Results: In all, 7 of 12 (58%) consecutive patients with MFS were found to have IgG antibodies to GSCs containing GQ1b, of whom 5 had IgG antibodies to GQ1b-GM1 complex (GQ1b/GM1) and 2 had antibodies to GQ1b/GD1a; 4 of 5 patients without sensory symptoms had anti-GQ1b/GM1 antibodies.
Conclusions: At least three different specificities in MFS-associated antibodies, GQ1b-specific, anti-GQ1b/GM1-positive and anti-GQ1b/GD1a-positive, were observed. In patients with MFS not only GQ1b itself but also clustered epitopes of GSCs, including GQ1b, may be considered to be prime target antigens for serum antibodies. A tendency to escape sensory disturbances is shown by anti-GQ1b/GM1-positive MFS.

We recently reported that some ganglioside complexes (GSCs) are target antigens for serum antibodies in patients with Guillain–Barré syndrome (GBS), an acute immune-mediated polyradiculoneuropathy, and suggested that anti-GSC antibodies may be associated with particular clinical features of GBS.[1] Because glycolipids including gangliosides tend to form clustered complexes with cholesterols in lipid rafts in the plasma membrane,[2] anti-GSC antibodies are likely to cause nerve dysfunction through binding to GSCs in lipid rafts in neuronal membranes.

Miller Fisher syndrome (MFS) is characterised by a clinical triad of ophthalmoplegia, ataxia and areflexia, and is considered to be a variant of GBS.[3] The presence of the IgG anti-GQ1b antibody in serum is an excellent diagnostic marker for MFS.[4] This antibody often cross reacts with GT1a[4 5] and is pathophysiologically associated with ophthalmoplegia or ataxia in MFS and GBS.[5–7] Thus, MFS is a clinically and serologically well-defined syndrome with a pathophysiological mechanism similar to that of GBS, which suggests that patients with MFS may also have anti-GSC antibodies. Here, we examined the serum samples of patients with MFS and found antibodies specific for a mixture of two gangliosides, including GQ1b or GT1a.

METHODS

ELISA for anti-GSC antibodies in serum from patients with MFS

Antibodies to GSC were investigated in acute-phase serum samples collected from consecutive patients with MFS, who were diagnosed at the National Defense Medical College hospital, Saitama-Ken, Japan, between April 1994 and December 2004. The diagnosis of MFS was based on acute self-limited ophthalmoplegia, ataxia and areflexia without marked limb weakness, the involvement of CNS or other neurological diseases. The ELISA was carried out for antibodies to the gangliosides GM1, GM2, GD1a, GD1b, GT1a, GT1b and GQ1b, as described previously.[8 9] When the corrected optical density was >0.1, the serum was considered to be positive. The ELISA for anti-GSC antibodies was carried out as described in our previous report.[1] GSCs used in the ELISA contained two of the above seven ganglioside antigens. Gangliosides were mixed for 30 min before their application to the ELISA. Anti-GSC antibody-positive samples were overlaid for thin-layer chromatography immunostaining, as described previously,[1] and the clinical features of anti-GSC antibody-positive patients with MFS were analysed. The above procedures were carried out at room temperature.

Immunoabsorption of anti-GSC antibody-positive serum samples

Anti-GSC antibodies were absorbed in antigen-coated ELISA wells, as described previously.[8 9] Ganglioside antigens used for the absorption test were GSCs, a mixture of two gangliosides (250 ng each) or 500 ng of each ganglioside. Uncoated wells were used as controls. Anti-GSC antibody-positive serum diluted 1:40 with 1% bovine serum albumin in phosphate-buffered saline was used, and the residual activities of the supernatants on the GSCs were estimated with ELISA. The percentage absorption of anti-GSC antibody activity was calculated as described previously.[9]

RESULTS

Anti-ganglioside antibody assay and representative serum data

Acute-phase serum samples were collected from 12 patients with MFS, 10 (83%) of whom had IgG anti-GQ1b antibodies. The results from the ELISA showed that 7 of the 12 (58%) patients had serum antibodies to GSCs, such as GQ1b/GM1, GQ1b/GD1b, GQ1b/GD1a, GT1a/GM1, GT1a/GD1b, GT1a/GD1a and GQ1b/GT1b (table 1), but not to GSCs without GQ1b or GT1a. Antibodies to GQ1b/GM1, GT1a/GM1 and GT1a/GD1b were frequent. One patient (patient 7) had no

Abbreviations: GBS, Guillain–Barré syndrome; GSC, ganglioside complex; MFS, Miller Fisher syndrome

Table 1 Anti-ganglioside complex antibodies in 12 consecutive patients with Miller Fisher syndrome

Patient number	Age (year)	Sex	Involved cranial nerves*	Sensory signs†	Corrected OD									Other anti-glycolipid antibodies
					Anti-GQ1b	Anti-GT1a	Anti-GQ1b/GM1	Anti-GQ1b/GD1b	Anti-GT1a/GM1	Anti-GT1a/GD1b	Anti-GQ1b/GD1a	Anti-GT1a/GD1a	Anti-GQ1b/GT1b	
1	32	M		+	0.13	0.72	(−)	(−)	(−)	(−)	(−)	(−)	(−)	
2	65	F	7	+	0.12	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	
3	38	M		+	0.86	0.55	(−)	(−)	(−)	(−)	(−)	(−)	(−)	
4	68	F		++‡	0.85	0.74	(−)	(−)	(−)	(−)	(−)	(−)	(−)	
5	27	M		+	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	
6	56	F		+	0.55	(−)	0.79	(−)	0.28	0.10	(−)	(−)	(−)	
7	12	F		−	(−)	(−)	0.11	(−)	0.55	(−)	(−)	(−)	(−)	
8	64	M	7	−	0.42	(−)	0.67	(−)	0.70	0.39	(−)	(−)	(−)	
9	69	M		−	0.77	(−)	1.08	(−)	1.02	0.56	(−)	(−)	(−)	
10	38	F		−	0.11	(−)	0.70	0.63	0.84	0.60	(−)	(−)	(−)	
11	35	M		++‡	0.20	(−)	(−)	(−)	(−)	(−)	0.40	0.22	0.42	
12	52	F	9, 10	+	1.28	1.76	(−)	(−)	(−)	(−)	1.56	(−)	1.68	IgM: GM1

OD, optical density; (−), antibody negative.
ELISAs were repeated twice, and the mean OD of the two experiments was calculated.
*Affected cranial nerves other than 3, 4, and 6. Because of the lack of anti-GT1a and GD1b in patient 6 and anti-GQ1b and GM1 in patient 7, anti-GT1a/GD1b and anti-GQ1b/GM1 in these patients were considered to be positive.
†Criteria for sensory signs: −, no sensory signs or symptoms; +, only paraesthesia or dysaesthesia; and ++, sensory deficits.
‡Deep sensory disturbance.

anti-GQ1b or anti-GT1a antibodies, but had antibodies to GQ1b/GM1 and GT1a/GM1. In contrast with anti-GSC antibodies in GBS, no antibodies to the GSCs consisting of two of the four major gangliosides, GM1, GD1a, GD1b and GT1b, were found in patients with MFS.

The specificity of the anti-GSC antibodies was investigated with a representative serum sample from an anti-GSC antibody-positive patient with MFS (patient 10). An ELISA showed that the serum had IgG antibody activities for GQ1b/GM1, GQ1b/GD1b, GT1a/GM1 and GT1a/GD1b, but little activity against GQ1b and GT1a (fig 1A–C). Thin-layer chromatography studies also showed specific immunoreactivity against the overlapping portion of two gangliosides, including GT1a/GM1, GQ1b/GM1 and GT1a/GD1b (fig 1D,E).

An immunoabsorption study with serum from patient 10 showed that the serum antibodies were specifically immunoreactive with GQ1b/GM1 and GT1a/GM1, but not with GM1 or GT1a, although the antibodies appeared to cross react with GQ1b. The percentage absorption of anti-GQ1b/GM1 antibody activity was 1.8% by the GM1 antigen, 43% by GQ1b, 8.8% by GT1a, 96% by GQ1b/GM1 and 83% by GT1a/GM1. The percentage absorption of anti-GT1a/GM1 antibody activity was 7.6% by GM1, 47% by GQ1b, 8.5% by GT1a, 94% by GQ1b/GM1 and 70% by GT1a/GM1.

Fine specificity of anti-GSC antibodies and clinical features of anti-GSC antibody-positive patients with MFS

The ELISA showed that the fine specificity of serum antibodies in patients with MFS was heterogeneous (table 1). 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. On the basis of the presence of anti-GSC antibodies, the 12 patients with MFS could be subdivided into the three groups: anti-GSC negative (patients 1–5), anti-GQ1b/GM1 positive (patients 6–10) and anti-GQ1b/GD1a positive (patients 11 and 12; table 1).

Sensory signs were infrequent in patients with MFS with antibodies to GQ1b/GM1 and GT1a/GM1 (table 1), but otherwise there were no remarkable differences in clinical features between anti-GSC antibody-positive and antibody-negative patients with MFS. All the patients had antecedent respiratory infections: *Haemophilus influenzae* was identified from a throat swab of patient 4 and influenza B virus was serologically proved to be a pathogen in the antecedent infection of patient 7.

DISCUSSION

This study confirmed that the anti-GQ1b antibody is a useful marker for MFS, but the fine specificity of anti-ganglioside antibodies in MFS was more diverse than expected. Antibodies to GSCs containing GQ1b or GT1a, and anti-GQ1b and anti-GT1a antibodies, may be crucial for the development of MFS. Antecedent respiratory infection in patients with MFS may be associated with production of antibodies to GSCs containing GQ1b or GT1a.

According to the results of the ELISA, serum antibodies in five patients (6–10) most strongly bound to GQ1b/GM1 and GT1a/GM1. Hence, a combination of [Galβ1–3GalNAc] and [NeuAcα2–8NeuAcα2–3Galβ1–3GalNAc] in the terminal residues of gangliotetraose structures may be important as an antigenic epitope for those anti-GSC antibodies (fig 1F). Serum antibodies in patients 11 and 12 bound most strongly to GQ1b/GD1a and GQ1b/GT1b, and a combination of [NeuAcα2–3Galβ1–3GalNAc] and [NeuAcα2–8NeuAcα2–3Galβ1–3GalNAc] in the terminal residues may be a target antigen in those patients. On the other hand, sera from patients 1–4 had antibodies to GQ1b or GT1a but not to GSCs, and showed attenuation of these activities on addition of another ganglioside antigen, suggesting that the serum antibodies had monospecific activities to GQ1b or GT1a. Thus, there are at least three different specificities in MFS-associated antibodies. Such differences in antibody specificity seem to have some influence on clinical features in MFS, because sensory signs were infrequent in patients with MFS with antibodies specific to GQ1b/GM1 and GT1a/GM1, but were commonly seen in patients with other antibodies. The clinical relevance of such anti-GSC antibodies, however, needs to be investigated in a larger number of patients with MFS before firm conclusions can be drawn.

Much evidence has indicated a pathophysiological role for the IgG anti-GQ1b antibody in the development of MFS. Immunohistochemical, ex vivo or in vitro studies with monoclonal anti-GQ1b antibody have shown not only specific localisation of GQ1b in the human peripheral nerves but also the neuroparalytic action of the anti-GQ1b

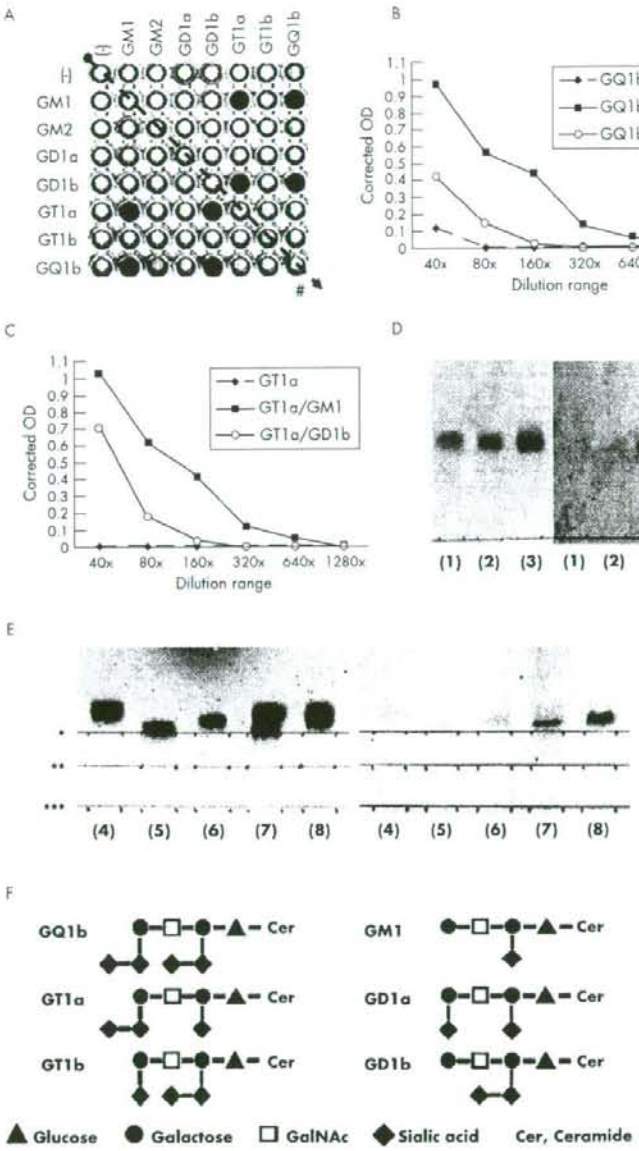

Figure 1 ELISA and thin-layer chromatogram (TLC) immunostaining of the serum of patient 10. (A) In the ELISA, reactions in wells containing GQ1b/GM1, GQ1b/GD1b, GT1a/GM1 and GT1a/GD1b (w/w = 0.1/0.1) were much stronger than those in wells containing only GQ1b. The number of control wells (#) on the plate is indicated by oblique dotted arrows. (B,C) Serum in the ELISA was diluted serially from 1:40 to 1:1280 and the antibody titres were as follows: IgG anti-GQ1b/GM1, 1:320; anti-GQ1b/GD1b, 1:80, anti-GQ1b, 1:40; anti-GT1a/GM1, 1:320; anti-GT1a/GD1b, 1:80, and anti-GT1a, negative results. (D,E) In TLC immunostaining, the developing solvent consisted of chloroform, methanol and 0.2% $CaCl_2 \cdot 2H_2O$ (50:45:10, v/v). The following ganglioside antigens were developed in each lane: (1) GT1a (2 μg), (2) GD1b (2 μg), (3) GT1a and GD1b (2 μg each), (4) GM1 (2 μg), (5) GT1a (2 μg), (6) GQ1b (2 μg), (7) GT1a and GM1 (2 μg each) and (8) GQ1b and GM1 (2 μg each). The start line for each antigen is indicated by asterisks: (*) for GQ1b, (**) for GT1a and (***) for GM1. Serum was diluted 1:100. Peroxidase-conjugated goat anti-human IgG Fc antibody (ICN Biomedicals, Aurora, Ohio, USA; diluted 1:200) was used as the secondary antibody. Left panel: TLC results visualised by orcinol reagent; right panel: TLC immunostaining. Positive immunostaining in lane (3) on the right panel indicates an antibody reaction to the GT1a–GD1b complex (GT1a/GD1b) (D). Positive staining in lanes (7) and (8) on the right panel indicates antibody reactions to GT1a/GM1 and GQ1b/GM1, respectively (E). Antibody reactions to GT1a, GD1b, GM1, GT1a and GQ1b were negative. (F) Carbohydrate structures of GQ1b, GT1a, GD1b, GM1, GT1b and GD1a are shown. OD, optical density.

antibodies, such as conduction block at motor nerve terminals.[3 9 10-11] Similar studies with antibodies monospecific to GSCs comprising GQ1b and GT1a may be useful for elucidation of the pathogenetic mechanisms of MFS.

The characteristic formation of clusters of gangliosides in the plasma membrane may result in anti-GSC antibodies causing nerve dysfunction more efficiently than monospecific anti-GQ1b antibody. Whether anti-GSC and anti-GQ1b antibodies bind to identical sites in neuronal membranes remains unclear, and future investigations on the localisation and possible roles of GSCs in the plasma membrane are required to deal with this issue.

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