剤が使用されていることだけを診断根拠にしてはならない．薬剤性ニューロパチーは見逃されることも多いが，一方で安易な根拠でoverdiagnosisされやすいことを再度強調したい．

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Review article

# Antibodies to gangliosides and ganglioside complexes in Guillain–Barré syndrome and Fisher syndrome: Mini-review

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ABSTRACT

Antiganglioside antibodies play a pathogenic role in the pathophysiology of Guillain–Barré syndrome (GBS) and Fisher syndrome (FS). Antiganglioside antibody-mediated nerve injury is likely to result from nerve damage through complement activation or dysfunction of molecules such as voltage-gated sodium and calcium channels. Clustered epitopes of complexes of two gangliosides in the cell membrane can be targeted by serum antibodies in GBS and FS and may regulate the accessibility and avidity of antiganglioside antibodies. The glycolipid environment or the specific distribution of target gangliosides in the peripheral nervous system may also influence the pathogenic effect of antiganglioside antibodies in GBS and FS. Structural and functional analyses of glycoepitopes of ganglioside complexes in membranes will provide new vistas on antibody–antigen interaction in GBS and shed light on microdomain function mediated by carbohydrate–carbohydrate interactions, which may lead to novel treatments for GBS and FS.



**Contents**



## 1. Introduction

Gangliosides are *N*-acetylneuraminic acid (sialic acid)-bearing glycosphingolipids that are concentrated in the outer leaflet of neuronal membranes with exposure of their oligosaccharides on the cell surface (Hakomori, 2000). Gangliosides are believed to reside in clusters within membrane microdomains that are referred to as lipid rafts or detergent-resistant membranes, together with other sphingolipids, cholesterol, and glycosylphosphatidylinositol (GPI)-anchored proteins (Simons and Toomre, 2000). Through molecular interactions with plasma membrane proteins at cell surfaces, the ganglioside glycans are involved in cell adhesion and intracellular

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signaling, myelin–axon interactions via Siglec (sialic acid-binding immunoglobulin-like lectin)-4, modulation of natural killer cell function, and inflammation through E-selectin, as addressed in a recent review (Lopez and Schnaar, 2009).

Gangliosides in the peripheral nervous system (PNS) can be targeted by serum antibodies in acute immune-mediated polyradiculoneuropathy, including Guillain–Barré syndrome (GBS) and variants such as Fisher syndrome (FS) (Chiba et al., 1992; Willison and Yuki, 2002); however, the pathogenic action of antiganglioside antibodies is not ubiquitously exerted in the PNS. Antiganglioside antibody-mediated nerve injury originates from antibody binding at specific loci in peripheral nerves, and is fundamentally regulated by antibody specificity and the specific distribution of target gangliosides (Chiba et al., 1993; Willison and Yuki, 2002; Kaida et al., 2009). Single ganglioside antigens have hitherto been utilized for conventional ELISA screening of antiganglioside antibodies. Recent studies have shown the presence of serum antibodies to ganglioside complexes (GSCs) consisting of two different gangliosides in GBS and FS (Kaida et al., 2004, Kaida et al., 2006, 2007), thereby emphasizing the significance of screening for antibodies to GSCs. Anti-GSC antibody-positive sera have no or little reactivity with constituent gangliosides, indicating that the sera react specifically with clustered glycoepitopes of GSCs. In this review we highlight the clinical and immunobiological aspects of the pathogenic action of antibodies to gangliosides and GSCs.

## 2. Correlation of clinical features with antiganglioside antibodies

Antiganglioside antibodies are often closely associated with clinical phenotype and specific symptoms (Willison and Yuki, 2002). This association is likely to depend upon the diverse distribution of ganglioside antigens in the peripheral nervous system.

### 2.1. Pure motor variant of GBS

The pure motor variant of GBS is characterized by no sensory loss, sparing of the cranial nerves, and predominant distal weakness, with frequent electrodiagnostic findings of acute motor axonal neuropathy (AMAN). This clinical phenotype is closely associated with antibodies to gangliosides such as GM1, GalNAc-GD1a, GD1a, and GM1b (Visser et al., 1995; Jacobs et al., 1996; Hao et al., 1999; Ang et al., 1999; Ho et al., 1999; Kaida et al., 2000; Yuki et al., 2000). The precise localization of GM1-like epitopes targeted by pathogenic anti-GM1 antibodies in human peripheral nerves has yet to be revealed, but recent analyses of a rabbit model of AMAN indicated that GM1 antigens are distributed at the nodes of Ranvier in motor nerves (Yuki et al., 2001; Susuki et al., 2003; Yuki et al., 2004). Using β1,4-N-acetylgalactosaminyltransferase (GalNAcT; GM2/GD2 synthase)-knockout mice, GM1 has been shown to play a role in maintaining the paranodal architecture and clusters of voltage-gated sodium channels (Susuki et al., 2007a).

GalNAc-GD1a is a minor ganglioside in the human brain and peripheral nerves (Svennerholm et al., 1973; Ilyas et al., 1988). An immunohistochemical study using rabbit anti-GalNAc-GD1a antibodies revealed that GalNAc-GD1a localizes in the vicinity of the nodes of Ranvier in human motor nerves, especially in the nodal and paranodal axolemmae (Kaida et al., 2003). An inner part of compact myelin and a periaxonal axolemma in the intramuscular nerves are also candidates for the target region of the anti-GalNAc-GD1a antibody (Kaida et al., 2003). Human motor and sensory nerves both contain GD1a, but the precise location of GD1a is unknown. As described below, structural differences of glycoepitopes of GD1a between motor and sensory nerves may explain the predisposition of the motor nerves for selective breakdown. The anti-GD1a antibody inhibits regeneration of damaged peripheral nerves, inducing delayed or poor recovery in patients with AMAN (Lehmann et al., 2007).

The tissue localization of GM1b in human PNS also remains to be determined. One study showed that among GBS patients with IgG anti-GM1b antibodies, 36% had IgG anti-GalNAc-GD1a antibodies and 32% had anti-GM1 antibodies, but the anti-GM1b antibodies were not associated with development of AMAN (Kusunoki et al., 1996a). In a collaborative study performed in Japan and the Netherlands, 56% of anti-GM1b-positive GBS patients had anti-GM1 antibodies and suffered from pure motor neuropathy, but there was no correlation between the presence of anti-GM1b antibodies and electrodiagnostic findings indicative of axonal neuropathy (Yuki et al., 2000).

### 2.2. Other phenotypes of GBS

IgG anti-GQ1b antibody has been identified as a diagnostic marker and a pathogenic factor in FS, and is often cross-reactive with GT1a (Chiba et al., 1992, 1993; Kusunoki et al., 1999b). An immunohistochemical investigation showed that GQ1b is densely localized in the paranodal regions of cranial nerves innervating the extraocular muscles and in a subpopulation of large neurons in dorsal root ganglia. Nerve terminals inside muscle spindles and in touch with intrafusal fibers can also be targeted by antibodies to GQ1b, GT1a, and GD1b (Liu et al., 2009). Therefore, GQ1b is likely to be a prime antigen in FS and the IgG anti-GQ1b antibody may cause ophthalmoplegia and ataxia through specific binding to these regions.

Acute neuropathy characterized by pharyngeal–cervical–brachial (PCB) weakness has been recognized as a variant of GBS, and a recent clinical study showed that PCB, GBS, FS, and Bickerstaff brainstem encephalitis form a continuous spectrum (Nagashima et al., 2007). A monospecific anti-GT1a antibody without GQ1b reactivity is essential for the development of bulbar palsy in patients with GBS (Nagashima et al., 2004). Human glossopharyngeal and vagal nerves contain both GQ1b and GT1a (Koga et al., 2002), but the localization of GT1a in human peripheral nerves has not been determined.

Monospecific anti-GD1b antibodies are likely to induce ataxia in GBS (Kusunoki et al., 1996b, 1999a; Kaida et al., 2008a). A recent analysis in a rabbit model of anti-GD1b-positive ataxic neuropathy indicated that an apoptotic mechanism in dorsal root ganglion cells is associated with development of ataxia (Takada et al., 2008), suggesting that activation of an apoptotic cascade plays a key role in development of ataxia in anti-GD1b-positive GBS.

## 3. Antiganglioside antibody-mediated pathophysiology in GBS and FS

### 3.1. Nerve injury through complement activation

Pathological studies on human specimens and recent experiments have shown that inappropriate activation of the complement cascade triggered by antiganglioside antibodies may induce nerve injury in GBS (Hafer-Macko et al., 1996a, 1996b; Lu et al., 2000; Putzu et al., 2000; Wanschitz et al., 2003; Willison et al., 2008). Especially, complement activation through the classical pathway is considered to be a key process in the development of GBS and FS (Willison et al., 2008). *Ex vivo* and *in vitro* experiments using mouse hemi-diaphragm preparations have shown that GQ1b-reactive monoclonal IgM antibodies and anti-GQ1b-positive sera impair neurotransmission at neuromuscular junctions (NMJs) through complement activation (Plomp et al., 1999; Goodyear et al., 1999). Among the classical, lectin, and alternative pathways of the complement activation system, activation of the classical pathway accompanied by MAC formation seems to play a central pathophysiologic role in experimental models of GBS and FS (Halstead et al., 2004; Halstead et al., 2005). In C6-deficient mice, monoclonal anti-GQ1b IgM antibodies do not provoke formation of MAC or increase MEPP frequency at NMJs. CD59-deficient ($CD59^{-/-}$) mice are unable to inhibit formation of MAC and are characterized by deposits of MAC and damage to perisynaptic

Schwann cells and neurofilament at nerve terminals (Halstead et al., 2004). Furthermore, this study demonstrated strong inhibition of MAC formation and loss of neurofilament under $Ca^{2+}$-free conditions, suggesting that activation of the classical pathway is essential for nerve injury since this pathway is $Ca^{2+}$ dependent, whereas the alternative pathway is $Ca^{2+}$ independent (Halstead et al., 2004). These observations indicate that nerve damage in GBS and FS occurs principally through antiganglioside antibody-mediated activation of the classical pathway.

### 3.2. Antibody-mediated dysfunction of ion channels in peripheral nerves

Recent *in vitro*, *in vivo*, and *ex vivo* studies suggest involvement of ion channels in the pathophysiology of GBS. The most potent molecules are ion channels associated with generation of muscle action potentials such as voltage-gated sodium channels (Navs). Dysfunction of Navs located and clustered at high density on the axonal membrane at the nodes of Ranvier may play an important role in the development of muscle weakness in GBS (Arasaki et al., 1993; Takigawa et al., 1995; Weber et al., 2000). GBS patients show marked refractoriness to axonal excitability in AMAN with IgG antibodies to GM1, GM1b, or GalNAc-GD1a (an increase in threshold current during the relative refractory period) followed by rapid normalization and a recovery of compound muscle action potentials (Kuwabara et al., 2002), suggesting that Nav dysfunction at the nodes of Ranvier is a primary cause of reversible conduction failure in GBS. AIDP patients without antiganglioside antibodies do not show similar refractoriness (Kuwabara et al., 2002). In view of localization of GM1-like epitopes and GalNAc-GD1a at high density at the nodes of Ranvier (Corbo et al., 1993; Sheikh et al., 1999; Kaida et al., 2003), anti-GM1 and anti-GalNAc-GD1a antibodies may directly or indirectly alter the regulatory function of Navs via antibody binding to antigens on the axonal membrane at the nodes.

Several studies have shown that anti-GM1 antibodies can exert a blocking effect on Navs at the nodes of Ranvier through complement activation (Arasaki et al., 1993; Takigawa et al., 1995; Weber et al., 2000; Santoro et al., 1992), but others have not found this blocking effect (Hirota et al., 1997; Dilley et al., 2003). It is intriguing that reversible disruption of Nav clusters with structural changes of the nodes was observed in ventral roots in a rabbit AMAN model immunized with a bovine brain ganglioside mixture including GM1 (Susuki et al., 2007b). Lengthened nodes and complement-mediated impairment of paranodal and nodal structures were also observed in the anti-GM1-positive rabbit model, with gradual recovery of these changes (Susuki et al., 2007b). Taken together, these findings suggest that antiganglioside antibody-mediated dysfunction of Navs is a principal pathogenesis in the AMAN variant of GBS. The prompt recovery (within one day) after immunomodulatory therapy that is often seen in clinical practice may be explained by functional blockage of Navs with little or no structural destruction of nodes.

Calcium (Ca) channels have been shown to be involved in the pathophysiology of GBS. In a co-culture of rat muscle-spinal cord cells, human and rabbit IgG anti-GalNAc-GD1a antibodies exerted a complement-independent inhibitory effect on acetylcholine (Ach) release at NMJs (Taguchi et al., 2004). Similarly, rabbit anti-GalNAc-GD1a-positive sera reversibly inhibits voltage-gated Ca channel currents of PC12 pheochromocytoma cells (Nakatani et al., 2007), and the Cav2.1 voltage-gated Ca channel current in cerebellar Purkinje cells is inhibited by sera containing IgG antibodies to GM1, GalNAc-GD1a, or GD1a (Nakatani et al., 2009). Such complement-independent inhibition of voltage-gated Ca channel current has also been observed in other *ex vivo* and *in vitro* studies using anti-GM1 or anti-GD1a monoclonal antibodies (Buchwald et al., 2007). Antibody–antigen interaction in the presynaptic membrane may cause inhibition of depolarization-induced calcium influx. The presynaptic membranes are likely to be susceptible to antiganglioside antibody attack because the blood–nerve barrier is absent and gangliosides are abundant in these membranes (Martin, 2003), but how target gangliosides interact with Ca channels in the presynaptic membrane remains to be elucidated. Taken together, the results showing complement-independent inhibition of voltage-gated Ca channel current at the presynaptic membrane may reflect an alternative pathophysiology in GBS, although clinical and electrophysiological examinations in GBS patients with antibodies to GM1, GD1a, or GalNAc-GD1a have not shown neuromuscular transmission failure.

## 4. Antibodies to ganglioside complexes in GBS

### 4.1. Clinical correlates of anti-GSC antibodies in GBS

Conventional measurement of antiganglioside antibodies has been done for purified single ganglioside antigens using enzyme-linked immunosorbent assays (ELISAs) or thin-layer chromatogram (TLC)-immunostaining. However, a mixture of two gangliosides can generate new epitopes that differ from those of the constituents and may be targeted by serum autoantibodies from GBS patients (Kaida et al., 2004). Such a mixture of gangliosides is referred to as a ganglioside complex (GSC). Antibodies to the GD1a-GD1b complex (GD1a/GD1b) were first found in GBS sera by ELISA and TLC immunostaining (Kaida et al., 2004). When GD1a and GD1b were developed such that they overlapped in the same lane on the TLC plate, the serum IgG reacted strongly with the overlapping portion (Fig. 1). With another developing solvent that produced completely separate positions of GD1a and GD1b, the reaction disappeared. In ELISA with GD1a, GD1b, and a mixture of the two, the serum IgG had a positive reaction only in a well coated with the mixture, with an optimal reaction at a GD1a to GD1b ratio of approximately 1 to 1. These findings indicate that a mixture of GD1a and GD1b induces formation of a GD1a/GD1b complex with a novel glycoepitope that differs from that of GD1a or GD1b.

We next investigated IgG antibodies to GSCs consisting of two of the four major gangliosides (GM1, GD1a, GD1b, and GT1b) using 234 GBS sera, and demonstrated that 39 sera (17%) had IgG antibodies to at least one GSC, including GD1a/GD1b, GM1/GD1a, GD1b/GT1b, GM1/GT1b, or GM1/GD1b (Table 1) (Kaida et al., 2007). All 39 anti-GSC-positive sera reacted with GM1/GD1a, 27 reacted with GM1/GT1b, 16 with GD1a/GD1b, 13 with GD1b/GT1b, and 6 with GM1/GD1b. Anti-GD1a/GT1b antibodies were not found in the sera. Since a particular combination of gangliosides is recognized by serum

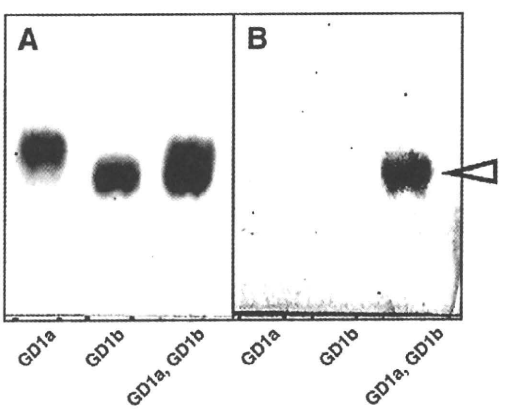


**Fig. 1.** Results from thin-layer chromatography (TLC). (A) TLC bands visualized with orcinol reagent. (B) TLC immunostaining using a representative anti-GD1a/GD1b-positive serum, showing that the overlapping region between GD1a and GD1b is strongly stained (arrowhead). Serum is diluted to 1:100.

**Table 1**
Antiganglioside complex IgG antibodies and the associated clinical features.

| GSC antigens | Disorders (frequency)[a] | Clinical features |
|---|---|---|
| GM1/GD1a | GBS (17%) | |
| GM1/GT1b | GBS (12%) | |
| GD1a/GD1b | GBS (7%) | Severe disability, need for artificial ventilation, impairment of lower cranial nerves |
| GD1b/GT1b | GBS (6%) | Severe disability, need for artificial ventilation, impairment of lower cranial nerves |
| GM1/GalNAc-GD1a | GBS (3–11%) | Pure motor, AMCBN |
| GM1/GQ1b, GM1/GT1a, GD1b/GQ1b, GD1b/GT1a | FS (41%), GBS with OP (28%) | Infrequent sensory dysfunction |
| GD1a/GQ1b, GD1a/GT1a, GT1b/GQ1b, GT1b/GT1a | FS (6%), GBS with OP (19%) | |
| GA1/GQ1b, GA1/GT1a | FS, GBS, BBE | |

GSC = ganglioside complex, GBS = Guillain–Barre syndrome, FS = Fisher syndrome, AMCBN = acute motor conduction block neuropathy, OP = ophthalmoplegia, BBE = Bickerstaff brainstem encephalitis.
[a] "Frequency" indicates frequency of anti-GSC antibodies in the disorder.

antibodies, an epitope formed by a combination of [Galβ1-3GalNAc] and [NeuAcα2-3Galβ1-3GalNAc] in the terminal residues of gangliotetraose structures is essential for antibody binding (Fig. 2). Most anti-GD1a/GD1b- or anti-GD1b/GT1b-positive sera also reacted with GM1/GD1a and GM1/GT1b, suggesting that they are more multivalent than the antibodies reacting only with GM1/GD1a or GM1/GT1b, or with a single ganglioside antigen. Predisposition to severe disability in patients with anti-GD1a/GD1b or anti-GD1b/GT1b antibodies may be associated with this multivalency. Whether GSCs consisting of three or more different gangliosides can be target antigens in GBS and its variants remains unclear. When mixtures of three or four gangliosides were used as antigens in ELISA, antibodies to GSCs consisting of two different gangliosides often decreased the antibody activities (Kaida et al., 2007). These results suggest that combinations of two gangliosides appear to form target epitopes in biological membranes.

Anti-GM1 and anti-GalNAc-GD1a antibodies are associated with a pure motor variant of GBS (Visser et al., 1995; Rees et al., 1995; Jacobs et al., 1996; Hao et al., 1999; Ang et al., 1999; Kaida et al., 2000, 2001). Pathological studies using peripheral nerve specimens from patients with AMAN suggest that AMAN-associated antigens are likely to be expressed in the axolemma of motor nerves, especially at the nodes of Ranvier (Hafer-Macko et al., 1996b). GM1-like epitopes are present in the axolemma at the nodes of Ranvier (Sheikh et al., 1999), although immunohistochemical studies of normal human peripheral nerves have not provided conclusive evidence for the distribution of the GM1 antigen (Kusunoki et al., 1993). GalNAc-GD1a is found in the vicinity of the nodes of Ranvier in human motor nerves; at nodal and paranodal axolemmae in the ventral roots and in a periaxonal axolemma-related region in intramuscular nerves (Kaida et al., 2003). From these findings, it can be speculated that GM1 and GalNAc-GD1a colocalize in the motor axolemma, and that antibodies to GSCs containing GM1 or GalNAc-GD1a may be associated with pure motor GBS. Actually, we found an antibody to a GSC consisting of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a) in 10 of 224 GBS sera (Kaida et al., 2008b), and the anti-GM1/GalNAc-GD1a-positive patients suffered from a pure motor variant of GBS, as expected. However, their electrophysiological findings featured early conduction block at intermediate nerve segments of motor nerves. In serial nerve conduction studies, the conduction block promptly improved and there were no findings indicative of remyelination or axonal degeneration. From these observations, we inferred that the conduction block results from reversible conduction failure on the axolemma at the nodes of Ranvier (Kuwabara et al., 1998; Kaida et al., 2008b). In view of the dense cluster of Navs at the nodes, antibody binding to GM1/GalNAc-GD1a at these nodes can cause reversible conduction block through alteration of the regulatory function of Nav. The prompt recovery after immune-mediated treatment such as IVIG may result from functional block with little or no pathological changes of the nodes. It remains to be determined whether the antibody–antigen interaction causes Nav dysfunction through complement activation or direct breakdown of Nav function, or both. Regardless, GM1, GalNAc-GD1a, and Nav may assemble in microdomains at the nodes of Ranvier.

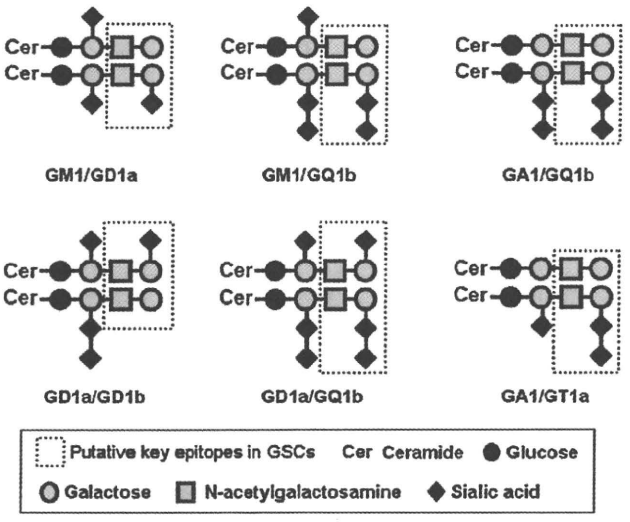


**Fig. 2.** Pattern diagrams of glycolipid complexes GM1/GD1a, GD1a/GD1b, GM1/GQ1b, GD1a/GQ1b, GA1/GQ1b, and GA1/GT1a. Squares with dotted lines indicate putative antigenic epitopes for antiganglioside complex antibodies.

### *4.2. Induction of anti-GSC antibodies*

Analyses of the molecular structure of *C. jejuni* lipooligosaccharide (LOS) showed molecular mimicry between the LOS and GSCs targeted by serum antibodies from GBS patients (Kuijf et al., 2007). Inhibition ELISA using GBS sera with antibodies to such GSCs as GM1/GD1a, GD1a/GD1b, GD1a/GQ1b, and GD3/GQ1b revealed that each anti-GSC antibody cross-reacted with the LOS from the autologous *C. jejuni* strains, indicating that the LOS contained GSC-like structures. Interestingly, ganglioside-like structures expressed in some LOS of *C. jejuni* strains were not in accord with those expected from anti-GSC antibodies. Strains isolated from GBS patients with anti-GD1a/ GQ1b antibodies expressed a homogeneous LOS with only a GD1c-like structure (Kuijf et al., 2007). Further studies on the structures of GSCs may explain the unexpected antibody–antigen interactions, such as the cross-reaction between the anti-GD1a/GQ1b antibodies and GD1c-like moieties.

## 5. Antibodies to ganglioside complexes in FS and GBS with ophthalmoplegia

Analysis of FS sera for antibodies to GSCs containing GQ1b or GT1a revealed that a half of FS patients had antibodies to GSCs such as GM1/

GQ1b and GD1a/GQ1b (Table 1) (Kaida et al., 2006; Kanzaki et al., 2008). Based on antibody specificity, the FS-associated antibodies were subdivided into three types: GQ1b-specific, GM1/GQ1b-reactive, and GD1a/GQ1b-reactive (Kaida et al., 2006). Given the combination of GQ1b and other gangliosides in the targeted GSCs, the conformation of terminal residues containing sialic acids is likely to regulate the antibody binding. A combination of [Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues of ganglio-N-tetraose structures is essential for binding of the anti- GM1/GQ1b-antibody, whereas a combination of [NeuAcα2-3Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues is targeted by the anti-GD1a/GQ1b antibody (Fig. 2) (Kaida et al., 2006; Kanzaki et al., 2008). Such diversity of antibody specificity may produce clinical difference among FS patients, and sensory function was preserved in FS patients who had anti-GM1/GQ1b-reactive sera (Kanzaki et al., 2008). However, patients with FS displayed the clinical triad regardless of the presence of such anti-GSC antibodies, suggesting that molecules targeted by GQ1b-specific, GM1/GQ1b-reactive, or GD1a/GQ1b-reactive antibody are in the vicinity of the nerve membrane. GQ1b must be a key molecule in the immunobiology of FS, and GSCs containing GQ1b appear to be preferential antigens in most FS patients. Anti-GQ1b IgG antibody remains as an excellent diagnostic marker of FS.

IgG anti-GQ1b antibody is also associated with development of ophthalmoplegia in GBS, acute ophthalmoplegia without ataxia, and Bickerstaff brainstem encephalitis, as well as FS (Chiba et al., 1993; Odaka et al., 2001). A recent study of anti-GSC antibodies in GBS revealed that IgG antibodies to GSCs containing GQ1b or GT1a were present in 47% of GBS patients with ophthalmoplegia, whereas no such anti-GSC antibodies were found in those with GBS without ophthalmoplegia (Kanzaki et al., 2008). This indicates that the antibodies to GSCs containing GQ1b or GT1a are closely associated with development of ophthalmoplegia in GBS. Our recent study on antibodies to glycolipid complexes consisting of asialo-GM1 (GA1) and GQ1b have made us reconsider the conformational structure of the glycoepitopes targeted by the FS-associated anti-GSC antibodies (Ogawa et al., 2009). Some anti-GM1 antibodies in GBS sera are cross-reactive with GA1 and probably bind to the terminal N-acetylgalactosamine-galactose moiety (Koga et al., 2001). Because terminal residues with a gangliotetraose structure in GA1 are shared with GM1 or GD1b, the terminal residues of a glycolipid complex, GA1/GQ1b should be analogous to those of GM1/GQ1b or GD1b/GQ1b. However, approximately 70% of anti-GA1/GQ1b or anti-GA1/GT1a positive sera did not react with GM1/GQ1b and GD1b/GQ1b (Ogawa et al., 2009). In view of the terminal residues of such glycolipid complexes, the specificity of antibodies to GSC containing GQ1b or GT1a may be regulated not only by sialic acids in the terminal residues but also by those attached to an internal galactose. Conformational analyses of glycoepitopes in the GSCs are required for identification of the exact target antigens and understanding of the antibody-mediated pathophysiology in GBS and its variants.

## 6. Glycolipid environment and avidity of antiganglioside antibodies

Ataxia is a well-known symptom in GBS that is thought to be closely associated with IgG anti-GD1b antibodies. This is supported by studies showing that IgG GD1b-specific antibodies induce experimental ataxic neuropathy (Kusunoki et al., 1996b, 1999a). GD1b has been shown to be localized in large neurons in dorsal root ganglia (Kusunoki et al., 1993), indicating that anti-GD1b antibodies cause ataxia by binding to large primary sensory neurons that mediate deep sensation. However, only half of GBS patients with IgG anti-GD1b antibody present with ataxia (Miyazaki et al., 2001). To unveil the reason for this discrepancy, we examined the specificity of IgG anti-GD1b antibodies using GSC antigens containing GD1b and analyzed the association of the antibody specificity with ataxia (Kaida et al., 2008a). We found that anti-GD1b activities were strongly inhibited by the addition of gangliosides with two or more sialic acids to GD1b in patients with GBS with ataxia, compared to those with GBS without ataxia (Kaida et al., 2008a). These results suggest that target epitopes of GD1b can be masked or modified by colocalization of gangliosides with two or more sialic acids, such as GD1a. Thus, IgG antibodies with high specificity for GD1b may play a critical role in development of ataxia in GBS and colocalization of another ganglioside with GD1b may influence the accessibility of the anti-GD1b antibodies (Fig. 3).

*Cis*-interaction of the sugar chain of gangliosides in membrane microdomains may modify the conformation of the glycoepitopes. Such complex glycolipid environments in the cell membrane may govern the accessibility and avidity of antiganglioside antibodies for target gangliosides. A recent intriguing study using GalNAc transferase-deficient ($GalNAcT^{-/-}$) and GD3 synthase-deficient ($GD3s^{-/-}$) mice supports this hypothesis (Greenshields et al., 2009). The binding ability of the pathogenic anti-GM1 antibody to GM1-like epitopes is dependent upon which gangliosides are in the vicinity of GM1 on the

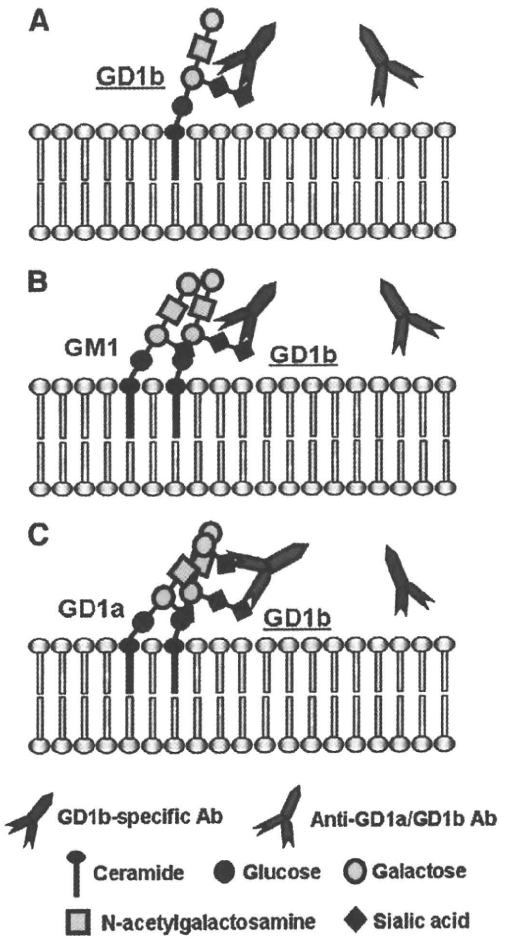


**Fig. 3.** Schematic diagram depicting proposed antigen–antibody interactions between GD1b-specific antibodies and GSCs containing GD1b in the nerve cell membrane. (A) The GD1b-specific antibody binds to antigenic epitopes of GD1b that are exposed and unmasked in the cell membrane. (B) GD1b and GM1 colocalize and *cis*-interact together in the membrane. The GD1b-specific antibody can access antigenic epitopes of GD1b in the GM1–GD1b complex. Colocalization and *cis*-interaction between GD1b and monosialogangliosides do not interrupt the binding of the GD1b-specific antibody to GD1b in the membrane (for details, see text). (C) GD1b and GD1a colocalize and *cis*-interact in the membrane. The GD1b-specific antibody cannot access antigenic epitopes of GD1b in the GD1a/GD1b complex, while the anti-GD1a/GD1b antibody can bind to glycoepitopes formed in the GD1a/GD1b.

cell membrane and whether the GM1-like epitopes are unmasked. Colocalization and *cis*-interaction of gangliosides may either enable or inhibit antibody binding to the neuronal membrane or have no effect (Greenshields et al., 2009). In our recent study, the epitope targeted by monoclonal anti-GA1 antibody was masked in a glycolipid complex GA1/GQ1b, whereas that recognized by the monoclonal anti-GQ1b antibody was preserved (Ogawa et al., 2009). Therefore, even if GA1 and GQ1b actually form complexes in the biological membrane, the anti-GQ1b antibody can access GQ1b epitopes in GA1/GQ1b but the anti-GA1 antibody cannot access GA1 epitopes in the same complex. Thus, the local glycolipid environment in the plasma membrane may regulate the pathogenic effect of antiganglioside antibodies, and it should be borne in mind that the antibody–antigen interaction depends not only upon the fine specificity of individual antibodies but also upon the conformation of glycoepitopes formed in glycolipid environments in the nerve cell membrane.

## 7. Putative factors influencing antibody binding to target epitopes

Certain specific conditions of glycoepitopes in the cell membrane are essential for exertion of the pathogenic action of antiganglioside antibodies. First, complex glycolipid environments in the cell membrane may influence the accessibility and avidity of antiganglioside antibodies for target gangliosides, as described above (Fig. 3) (Kaida et al., 2008a; Greenshields et al., 2009). Analyses of the reactivity of antiganglioside antibodies against various GSCs are useful for evaluation of the accessibility of the antibodies. Second, the large amount of targeted gangliosides in particular loci of peripheral nerves is closely associated with antibody-mediated injury and specific clinical features. GQ1b is abundantly distributed in human oculomotor, trochlear, and abducens nerves, leading to predisposition to binding of anti-GQ1b antibodies (Chiba et al., 1993, 1997). Anti-GD1a antibody-mediated nerve injury is observed in GD3-synthase knockout mice that overexpress GD1a, but not in normal mice, probably because the abundant expression of GD1a at a particular region is critical for development of anti-GD1a-mediated nerve damage (Goodfellow et al., 2005). Third, the conformational difference of glycoepitopes between motor and sensory nerves may influence antibody binding and development of nerve injury. Ganglioside analysis of human motor and sensory nerves has shown that the amount of GM1 and GD1a is almost equal in both nerves, but that the ceramide compositions differ between the motor and sensory nerves (Ogawa-Goto et al., 1990): the gangliosides from sensory nerves are abundant in long-chain fatty acids, in contrast to those from motor nerves. In a binding assay using derivatives of GD1a bearing very long chain fatty acids, the difference in length of fatty acids in the ceramide reduced the binding ability of monoclonal anti-GD1a antibodies with GD1a derivatives, indicating that the ceramide composition can modify the steric structure of gangliosides in membranes (Tagawa et al., 2002). These findings may partly explain the preferential binding of anti-GD1a antibodies from AMAN patients to GD1a in motor nerves (Gong et al., 2002).

Finally, the conformational microstructure of sialic acids in gangliosides may regulate the binding ability of antiganglioside antibodies. In a recent immunohistochemical study using GD1a derivatives with chemically modified sialic acid residues, anti-GD1a monoclonal antibodies that preferentially stained motor axons specifically bound to GD1a-1-ethyl ester, GD1a-1-alcohol, and GD1a-1-methyl ester, in contrast to other anti-GD1a monoclonal antibodies that stained both motor and sensory axons (Lopez et al., 2008). There were no differences in binding to GD1a derivatives between anti-GD1a antibodies from AMAN patients and motor-specific anti-GD1a monoclonal antibodies. Thus, ganglioside exposure in the nerves and the fine specificity of antiganglioside antibodies is likely to regulate their accessibility to target gangliosides. The effects of phospholipids should also be considered because the presence of several kinds of phospholipids influences antibody binding to gangliosides (Hirakawa et al., 2005).

## 8. Perspective

Recent progress on the immunobiological mechanism in GBS has contributed to the precise understanding of antiganglioside antibody-mediated nerve dysfunction, and has encouraged development of novel therapeutic strategies for patients with GBS and its variants (Willison et al., 2008; Kaida and Kusunoki, 2009). Consideration of GSCs will provide new avenues of research on antibody–antigen interactions in GBS. Examination of anti-GSC antibodies may expand the spectrum of antiganglioside antibodies in GBS, enhancing their value as diagnostic markers and expediting understanding of the pathophysiology underlying antiganglioside antibody-mediated nerve dysfunction. New techniques such as combinatorial glycoarrays are beneficial for studies on anti-GSC antibodies (Rinaldi et al., 2009). The understanding of GSCs will also shed light on microdomain function mediated by carbohydrate–carbohydrate interactions in biological membranes. Microdomain function is controlled by carbohydrate-binding proteins such as selectins and Siglecs and is based on *cis*- or *trans*-carbohydrate–carbohydrate interactions (Hakomori, 2004; Varki, 2007). In the microdomain, complex glycoconjugates such as GSCs with clustered sialic acid epitopes may form rigid rodlike structures with multivalency and strict binding specificity, and are likely to function in cell–cell recognition or immune-mediated events in a more effective manner than a solo glycoepitope of an isolated ganglioside. This hypothesis is supported by a recent study demonstrating that a GSC, GM2/GM3, provides more efficient suppression of cell motility through blocking of cMet activation compared to GM2 or GM3 alone (Todeschini et al., 2008).

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MINI-REVIEW

# Antibodies against ganglioside complexes in Guillain–Barré syndrome and related disorders

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**Abstract**

Guillain-Barré syndrome (GBS) is acute autoimmune neuropathy, often subsequent to an infection. Serum anti-ganglioside antibodies are frequently elevated in titer. Those antibodies are useful diagnostic markers and possible pathogenetic factors. Recent data demonstrated that sera from some patients with GBS react with ganglioside complexes (GSCs) consisting of two different gangliosides, but not with each constituent ganglioside. Those antibodies may specifically recognize a new conformational epitope formed by two gangliosides. In particular, the antibodies against GD1a/GD1b and/or GD1b/GT1b complexes are associated with severe GBS requiring artificial ventilation. The antibodies to GM1/GalNAc–GD1a and those to GSCs containing GQ1b or GT1a are associated with pure motor GBS and Fisher syndrome, respectively. In contrast, the binding activities of the antibodies highly specific to GD1b are strongly inhibited by the addition of GD1a to GD1b. Gangliosides along with other components as cholesterol are known to form lipid rafts, in which two different gangliosides may form a new conformational epitope. Future investigation is necessary to elucidate the roles of GSCs in the plasma membrane and of the clinical relevance of the anti-GSCs antibodies.

**Keywords:** ganglioside, Guillain-Barré syndrome, membrane microdomain, peripheral nerve.

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Anti-ganglioside antibodies, mostly IgG type, are present in the sera from approximately 60% of patients with Guillain-Barré syndrome (GBS), acute immune-mediated polyradiculoneuropathy (Willison and Yuki 2002; Kusunoki *et al.* 2008; Van Doorn *et al.* 2008). Because the presence of anti-ganglioside antibodies in the acute-phase sera is a characteristic feature of GBS, those antibodies can be used as diagnostic markers of GBS. There are many molecular species of gangliosides, named depending on the carbohydrate sequences. Each ganglioside has unique distribution within the PNS. Considering the gangliosides are localized in the plasma membrane with their carbohydrate portions extended to the extracellular spaces, the anti-ganglioside antibodies may function in the pathogenesis of GBS through antibody-antigen interaction in PNS.

IgG anti-GQ1b antibody is one of the best studied antibodies. Ig anti-GQ1b antibodies are specifically associated with a variant of GBS, Fisher syndrome (FS) characterized by ophthalmoplegia and ataxia (Chiba *et al.* 1992). Anti-GQ1b monoclonal antibody specifically immunostains paranodal myelin of human cranial nerves innervating extraocular muscles (Chiba *et al.* 1993) and some large neurons in dorsal root ganglia (Kusunoki *et al.* 1999). It has recently been reported that the neuromuscular junctions of human extraocular muscles are richly bound by the antibodies against GQ1b and GT1a (Liu *et al.* 2009). Thus, the anti-GQ1b antibodies may cause opthalmoplegia and ataxia by binding to the regions where GQ1b is densely localized.

Measurement of anti-ganglioside antibodies has been conducted with ELISA or TLC-immunostaining by the use of purified single ganglioside antigens. Gagliosides have characteristics of forming clusters in the plasma membrane

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*Abbreviations used*: AMCBN, acute motor conduction block neuropathy; FS, Fisher syndrome; GBS, Guillain-Barré syndrome; GSC, ganglioside complex; LOS, lipooligosaccharides.

(Hakomori 2002). In the clusters, the carbohydrate structure of a ganglioside may interact with each other to form a novel epitope. We recently demonstrated that some GBS patients had serum antibodies that specifically recognize the novel glycoepitopes formed by two individual ganglioside molecules and named such antibodies as 'anti-ganglioside complex (GSC) antibodies' (Kaida *et al.* 2004).

## Antibodies to ganglioside complexes in GBS

### Antibodies to GD1a/GD1b and GD1b/GT1b complexes in severe GBS

Anti-GD1a/GD1b complex antibodies are the first identified antibodies against GSCs. We investigated a serum from a GBS patient who showed acute severe flaccid tetraparesis and needed artificial ventilation. We found an unidentified immuno-reactive band in the position just below GD1a on TLC of a crude ganglioside fraction from bovine brain. The serum was not reactive with any of such purified gangliosides as GM1, GM2, GM3, GD1a, GD1b, GD3, GalNAc–GD1a, GT1b, and GQ1b. But the serum IgG bound strongly to the well coated with the mixture of GD1a and GD1b gangliosides (GD1a/GD1b complex). When GD1a and GD1b were developed in the same lane on TLC using a developing solvent, chloroform/methanol/0.2%$CaCl_2 \cdot 2H_2O$ (50 : 45 : 10), the serum IgG strongly immunostained just the overlapping portion between GD1a and GD1b. When another developing solvent (C/M/0.2%$CaCl_2 \cdot 2H_2O$, 30/65/10) that completely separated the positions of GD1a and GD1b was used, no immunoreaction was identified. Those data indicate that mixing GD1a and GD1b may produce a new conformational glycoepitope which is different from that of GD1a or GD1b alone and the antibody in sera from the above patient may specifically recognize such a new glycoepitope.

We next investigated antibodies in sera from 234 GBS patients with ELISA using a mixture of two of the four major gangliosides (GM1, GD1a, GD1b and GT1b) (Kaida *et al.* 2007). The sera with anti-GSC antibodies often exhibited to some extent reactivity with constituent gangliosides of the GSCs. When optical density for the anti-GD1a/GD1b antibody was 0.2 higher than that corresponding to anti-GD1a or anti-GD1b antibody or it was more than the sum of those of anti-GD1a and anti-GD1b antibodies, the sera were judged to be anti-GD1a/GD1b-positive. The same criteria also were applied to the other GSCs. The cutoff value (0.2) for anti-GSC antibodies was decided arbitrarily. The results showed that 39 of 234 patients (17%) had antibodies against at least one of the mixture antigens. All the 39 patients had anti-GM1/GD1a antibodies, 27 had anti-GM1/GT1b antibodies, 16 had anti-GD1a/GD1b antibodies, and 13 had GD1b/GT1b antibodies. Most of anti-GD1a/GD1b or anti-GD1b/GT1b antibody reacted also with GM1/GT1b as well as GM1/GD1a. Immunoabsorption study suggested that anti-GSC antibodies specifically react with clustered glycoepitopes common to these GSCs, rather than individually with each GSC. An epitope formed by a combination of [Galβ1-3GalNAc] and [NeuAcα2-3Galβ1-3GalNAc] in the terminal moieties of ganglio-*N*-tetraose structures is likely to be essential for the antibody binding. Among them, antibodies against GD1a/GD1b and GD1b/GT1b complexes were significantly associated with severe GBS requiring artificial ventilation (Kaida *et al.* 2007). Those antibodies can be useful markers of severe GBS. Future study is needed to clarify why anti-GD1a/GD1b and GD1b/GT1b antibodies are associated with severe disabilities.

### Antibodies to ganglioside complexes including GQ1b

Because FS is considered to be a variant of GBS, we extended an investigation of anti-GSC antibodies to FS patients. Presence of anti-ganglioside complexes antibodies in FS therefore was investigated with ELISA using seven ganglioside antigens; GM1, GM2, GD1a, GD1b, GT1a, GT1b and GQ1b (Kaida *et al.* 2006).

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

The results of anti-GSCs antibody assay on larger number of patients with FS and those with GBS with opthalmoplegia indicated that the serum antibodies could be subdivided into the three groups (Kanzaki *et al.* 2008): (i) antibodies specific to GQ1b and/or GT1a without anti-GSCs reactivity; (ii) antibodies that recognize a combination of [Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues of ganglio-*N*-tetraose structures, such as antibodies to GQ1b/GM1, GQ1b/GD1b, GT1a/GM1, GT1a/GD1b (Fig. 1); and (iii) antibodies that recognize a combination of [NeuAcα2-3Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues, such as antibodies to GQ1b/GD1a, GT1a/GD1a, GQ1b/GT1b, GT1a /GT1b. In addition, recent report showed that some patients have the antibodies specific to GQ1b/GA1 (Ogawa *et al.* 2009).

Sensory signs were infrequent in FS patients with antibodies to GQ1b/GM1 but were frequent in patients with other types of antibodies. However, the clinical relevance of such anti-GSC antibodies needs to be investigated in future.

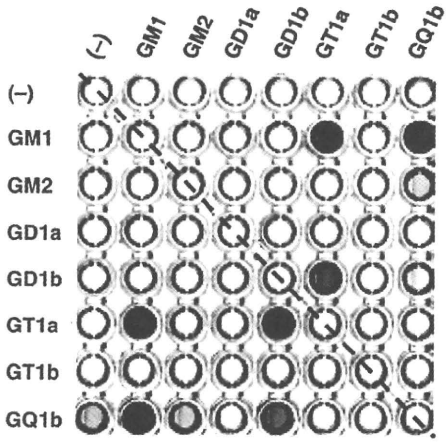


**Fig. 1** An ELISA plate showing the binding activities of a serum antibody that recognizes a combination of [Galβ1-3GalNAc] and [NeuAcα2-8NeuAcα2-3 Galβ1-3GalNAc] in the terminal residues. All the wells in each line and column were coated with a respective ganglioside (e.g. the wells in the first line and column were coated only with a single ganglioside, the well in the eighth line and the second column was coated with GQ1b and GM1), except for those on the oblique dotted line that were uncoated control wells. The antibody binds strongly to GQ1b/GM1, GQ1b/GD1b, GT1a/GM1 and GT1a/GD1b but only weakly to GQ1b.

### Antibodies to GM1/GalNAc–GD1a complex in pure motor GBS

IgG antibodies against GM1 or those against GalNAc–GD1a are known to closely correlate with acute motor axonal neuropathy (Kaida *et al.* 2000; Willison and Yuki 2002). We investigated antibody activities against the mixture of GM1 and GalNAc–GD1a (GM1/GalNAc–GD1a complex) in a large population of patients with GBS. The results showed that ten of 224 GBS patients had IgG antibodies to the GM1/GalNAc–GD1a complex (Kaida *et al.* 2008a).

We then analyzed the clinical and electrophysiologic findings of those 10 anti-GM1/GalNAc–GD1a-positive patients. Respiratory infections preceded the neurological onset in six cases and gastrointestinal infections in two cases. Therefore, although *Campylobacter jejuni* is an infectious agent that frequently causes the antecedent infection of GBS cases with anti-GM1 and anti-GalNAc–GD1a antibodies, *C. jejuni* may not be the major infectious agent inducing anti-GM1/GalNAc–GD1a complex antibodies. Cranial nerve involvement and sensory signs are infrequent. Early motor conduction block at intermediate nerve segments was found in five patients. Generally, the response to therapy was good. According to the criteria established by Hadden *et al.* (1998), four were categorized as demyelinative and two were axonal. When judged by other criteria (Ho *et al.* 1995), four were demyelinative and three were axonal.

**Table 1** Representative anti-GSCs antibodies in GBS and FS

| Antigen | Associated disease | Frequency (%) | Clinical features |
|---|---|---|---|
| GD1a/GD1b | GBS | 7 | Severe GBS |
| GD1b/GT1b | GBS | 6 | Severe GBS |
| GM1/GalNAc–GD1a | GBS | 4 | Pure motor GBS AMCBN |
| GQ1b/GM1 and related GSCs | FS | 41 | Infrequent sensory dysfunction |
| | GBS with OP | 28 | |
| GQ1b/GD1a and related GSCs | FS | 6 | |
| | GBS with OP | 19 | |

GSC, ganglioside complex; GBS, Guillain-Barré syndrome; FS, Fisher syndrome; AMCBN, acute motor conduction block neuropathy; OP, ophthalmoplegia.
GQ1b/GM1 and related GSCs, GQ1b/GM1, GQ1b/GD1b, GT1a/GM1, GT1a/GD1b; GQ1b/GD1a and related GSCs, GQ1b/GD1a, GT1a/GD1a, GQ1b/GT1b, GT1a /GT1b.

The clinical findings of the 10 GBS patients were consistent with a pure motor variant of GBS. Clinical features of anti-GM1/GalNAc–GD1a IgG-positive GBS resemble those of acute motor conduction block neuropathy (AMCBN), in view of preserved sensory function, early conduction block at intermediate nerve segments and good recovery (Capasso *et al.* 2003). IgG anti-GM1 antibody (and sometimes anti-GalNAc–GD1a antibody) was reported in their sera. However, IgG anti-GM1 or anti-GalNAc–GD1a antibodies are frequently detected in sera of acute motor axonal neuropathy type GBS and conduction block is not common in such cases. Anti-GM1/GalNAc–GD1a antibody is likely to cause early reversible changes on the axolemma and may be more closely associated with AMCBN than the anti-GM1 or anti-GalNAc–GD1a antibody. GM1 and GalNAc–GD1a may form a complex in the axolemma at nodes of Ranvier or paranodes of the motor nerves, and may be a target antigen in pure motor GBS; especially in the form of AMCBN.

Representative anti-GSCs antibodies in GBS and FS are listed in the Table 1.

## Antibodies against ganglioside complexes in chronic neuropathies

Nobile-Orazio *et al.* (2010) investigated serum IgM antibodies to GSCs in such chronic neuropathies as multifocal motor neuropathy, chronic inflammatory demyelinating polyradiculoneuropathy and IgM paraproteinemic neuropathy. As a result, one of 34 chronic inflammatory demyelinating polyradiculoneuropathy patients had IgM antibody activity to GT1b/GM1 and GT1b/GM2, and one of 23 IgM paraproteinemic neuropathy patients had IgM anti-GM2/GD1b activity.

## Production of antibodies against ganglioside complexes

In GBS and related disorders subsequent to *C. jejuni* infection, anti-ganglioside antibodies are shown to be induced by the immune reaction against lipo-oligosaccharides (LOS) of pathogens causing antecedent infection (Willison and Yuki 2002; Van Doorn *et al.* 2008). A similar mechanism can be speculated in the production of anti-GSC antibodies. Kuijf *et al.* (2007) recently reported that such anti-GSC antibodies as anti-GM1/GD1a and GQ1b/GD1a cross-reacted to LOS from the autologous *C. jejuni* strain, indirectly demonstrating that the LOS contained GSC-like structures. However, carbohydrate structures expressed in the LOS may not exactly be the combination of the two carbohydrate chains expected from the reactivity of the serum anti-GSC antibodies.

## Inhibition of the reactivity of the anti-ganglioside antibody by another coexistent ganglioside

If the interaction of two gangliosides creates a new epitope with conformational changes, the binding acitivity of the antibody highly specific to one ganglioside may be lessened by the addition of another ganglioside to make an antigen mixture.

We investigated sera from 17 GBS patients who had IgG antibody reactive only with GD1b in routine antibody assay. For those sera, antibody activity against a mixture of GD1b and another ganglioside was examined and compared the activity with that against GD1b alone. The results showed that the addition of GD1a, GT1a, GT1b, GQ1b and GalNAc–GD1a to GD1b caused marked decrease of the binding activity of anti-GD1b antibodies, suggesting that those gangliosides may interact with GD1b to make a novel epitope which cannot be easily recognized by the anti-GD1b antibodies (Kaida *et al.* 2008b).

In addition, the reduction rates of the binding activities caused by the addition of such gangliosides as GD1a, GT1b, GQ1b and GalNAc–GD1a were significantly more in the antibodies from ataxic patients than in those from non-ataxic patients. The addition of another ganglioside may cause conformational change. Therefore, the more specific the antibody is, the more affected its reactivity should be. It therefore suggests that the anti-GD1b IgG antibodies in ataxic patients may be more specific to GD1b than those in patients without ataxia. This may provide further evidence to the association between anti-GD1b antibody and ataxia (Kusunoki *et al.* 1996).

Thus, the antibodies specific to GD1a/GD1b complex are associated with severe GBS (Fig. 2) and those specific to GD1b itself are associated with the development of ataxia (Fig. 3).

A similar inhibitory effect of neighboring gangliosides has recently been reported in the case of anti-GM1 antibodies by

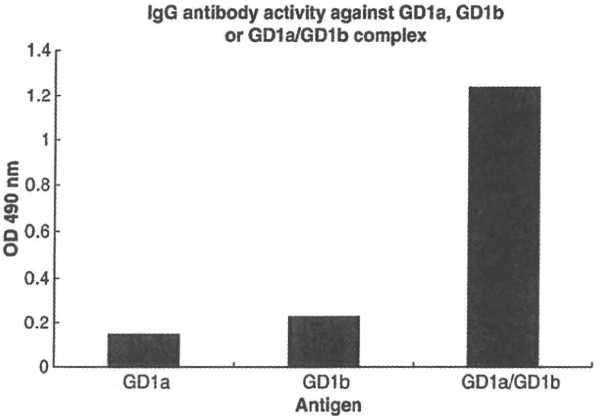


**Fig. 2** ELISA result on a serum sample from a patient with severe Guillain-Barré syndrome (Kaida *et al.* 2004). This patient's serum IgG shows strong reaction with a mixture of GD1a and GD1b (GD1a/GD1b) but reacts only weakly with GD1a or GD1b alone.

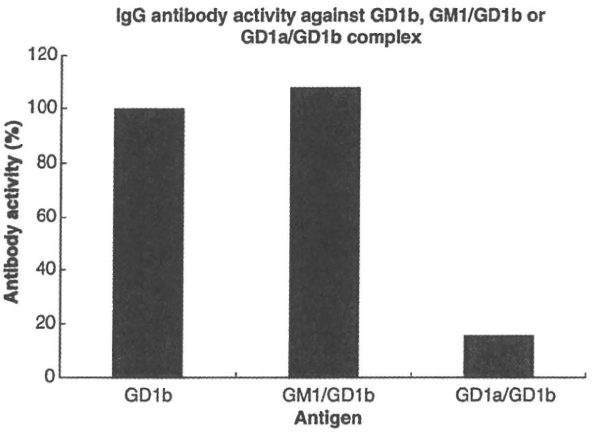


**Fig. 3** The IgG antibody activities to mixture antigens in sera from nine GBS patients with ataxia who had only IgG anti-GD1b antibody in routine antibody assay (Kaida *et al.* 2008b). Bars of GM1/GD1b and GD1a/GD1b showed the average activities of the nine patients. Compared with the antibody activity to GD1b alone (100%), the activity was markedly reduced because of the addition of GD1a to GD1b antigen whereas the addition of GM1 did not affect the antibody activity.

Greenshields *et al.* (2009). Negative effects by gangalioside complexes on the binding of IgM anti-GM1 antibodies in sera from patients with chronic immune-mediated neuropathies, particularly multifocal motor neuropathy, have also been reported (Nobile-Orazio *et al.* 2010).

## Future studies on the anti-GSC antibodies in the pathogenesis of autoimmune neuropathies

Gangliosides are located in the cell membranes with carbohydrate portions on the outer surfaces, and are preferentially

packaged with cholesterol, forming lipid rafts. Within rafts, gangliosides are considered to interact with important transmembrane receptors or signal transducers (Simons and Ikonen 1997; Hakomori 2002). Anti-GSC antibodies may cause dysfunction of the axon or Schwann cells through their binding to clustered epitopes of glycosphingolipids in the plasma membrane microdomains. Future study on the localization of each ganglioside complex is needed. Animal model of the autoimmune neuropathy mediated by anti-GSC antibodies should also be developed. Such investigations may lead to the understanding of the roles of GSCs in the plasma membrane and of the clinical relevance of the anti-GSCs antibodies.

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# Guillain-Barré 症候群

海田賢一　楠　進

## はじめに

運動神経障害優位の急性多発性神経炎である Guillain-Barré 症候群(GBS)は，何らかの免疫介在性機序により発症すると考えられている．細胞性免疫・液性免疫の両面から解析が行われてきているが，これまでの研究では自己抗体を発症因子とする液性免疫機序の解明が先行しており，現在の段階では液性免疫機序に関連する分子マーカーが診断および病態の解析に極めて有用である．本稿では，主要な液性免疫関連マーカーである抗ガングリオシド抗体を中心に解説する．

## Guillain-Barré 症候群における分子マーカー：抗ガングリオシド抗体

GBS 症例の約 60%に糖脂質抗原に対する自己抗体が上昇することが知られ，GBS の診断マーカーとして活用されている．その中でもシアル酸を含むスフィンゴ糖脂質であり神経系に豊富に存在するガングリオシドに対する自己抗体がほとんどである．特に IgG1，IgG3 のサブクラスの抗体が病的意義を持っていると考えられている．標的抗原であるガングリオシドは細胞膜の主要な構成成分の一つとして，糖鎖成分を細胞膜外に漂わせた状態で脂質二重層の外層に存在している(図 1)．細胞膜上では集簇して存在し(クラスター形成)，コレステロールや GPI アンカー蛋白(glycosylphosphatidylinositol-anchored protein)とともに脂質ラフト(lipid rafts)と呼ばれるマイクロドメインを形成して，細胞間の接着，シグナル伝達など様々な神経細胞機能に関わっていると考えられている[1]．このようなガングリオシドに対する抗体の産生機序，病的意義については未解明の部分も多いが，先行感染病原体の細胞膜リポオリゴ糖(LOS)に存在する糖鎖構造がヒト末梢神経上のガングリオシド糖鎖と共通しており，先行感染によって惹起された病原体 LOS 糖鎖に対する抗体が類似のヒト神経糖鎖に反応して神経障害をおこすといった分子相同性機序が最近の研究で明らかとなっている[2]．また，ガングリオシドはそれぞれ神経系における局在が異なることが知られており，抗ガングリオシド抗体の種類によって対応する神経症候が異なることが知られている[3]．実際，GBS はその神経症候によりいくつかの亜型に分類されるが，それぞれの亜型に関連する抗糖脂質抗体が知られている(表)．以下に，抗ガングリオシド抗体検出法と各抗ガングリオシド抗体とその病的意義について述べる．

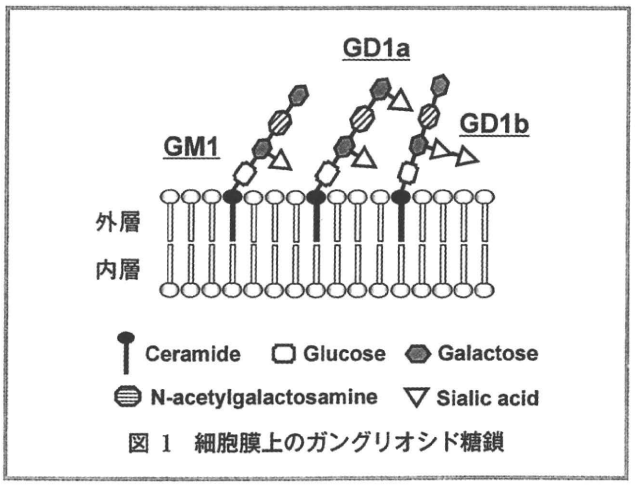


図 1　細胞膜上のガングリオシド糖鎖

### 1．抗ガングリオシド抗体検出法

ウシ脳から精製されたガングリオシドを抗原として ELISA(enzyme-linked immunosorbent assay)法を用いて患者血清中の抗体を測定する[4]．経過とともに力価が低下するため原則として治療前の急性期血清を用いるが，治療後でも検出されることが多い．ルーチンの抗体スクリーニングでは単一ガングリオシドが抗原として用いられている．未同定抗原の検索のために薄層クロマトグラフィー免疫染色法(thin-layer chromatogram immunostaining)を用いることもある．その際，ウシ脳ガングリオシドをシアル

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GBS における各種抗糖脂質抗体に関連する神経症状，標的抗原の局在

| 抗糖脂質抗体の標的抗原 | サブクラス | 先行感染 | 神経症状 | ヒト末梢神経における標的抗原の局在 |
|---|---|---|---|---|
| ＜単独抗原＞ | | | | |
| GM1 | IgG | *C. jejuni*, *H. Influenzae* | AMAN，純粋運動型 GBS | 不明(Ranvier 絞輪，傍絞輪部軸索膜：推定) |
| GD1a | IgG | *C. jejuni* | AMAN | |
| GalNAc-GD1a | IgG | *C. jejuni* | AMAN，純粋運動型 GBS | 運動神経 Ranvier 絞輪，傍絞輪部軸索膜 |
| | IgM | *C. jejuni* | AMAN，純粋運動型 GBS | 腓腹神経小径線維 |
| | IgM | サイトメガロウイルス | 顔面神経麻痺，感覚障害 | |
| GM1b | IgG | *C. jejuni* | 純粋運動型 GBS | |
| GD1b | IgG | 何らかの呼吸器感染 | GBS における感覚性運動失調(GD1b 特異的抗体の場合) | 後根神経節大型細胞，傍絞輪部ミエリン |
| GM2 | IgG, IgM | サイトメガロウイルス | AIDP，顔面神経麻痺，感覚障害 | |
| GQ1b | IgG | 何らかの呼吸器感染 *C. jejuni*, *H. Influenzae* | FS，眼球運動を伴う GBS | 動眼神経，滑車神経，外転神経の傍絞輪部ミエリン，後根神経節 |
| GT1a | IgG | | 嚥下障害，咽頭頸部上腕型 GBS | |
| GD3 | IgG | | AIDP | |
| LM1 | IgG | 何らかの呼吸器感染 | AIDP | ミエリン |
| Gal-C | IgG, IgM | マイコプラズマ肺炎 | | ミエリン |
| ＜GSC 抗原＞ | | | | |
| GM1/GD1a | IgG | 消化器感染(*C. jejuni*) | GBS(17%) | |
| GM1/GT1b | IgG | 消化器感染(*C. jejuni*) | GBS(12%) | |
| GD1a/GD1b | IgG | 消化器感染(*C. jejuni*) | GBS(7%)：重症，下位脳神経障害 | |
| GD1b/GT1b | IgG | 消化器感染(*C. jejuni*) | GBS(6%)：重症，下位脳神経障害 | |
| GM1/GalNAc-GD1a | IgG | 何らかの呼吸器感染 | GBS(3-11%)：純粋運動型，AMCBN | |
| GM1/GQ1b, GM1/GT1a, GD1b/GQ1b, GD1b/GT1a | IgG | | FS(41%)，眼球運動障害を伴う GBS(28%) | |
| GD1a/GQ1b, GD1a/GT1a, GT1b/GQ1b, GT1b/GT1a | IgG | | FS(6%)，眼球運動障害を伴う GBS(19%) | |

GBS：Guillain-Barré syndrome, *C. jejuni*：*Campylobacter jejuni*, *H. influenzae*：*Haemophilus influenzae*
AMAN：acute motor axonal neuropathy, AIDP：acute inflammatory demyelinating polyradiculoneuropathy, FS：Fisher syndrome
Gal-C：galactocerebroside, GSC：ganglioside complex, AMCBN：acute motor conduction block neuropathy

酸数によりいくつかのガングリオシド分画に分けて抗原として用いている．

最近われわれは2種類のガングリオシドを混合し作製した複合抗原が GBS および Fisher 症候群(FS)において標的抗原となることを見出した[5]．これは単独のガングリオシド抗原にはほとんど反応しないが，2種のガングリオシドを混合した際に初めて強く反応するものである．この抗体は複合抗原において形成される新たな複合エピトープを特異的に認識していると考えられる．われわれはこの複合抗原をガングリオシド複合体(ganglioside complex, GSC)と名付け，GBS およびその亜型において抗 GSC 抗体の病因的意義を調べている(図2)．この抗 GSC 抗体の発見以来，われわれの研究室では GSC に対する抗体も適宜測定している．また，フォスファチジン酸(phosphatidic acid, PA)をガングリオシド抗原に加えることによって，一部の抗ガングリオシド抗体活性が増強されることを見出し[6]，単独ガングリオシド抗原に PA を加えた検索も行っている．

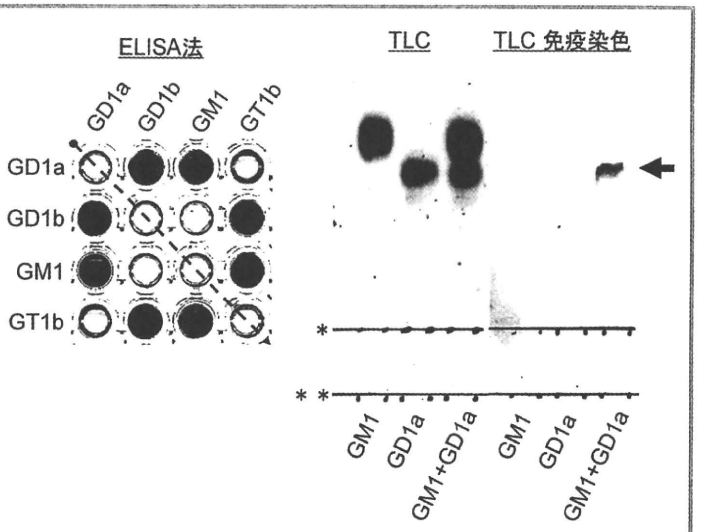


**図 2　ELISA と TLC 免疫染色による抗ガングリオシド複合体抗体測定**

ELISA(左)では，GD1a/GD1b, GM1/GD1a, GD1b/GT1b, GM1/GT1b に対する IgG 抗体が陽性である．TLC 免疫染色(右)では GM1，GD1a のオーバーラップした箇所に反応がみられ(矢印)，GM1/GD1a 抗体陽性である．＊，＊＊はそれぞれ GD1a，GM1 をアプライした基線．

## 2．各抗ガングリオシド抗体の病的意義

### A．抗 GM1 抗体

GBS で最も出現頻度の高い抗体である．感覚障害がなく，脳神経障害に乏しい純粋運動型 GBS に相関しており，運動軸索型 GBS（AMAN；acute motor axonal neuropathy）との関連も指摘されている．*Campylobacter jejuni* 腸炎を先行感染とすることが多く，実際，抗 GM1 抗体陽性 GBS 例の便から分離培養された *Campylobacter jejuni* の LOS に GM1 様構造が存在することが明らかにされている[7]．ヒト運動神経における抗 GM1 抗体の標的部位は明確にはなっていないが，AMAN 症例の病理学的検索や動物組織の検討から，運動神経 Ranvier 絞輪部の軸索膜上に GM1 は豊富に存在していると考えられている[8]．GM1 を含むガングリオシド混合物を感作した動物モデルの検討では，惹起された IgG 抗 GM1 抗体が運動神経 Ranvier 絞輪軸索膜に作用し，補体活性化を通じて Na チャンネルのクラスターを破壊することが観察されている[9]．抗 GM1 抗体は類似した糖鎖をもつ GD1b と反応することも多いが，GM1 に特異性の高い抗 GM1 抗体が重症度と相関する可能性が指摘されている[10]．

### B．抗 GalNAc-GD1a 抗体

GD1a の糖鎖末端に N-アセチルガラクトサミン基（N-acetylgalactosamine；GalNAc）をもつ GalNAc-GD1a は，ヒト脳，末梢神経に存在する微量ガングリオシドである．ヒト末梢神経では前根，筋内神経の Ranvier 絞輪部とその周辺の軸索膜およびミエリン最内側部に豊富に存在している．IgG 抗 GalNAc-GD1a 抗体陽性 GBS は先行感染に *Campylobacter jejuni* 腸炎が多く，遠位優位の筋力低下を呈する脳神経障害のない純粋運動型である[4]．電気生理学的に軸索障害型を示す．神経筋培養系における検討では，抗 GalNAc-GD1a 抗体が運動神経終末における電位依存性 Ca チャンネルを抑制する作用が観察されている[11]．

一部の GBS 例で IgG でなく IgM クラスの抗 GalNAc-GD1a 抗体のみ上昇している例があり，先行感染が *Campylobacter jejuni* 腸炎であれば抗体反応性が GalNAc-GD1a 特異的であり，臨床的に純粋運動型を示す．一方，先行感染がサイトメガロウイルスであれば GM2 と交叉反応を示す抗体であり，顔面神経麻痺，感覚障害を高頻度に示す[12]．

### C．抗 GD1a 抗体

GD1a もヒト神経系における主要なガングリオシドである．IgG 抗 GD1a 抗体陽性 GBS は運動軸索型 GBS（AMAN）を呈する．最近の研究で，抗 GD1a 抗体は運動神経終末だけでなく筋内運動神経線維の Ranvier 絞輪において補体依存性に軸索障害をきたすことが明らかにされている[13]．抗 GD1a 抗体は障害神経の再生を抑制する作用をもち，回復遅延をきたす可能性がある．

### D．抗 GM1b 抗体

多数例の解析で，抗 GM1b 抗体陽性 GBS は消化器感染が先行する純粋運動型 GBS であり，進行は速いものの免疫グロブリン静注療法への反応がよいことが示されている[14]．抗 GM1b 抗体陽性例の 36％に抗 GalNAc-GD1a 抗体が，32％に抗 GM1 抗体が同時に上昇している．

### E．抗 GQ1b 抗体

FS は外眼筋支配神経（動眼・滑車・外転神経）の麻痺，運動失調，腱反射消失を三徴とする GBS の亜型である．抗 GQ1b 抗体は FS の 90％以上の症例に陽性であり，免疫介在性ニューロパチーにおいてもっとも疾患特異的なマーカーである．一方，外眼筋麻痺を伴う GBS においても抗 GQ1b 抗体は有意に高頻度であり，同抗体は GBS において外眼筋麻痺と強い相関をもつ．また，外眼筋麻痺の明らかでない不全型 FS の検討で運動失調と抗 GQ1b 抗体の相関も認められている．ヒト末梢神経では外眼筋支配神経の傍絞輪部ミエリン，一部の後根神経節大型細胞に豊富に GQ1b が存在しており，このような GQ1b の独特の局在が FS 発症に強く関わっていると考えられている[15]．また，人工呼吸器装着を要する GBS では抗 GQ1b 抗体陽性例が有意に多く，GBS における呼吸筋麻痺にも何らかの影響を及ぼしている可能性がある．さらに，FS の特徴をもちながら中枢神経障害も伴う Bickerstaff 型脳幹脳炎でも本抗体が陽性となる．

### F．抗 GD1b 抗体

GBS にみられる抗 GD1b 抗体はその反応特異性から主に 2 つに分類される．すなわち，GM1，asialo-GM1（GA1）と交叉反応を示すタイプと，GD1b に特異的なタイプである．前者は GM1，GD1b に共通する糖鎖 Gal-GalNAc を認識するもので，抗 GM1 抗体とともに上昇することがほとんどである．GM1 と交叉反応する抗 GD1b 抗体をもつ例

の臨床像は抗GM1抗体陽性GBSと同じである．一方，GD1b特異的抗体は感覚失調症状と相関している．ヒト後根神経節の大型細胞にGD1bが局在すること，GD1b感作家兎においてGD1b特異的抗体が失調性ニューロパチーの発症に関与し，後索，後根神経節大型細胞の障害がみられることも感覚性運動失調との相関を支持している[16]．さらに，感覚性運動失調性ニューロパチーを発症した家兎の後根神経節では，深部感覚を担っていると考えられる大型細胞のアポトーシスが観察され，GD1b特異的抗体がこのアポトーシスの発生に関与している可能性がある[17]．最近われわれは，従来の単独ガングリオシド抗原を用いたスクリーニングでIgG抗GD1b抗体が単独陽性である血清を集め，GD1bを含むGSCを抗原としてその反応性を検討した．その結果，運動失調をきたすGBSの抗GD1b抗体はGD1bに極めて特異性が高いことが明らかになった[18]．以上のように，抗GD1b抗体の特異性を評価するためにはGSC抗原を用いた検討も必要である．

**G．抗GT1a抗体**

抗GQ1b抗体と同時に上昇することが多く，GQ1bと共通する糖鎖を認識していると考えられている．GBSの亜型，咽頭頸部上腕型GBSと抗GT1a抗体との関連を示唆する報告は多いが，明確な結論は出ていない．GQ1bと交叉反応を示さないGT1a特異的抗体はGBSの球麻痺症状の発症に重要であると考えられている．ヒト末梢神経におけるGT1aの局在は不明である．

**H．抗ガングリオシド複合体(GSC)抗体**

2種のガングリオシドの混合抗原(GSC)を特異的に認識する抗体が一部のGBSおよびFSにおいてみられる(表)．主要なガングリオシドGM1，GD1a，GD1b，GT1bの2つを組み合わせて抗GSC抗体を検索したところ，17%が陽性であった．抗GD1a/GD1b抗体あるいは抗GD1b/GT1b抗体陽性例は重症で高頻度に人工呼吸器装着を要し，下位脳神経障害が多いという特徴を示しており，これらの抗GSC抗体は重症化と相関する可能性がある[19]．GM1とGalNAc-GD1aの混合抗原に特異的に反応する抗体が数%のGBS例にみられる[20]．抗GM1/GalNAc-GD1a抗体陽性GBS 10例の検討では，ほとんどが純粋運動型GBSであり，生理的圧迫部位ではなく運動神経幹中間部に病初期から伝導ブロックがみられることが特徴であった．この伝導ブロックは治療後早期に回復し，経過を通じて再髄鞘化を示す所見に乏しいことから，Ranvier絞輪部における軸索機能障害による可逆性伝導障害であると推測されている．運動神経Ranvier絞輪部軸索膜に集簇して存在するGM1，GalNAc-GD1aが複合体を形成し，純粋運動型GBSの標的抗原となっている可能性がある．

FSでは上記の組み合わせのGSCに対する抗体はみられず，GQ1bあるいはGT1aを含むGSCに対する抗体を約半数に認める[21,22]．FSにおける抗ガングリオシド抗体は反応特異性の点から，GQ1b特異的抗体，GQ1b/GM1の糖鎖エピトープに反応する抗体，GQ1b/GD1aの糖鎖エピトープに反応する抗体の3つに分類され，抗GQ1b/GM1抗体陽性例では感覚障害の少ない傾向がみられている[21,22]．これまでGQ1bそのものが標的抗原であると考えられてきたが，実際の細胞膜上ではGQ1bはGM1あるいはGD1aとGSCを形成して標的抗原となっている可能性がある．

以上のように抗ガングリオシド抗体の病因的意義，診断マーカーとしての有用性を検討するにあたり，GSCに対する反応性を検証することが重要である．最近の研究で，GSCは単独ガングリオシドよりも細胞内情報伝達に強い影響を及ぼす可能性が指摘されている[23]．従って，抗GSC抗体による神経障害について，補体活性化を介する神経障害だけではなく神経細胞機能を直接障害するようなメカニズムも今後検証していく必要があろう．

**I．その他の抗糖脂質抗体**

マイコプラズマ感染後GBSにガラクトセレブロシド(galactocerebroside, Gal-C)に対する抗体が上昇することが多く，脱髄と関連すると考えられている．Gal-C感作家兎では脱髄型ニューロパチーがみられている．抗GM1抗体も同時に上昇する場合は軸索型を示す症例もあり，マイコプラズマ脂質分画と交叉反応する抗GM1抗体が病的意義をもつ可能性が指摘されている．サイトメガロウイルス感染後GBSでは抗GM2抗体の上昇がみられ，感覚障害，顔面神経麻痺を示す症例が多い．その他，末梢神経ミエリンに豊富に存在するLM1(sialosylneolactotetraosylceramide)に対するIgG抗体をもつGBSは脱髄型が多い．

## その他の分子マーカー

これまでGBSの病態に強い相関をもつミエリン蛋白分

子，軸索蛋白分子に対する自己抗体は報告されていない．Schwann 細胞の細胞骨格蛋白である glial fibrillary acidic protein が GBS の軸索障害と相関するマーカーとなる可能性が報告されているが議論もある[24]．

## むすび

近年の研究で，GBS の病態として補体活性化を介した神経障害，補体非依存性のイオンチャンネル障害，後根神経節細胞のアポトーシスなどが推測されており，抗ガングリオシド抗体がこれらの病態に強く関与していることが示されている．抗ガングリオシド抗体は GBS およびその亜型においてもっとも重要な分子マーカーとして広く認識されつつあるが，その病的作用の発現には様々な因子が関連していることを理解する必要がある[25]．抗体の反応特異性のほかに，標的ガングリオシド抗原の末梢神経における特異な局在とその量，細胞膜上の GSC 形成のような標的抗原周辺の複雑な糖脂質環境，標的抗原の糖鎖エピトープの立体構造なども抗原抗体反応に影響を及ぼすと考えられている．抗ガングリオシド抗体の病的意義を考えるとき，このような条件・因子を考慮する必要がある．今後抗 GSC 抗体や PA 添加抗原に対する抗体検索のほかに，細胞膜上に発現させた標的糖脂質抗原への結合を解析するなど，従来の抗体測定法を工夫することによって抗ガングリオシド抗体の病的作用のメカニズムがさらに明らかとなることが期待される．

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# Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica

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**Neuromyelitis optica (NMO) is an inflammatory disease affecting the optic nerve and spinal cord, in which autoantibodies against aquaporin 4 (AQP4) water channel protein probably play a pathogenic role. Here we show that a B-cell subpopulation, exhibiting the $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ phenotype, is selectively increased in the peripheral blood of NMO patients and that anti-AQP4 antibodies (AQP4-Abs) are mainly produced by these cells in the blood of these patients. These B cells showed the morphological as well as the phenotypical characteristics of plasmablasts (PB) and were further expanded during NMO relapse. We also demonstrate that interleukin 6 (IL-6), shown to be increased in NMO, enhanced the survival of PB as well as their AQP4-Ab secretion, whereas the blockade of IL-6 receptor (IL-6R) signaling by anti–IL-6R antibody reduced the survival of PB in vitro. These results indicate that the IL-6–dependent B-cell subpopulation is involved in the pathogenesis of NMO, thereby providing a therapeutic strategy for targeting IL-6R signaling.**

neuroinflamatory disease | autoimmunity | multiple sclerosis | central nervous system | IL-6 receptor blockade

Neuromyelitis optica (NMO) is an inflammatory demyelinating disorder characterized by recurrent attacks of severe optic neuritis and myelitis. Unlike the conventional form of multiple sclerosis (CMS), the lesions of NMO tend to spare the cerebral white matter, especially during the early stage (1), and even a single episode of attack can cause serious neurological deficits such as total blindness and paraplegia. Accordingly, accumulation of irreversible damage to the central nervous system (CNS) along with rapid progression of disability is more frequently found in NMO compared with CMS (2).

NMO can be distinguished from CMS by clinical, neuroimaging, and serological criteria (3). It is now known that serum anti-aquaporin 4 (AQP4) autoantibodies can be used as a disease marker of NMO (1, 2). AQP4 is the most abundantly expressed water channel protein in the CNS and is highly expressed in the perimicrovessel astrocyte foot processes, glia limitans, and ependyma (4). Emerging clinical and pathological observations suggest that anti-AQP4 antibodies (AQP4-Abs) play a key role in the pathogenesis of NMO. Prior studies have documented a significant correlation of serum AQP4-Ab levels with the therapeutic efficacy of plasma exchange during clinical exacerbations of NMO (2, 5). In the CNS lesions of NMO, reduced expression of AQP4 on astrocytes is evident even during the early stage (6), which is followed by the occurrence of vasculocentric destruction of astrocytes associated with perivascular deposition of complement and IgG (7).

On the other hand, recent studies have suggested that AQP4-Abs alone are incapable of causing the clinical and pathological features of NMO. In fact, Hinson et al. emphasized the role of cellular immunity in combination with AQP4-Abs by showing that the attack severity of NMO was not correlated with serum AQP4-Ab levels (8). It was also demonstrated that direct injection of IgGs derived from NMO patients into the brains of naïve mice did not cause NMO-like lesions, although brain tissue destruction associated with leukocyte infiltration was elicited by coinjecting human complement (9). Other groups have shown that the passive transfer of IgGs from NMO patients to rats challenged with induction of experimental autoimmune encephalomyelitis (EAE) may cause a decrease in the expression of AQP4 in astrocytes along with worsening of clinical EAE (10–12). In contrast, the transfer of IgGs to unimmunized rats did not cause any pathology. These results suggest that induction of AQP4-Ab–mediated pathology in NMO depends on the presence of complement, leukocytes, and T cells.

Although AQP4-Ab–secreting cells are a potential target for therapy, detailed characteristics of AQP4-Ab–producing cells have not been clarified yet. Because some NMO patients have elevated serum anti-nuclear and anti–SS-A/SS-B Abs (1), as found in patients with systemic lupus erythematosus (SLE) or Sjögren syndrome, NMO might share common pathological mechanisms with these autoimmune diseases. Kikuchi et al. previously reported that $CD180^{-}$ B cells are activated B cells capable of producing autoantibodies in SLE (13). CD180 is a member of the leucine-rich repeat family of molecules with homology to Toll-like receptor 4 (14), which is highly expressed by naïve and memory B cells but not by plasma cells (15). Odendahl et al. demonstrated that $CD27^{high}CD38^{+}$ B cells, capable of producing high-affinity IgG (16), are increased in the peripheral blood of SLE patients with some correlation to disease activity (17). Considering the phenotypes of autoantibody-producing cells reported in SLE, we analyzed the expression of CD27, CD38, and CD180 on $CD19^{+}$ B cells in the peripheral blood of NMO patients. We found that $CD27^{high}CD38^{high}CD180^{-}$ B cells were significantly increased in AQP4-Ab seropositive patients diagnosed with NMO or NMO spectrum disorder (1) compared with healthy subjects (HS) or CMS patients. Notably, this B-cell subpopulation was found to be a major source of AQP4-Abs in the peripheral blood of AQP4-Ab seropositive patients and depended on interleukin-6 receptor (IL-6R) signaling for survival.

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