Ⅲ．研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

NEUROLOGY

Single nucleotide polymorphism of TAG-1 influences IVIg responsiveness of Japanese patients with CIDP

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Single nucleotide polymorphism of TAG-1 influences IVIg responsiveness of Japanese patients with CIDP

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ABSTRACT

Objective: Chronic inflammatory demyelinating polyneuropathy (CIDP) is characterized by immune-mediated peripheral demyelination. Although corticosteroid, IV immunoglobulin (IVIg) and plasma exchange have been established as the most effective therapeutics, subpopulations of patients show little or no response to either of these therapies. In this study, we examined whether particular genetic factors influence the therapeutic responsiveness of patients with CIDP.

Methods: One hundred Japanese patients categorized as responders or nonresponders to IVIg therapy participated in our study. We performed an association analysis with single nucleotide polymorphisms (SNPs) and haplotype studies between the IVIg responders and nonresponders.

Results: Two separate SNPs, corresponding to TAG-1 (transient axonal glycoprotein 1) and CLEC10A (C-type lectin domain family 10, member A), showed strong significant differences between responders and nonresponders. Haplotype analysis of a series of expanded SNPs, from TAG-1 or CLEC10A, showed that only TAG-1 included a significant haplotype within 1 linkage disequilibrium block, which accommodates IVIg responsiveness. Diplotype analysis of TAG-1 also supported this observation.

Conclusions: Transient axonal glycoprotein 1 is a crucial molecule involved in IV immunoglobulin responsiveness in Japanese patients with chronic inflammatory demyelinating polyneuropathy.
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GLOSSARY

CIDP = chronic inflammatory demyelinating polyneuropathy; **CMAP** = compound muscle action potential; **dbSNP** = Single Nucleotide Polymorphism database; **IVIg** = IV immunoglobulin; **LD** = linkage disequilibrium; **PNS** = peripheral nervous system; **SAP** = shrimp alkaline phosphatase; **SNP** = single nucleotide polymorphism.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an immune-mediated demyelinating polyneuropathy characterized by a relapsing or chronic progression during its clinical course, and symmetric-dominant motor and sensory impairments.[1-3] Although several heterogenic pathogenic factors are thought to be involved in the pathogenesis of CIDP, most of them remain to be elucidated. The therapeutic efficacy of corticosteroid, IV immunoglobulin (IVIg) and plasma exchange has been established by randomized clinical trials.[2,4-6] However, approximately 20% to 40% of patients show little or no response to each of these therapies, suggesting extensive heterogeneity in therapeutic responsiveness.[7,8] We have reported that the IVIg unresponsiveness of patients with CIDP is associated with a concomitant increase of axonal dysfunction that manifests as muscle atrophy or decreased compound muscle action potentials (CMAPs).[8] In accord with these findings, several reports have emphasized that damage to axons or axon–Schwann cell interactions are associated with the long-term prognosis and therapeutic responsiveness in CIDP.[8-11] Furthermore, the pathology of the Ranvier node, paranode, or juxtaparanode has been considered to be the main factor involved in the axonal damage.[12,13] Hence, IVIg responsiveness would be closely related to the pathologic features of the node, paranode, or juxtaparanode.

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Single nucleotide polymorphisms (SNPs) are genetic polymorphisms that may underlie the clinical phenotypes and therapeutic responsiveness in a wide range of diseases, including immune-mediated neurologic disorders. In this study, we assessed whether SNPs of candidate genes, which are particularly related to the function of the Ranvier node, paranode, or juxtaparanode, influence the therapeutic responsiveness of patients with CIDP. We observed that SNPs in TAG-1, which is a key molecule for axon–Schwann cell interactions and is distributed at the juxtaparanode, are related to IVIg responsiveness.[12,14-17]

METHODS

Subjects. DNA samples from 100 unrelated patients with CIDP, consisting of 56 men and 44 women aged 36 to 86 years, were analyzed in this study. The patients were those seen in Nagoya University Hospital and its affiliated hospitals during 1 year from 2005 to 2006 who fulfilled the diagnostic criteria for CIDP of the ad hoc subcommittee of the American Academy of Neurology AIDS Task Force.[1] All patients with positive M protein, a history of toxic exposure, and hereditary diseases were excluded from this study. All patients received IVIg therapy (400 mg/kg/day, 5 days in a row) as the first treatment after the onset of the disease. The ethnic background of all the subjects was Japanese.

Standard protocol approvals, registrations, and patient consents. The study was approved by the ethics committee of Nagoya University Graduate School of Medicine. Before participating in the study, informed consent was obtained from all patients.

Clinical measurement of IVIg efficacy. The overall disability sum score was assessed with the Inflammatory Neuropathy Cause and Treatment scale for the upper and lower limbs 1 day to 2 weeks before and 6 weeks after IVIg therapy.[18] Based on our previous study on responsiveness to IVIg therapy, patients who showed an improvement in their score of 1 or more in either limb after therapy were defined as responders to IVIg therapy, and those with no improvement or an exacerbation of symptoms were defined as nonresponders.[8] By this assessment, we defined 72 patients (male:female numbers = 41:31) as responders and 28 (male:female numbers = 15:13) as nonresponders. The clinical information of both responders and nonresponders was also collected and showed that axonal involvement, such as concomitance of muscle atrophy and decreased CMAPs, is an important feature of nonresponders (table e-1 on the *Neurology*® Web site at www.neurology.org).

Selection of candidate genes and SNPs. Because we have previously demonstrated that axonal impairment related to the Ranvier node, paranode, and juxtaparanode is likely to be associated with IVIg responsiveness, we selected molecules distributed on the axon or the myelin sheath as candidate genes.[19] In addition, we screened molecules that are suspected to be participants in cellular or humoral immunity in CIDP pathogenesis.[20] In addition, we only selected candidate genes that had SNPs located within their coding or regulatory regions by referring to the Single Nucleotide Polymorphism database (dbSNP; National Center for Biotechnology Information, NIH, Bethesda, MD), with a minor allele frequency over 10% in dbSNP, and that had nonsynonymous SNPs. Under these screening processes, we excluded the candidate genes *PMP22*, MPZ, Cx32, KCNA1, KCNA2, CNTN1, Caspr, and Caspr2 from this allelic and genotype association study. Thus, we finally adopted the SNPs of TAG-1, CLEC10A, BDNF, NRG3, TTRAP, TTPBP, CTLA4, and KIF1B for allelic and genotype association analyses (table e-2).

Genotyping for polymorphisms. Genotyping of candidate SNPs was performed by the MassARRAY system (Sequenom, Inc., San Diego, CA), chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of primer extension products after the PCR amplification.[21,22]

Extension primers, extended across the SNP site, were designed using SpectroDESIGNER software (Sequenom, Inc.). Either the forward or the reverse strand was chosen based on criteria such as appropriate GC content, avoidance of primer dimmer and hairpin structure, and the absence of any other SNP site. The extension reaction is controlled by a mixture of dideoxy-terminated nucleotides, such that 1 single-base extension product is created and 1 double-base extension product is created corresponding to an SNP allele. This scheme creates 2 peaks in the mass spectrometer that are separated by approximately 300 Da.

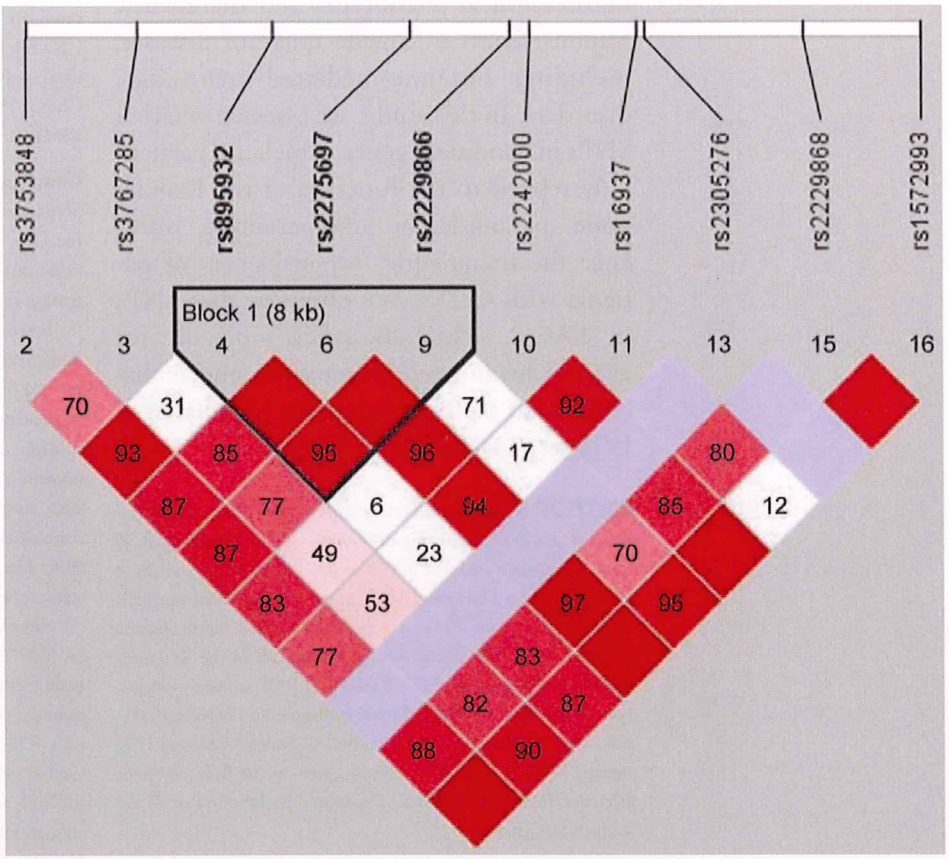
Sample preparation for MassARRAY was performed using an hME (homogeneous mass extend) assay according to the manufacturer's standard procedure. PCR was performed under standard conditions for all assays except the annealing temperature. The volume was 5 μL, including HotStarTaq (Qiagen GmbH, Hilden, Germany), deoxyribonucleotide-triphosphate (dNTP), and PCR primers. Less than 2.5 ng of genomic DNA is enough for a reaction. The samples were denatured at 95°C for 15 minutes and then subjected to 45 cycles of 95°C (20 seconds) for denaturation, optimum temperature for each primer set (56°C in standard; 30 seconds) for annealing, and 72°C (60 seconds) for elongation, followed by 5 minutes at 72°C.

Unincorporated dNTPs are deactivated using 0.3 U of shrimp alkaline phosphatase (SAP) followed by primer extension (MassEXTEND) reaction. Samples including SAP are incubated for 20 minutes at 37°C followed by 85°C for 5 minutes to inactivate the SAP.

MassEXTEND reactions were performed after PCR in several groups of different termination mixtures [e.g., dideoxyadenosine-triphosphate (ddATP), dideoxycytidine-triphosphate (ddCTP), dideoxythymidine-triphosphate (ddTTP), deoxyguanosine-triphosphate (dGTP)] according to each assay design.[21,22] The total reaction volume was 9 μL, including extension primer, Thermosequenase (Amersham Pharmacia Biotech, Buckinghamshire, UK), and the appropriate termination mix. Universal thermal cycling was applied (94°C for 2 minutes, after 40 cycles of 94°C for 5 seconds, 52°C for 5 seconds, and 72°C for 5 seconds). After desalting of the MassEXTEND reaction products with SpectroCLEAN reagent (Sequenom, Inc.), approximately 10 nL was loaded onto SpectroCHIPs (Sequenom, Inc.) preloaded with matrix. SpectroCHIPs were analyzed in fully automated mode by a MassARRAY mass spectrometer (Bruker-Sequenom, San Diego, CA).

SpectroTYPER software (Sequenom, Inc.) performs genotype calling automatically using a set of digital filters optimized for mass spectra of oligonucleotide. Quality values are attached to each genotyping result, such as conservative (high quality), moderate, aggressive (not so good), and low probability. Only conservative and moderate calls were adopted in this study. Samples with low quality value were reanalyzed.

Figure Linkage disequilibrium plot of a series of single nucleotide polymorphisms in TAG-1 and CLEC10A



Haploview program between IV immunoglobulin (IVIg) responders (n = 72) and nonresponders (n = 28) indicates 1 specific linkage disequilibrium (LD) block (8 kb) constructed by a series of single nucleotide polymorphisms in TAG-1, whereas CLEC10A has no LD block in the whole sequence (data not shown). This LD block of TAG-1 is associated with IVIg responsiveness.

Statistical analysis. The allelic and genotype distributions were tested for association by the Fisher exact test. To estimate the degree of linkage disequilibrium, the standardized disequilibrium coefficient (D′) was calculated, and a linkage disequilibrium (LD) block was defined by the program Haploview (version 4.1, Broad Institute, Cambridge, MA).[23] To check Hardy-Weinberg equilibrium, the linkage disequilibrium block, and case-control association, we used Haploview.[23,24] The method of LD definition was due to confidence intervals.[22,25] For a multiple regression test for SNPs, we applied permutation tests of haplotype in LD block because the Bonferroni correction is rather conservative and too restrictive.[26,27]

RESULTS **Allelic and genotype association study.** Among the candidate SNPs, 2 SNPs (rs2275697, rs90951) from 2 genes (TAG-1, CLEC10A) showed a highly significant association to responders and nonresponders (table e-2). We further selected a series of SNPs that covered the whole sequence of each gene, including both nonsynonymous and synonymous SNPs: 16 SNPs from TAG-1 and 7 SNPs from CLEC10A (table e-3). Because some SNPs were extremely rare and showed only 1 allele type, we excluded them, leaving 9 SNPs from TAG-1 and 4 SNPs from CLEC10A that were applied for further haplotype analysis (table e-3).

Construction of linkage disequilibrium blocks. In linkage disequilibrium analysis, D′ values were close to 1 in one segment and were defined as a linkage disequilibrium block. We applied Haploview to confirm whether a specific LD block existed (figure). As a result, no specific block was detected in CLEC10A, whereas TAG-1 showed a significant LD block (rs895932-rs2275697-rs229866) of approximately 8 kb, longer than would be expected given the high local recombination rate (figure). Hardy-Weinberg equilibrium was also examined for each genotyped SNP of TAG-1, and no significance was identified (table e-4).

Haplotype and diplotype analysis of TAG-1. Haplotype analysis of each genotyped SNP of TAG-1 was performed with Haploview, and we also analyzed the significance of the haplotype (rs895932-rs2275697-rs229866) within the LD block between responders and nonresponders. Two haplotypes within 4 major haplotypes showed a strong association to IVIg responsiveness. Haplotype G-A-T frequently occurred in responders (56.5% of responders vs 33.9% of

Table TAG-1 haplotype associations between IVIg responders and nonresponders, with permutation *p* values

Haplotype	Frequency	Responder, nonresponder frequencies	χ^2	*p* Value	Permutation *p* value
GAT	0.501	0.565, 0.339	8.185	0.0042	0.0106
GGT	0.258	0.185, 0.446	14.31	0.0002	0.0003
AGC	0.140	0.153, 0.107	0.707	0.4005	0.8203
AGT	0.095	0.097, 0.090	0.027	0.8683	1

From the haplotype association analysis, the haplotype GAT significantly correlated to IV immunoglobulin (IVIg) responders, whereas the haplotype GGT strongly correlated to nonresponders.

nonresponders, $p = 0.0042$), whereas haplotype G-G-T was overrepresented in nonresponders (18.5% of responders vs 44.6% of nonresponders, $p = 0.0002$) (table). Both the haplotypes maintained the significance with the permutation test of Haploview (table).

Next, we performed diplotype analysis focused on the haplotypes G-A-T (H1) and G-G-T (H2) and the other identified haplotypes (Hx) between the IVIg responders and nonresponders. Among the total patients with CIDP (n = 100), we could generate 5 types of diplotypes, such as H1/H1 (responders 84.0%, nonresponders 16.0%), H1/Hx (none), Hx/Hx (responders 100%, nonresponders 0%), H1/H2 (responders 75.0%, nonresponders 25.0%), H2/Hx (responders 69.2%, nonresponders 30.8%), and H2/H2 (responders 22.2%, nonresponders 77.8%). From the diplotype analysis, a haplotype G-G-T recessive model was well correlated with the IVIg nonresponders (Pearson, $p < 0.005$).

DISCUSSION TAG-1, a nerve-specific adhesion molecule, is distributed not only on the axon but also on the myelin sheath of the juxtaparanode and belongs to the immunoglobulin superfamily.[12,14,28] Though the functional role of TAG-1 is not fully understood, TAG-1 on the myelin sheath is supposed to interact with another TAG-1 molecule on the opposite side of the axon surface acting as a trans axon-myelin interaction and with other molecules on the axon, such as Caspr2, which also interacts with voltage-dependent potassium channels (Kv1.1 and Kv1.2) acting as cis axonal maintenance on the axon.[12,14,16,29-31] Our data indicated that TAG-1 could have a crucial role in IVIg responsiveness in CIDP through the role of TAG-1 in axonal maintenance, via TAG-1 itself or through molecules that are involved in the IVIg responsiveness in CIDP. Interestingly, within the significant haplotype, rs2275697 has nonsynonymous characteristics and therefore generates heterogeneity of TAG-1 molecules. We have reported that the IVIg nonresponders showed more extensive axonal damage as indicated by significant muscle atrophy and a decrease in CMAPs.[8] Because one of the primary target sites in the pathogenesis of CIDP is considered to be the nodal or paranodal regions, axonal damage in these regions would affect the nonresponsiveness to IVIg therapy.[8] Knockout of TAG-1 can induce the disruption of juxtaparanodal molecules such as Caspr2 and Kv channels and finally decrease the number of Kv1.1 and 1.2 channels in the proper axonal region.[12,14] There are some reports describing TAG-1 and related molecules in the peripheral nervous system (PNS) compared with the CNS, and the TAG-1 knockout mouse did not show abnormalities in myelin thickness or nerve conduction velocity in sciatic nerves.[12,14] However, another TAG-1 knockout mouse possessed shortened internodes in the CNS.[29,32] It is supposed that TAG-1 has a role in inducing the proper distribution of potassium channels on the juxtaparanodes and thereby maintains axonal function in the PNS in the presence of Schwann cells or the myelin sheath.[28,33-36] Taken together, the immune-mediated region at the juxtaparanode would differentially influence axonal function through the different nonsynonymous SNPs of the TAG-1 gene with inflammatory demyelination caused by CIDP.

AUTHOR CONTRIBUTIONS

Dr. M. Iijima completed the statistical analysis.

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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

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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

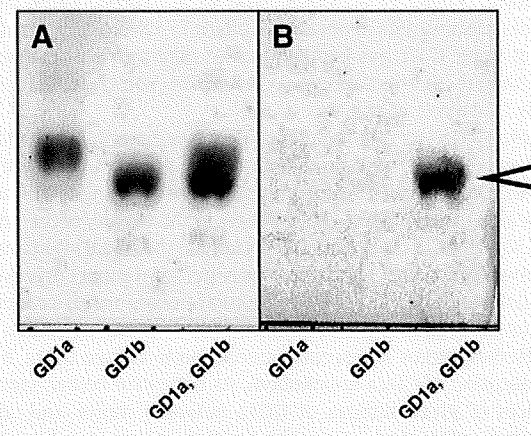

Fig. 1. Results from thin-layer chromatography (TLC). (A) TLC bands visualized with orcinol reagent. (B) TLC immunostaining using a representative anti-GD1a/GD1b-positive serum, showing that the overlapping region between GD1a and GD1b is strongly stained (arrowhead). Serum is diluted to 1:100.

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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.

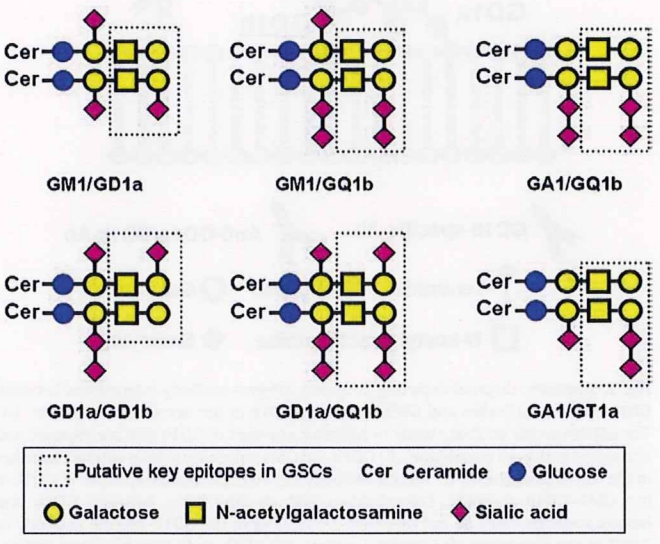

Fig. 2. Pattern diagrams of glycolipid complexes GM1/GD1a, GD1a/GD1b, GM1/GQ1b, GD1a/GQ1b, GA1/GQ1b, and GA1/GT1a. Squares with dotted lines indicate putative antigenic epitopes for antiganglioside complex antibodies.

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/

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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

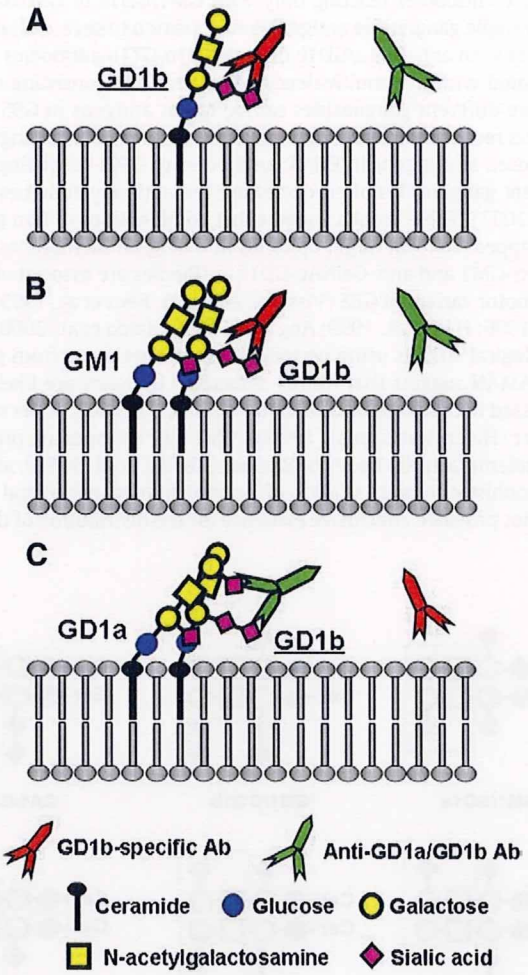

Fig. 3. Schematic diagram depicting proposed antigen–antibody interactions between GD1b-specific antibodies and GSCs containing GD1b in the nerve cell membrane. (A) The GD1b-specific antibody binds to antigenic epitopes of GD1b that are exposed and unmasked in the cell membrane. (B) GD1b and GM1 colocalize 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.

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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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Editorial

Put the right person in the right place: Segmental evaluation of the peripheral nerve for a diagnosis of CIDP ☆

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In every field, we must put the right person in the right place in order to make satisfactory progress in the work. The concept is exactly the case in a busy EMG clinic. Chronic inflammatory demyelinating polyneuropathy (CIDP) should be considered in any patients with progressive symmetric (or even asymmetric) polyneuropathy in whom the clinical course is progressive or relapsing–remitting for more than 2 months (Hughes et al., 2006). Particularly if there is proximal as well as distal muscle weakness, typical CIDP is strongly suggested. The distal nerve terminals and nerve roots, where the blood–nerve barrier is anatomically deficient (Olsson, 1990), are preferentially affected by immune attack in CIDP (Bromberg and Albers, 1993; Kuwabara et al., 2002), and this pattern of demyelinating lesions is presumably responsible for the "non-length-dependent" distribution of muscle weakness in typical CIDP.

Therefore, electrodiagnostic investigation for CIDP should focus on the nerve conduction abnormalities in the distal nerve terminal and nerve roots, in addition to examination of the nerve trunk. For the nerve terminal lesion, motor nerve conduction studies frequently show prolonged distal latencies and temporal dispersion of distally-evoked compound muscle action potential (CMAP), suggesting demyelination in the distal nerve segments (Thaisetthawatkul et al., 2002). In current electrodiagnostic criteria proposed by the European Federation of Neurological Societies and the Peripheral Nerve Society (EFNS/PNS), prolonged duration of distally-evoked CMAP (temporal dispersion of distal CMAP) has been added to detect nerve terminal demyelination, in addition to prolonged motor distal latency (Hughes et al., 2006).

On the other hand, it is sometimes difficult to evaluate the nerve roots and proximal portion of the peripheral nerves. F-waves are usually included in electrodiagnostic criteria, but the nerve root lesions can be reliably evaluated only when nerve conduction in the nerve trunk and distal portion is relatively preserved. F-waves may be absent due to motor axonal loss without demyelination, or to decreased excitability of anterior horn cells. Magnetic root stimulation may be used for the detection of proximal motor conduction in CIDP, but again, distal nerve lesion largely affects the results. Moreover, supramaximal stimulation is not guaranteed. The EFNS/PNS guideline recommends MRI of the spinal roots, and brachial or lumbar plexus (Hughes et al., 2006). Such MRI may demonstrate a prominent enlargement or abnormal contrast-enhancement of the nerves, suggestive of a diagnosis of CIDP, but findings could be equivocal, and the protocol of systematic MRI examination of the nerve roots needs to be established.

In the current issue of Clinical Neurophysiology, a paper by Tsukamoto and colleagues (Tsukamoto et al., 2010) nicely demonstrates proximal-dominant involvement in typical CIDP patients, by using the N8 and P15 components in tibial SEPs. Whereas N8 is a near-field potential recorded from the knee after tibial nerve stimulation at the ankle, P15 is a junctional potential that is generated at the greater sciatic foramen just distal to the dorsal root ganglion (Sonoo et al., 1992). Tibial nerve stimulation at the ankle elicits a stationary, dipolar potential P15 over the buttock with a reference electrode at the contralateral greater trochanter. P15 is distributed in the rostral and contralateral region. The derivation from the contralateral iliac crest to the ipsilateral greater trochanter registers a large P15 potential which is well free from artifacts. Sequential bipolar recording along the course of the sciatic nerve indicated that P15 is generated around the greater sciatic foramen. Comparison with a simulation study suggested that P15 is a junctional potential which is generated when the sciatic nerve enters the bone at the greater sciatic foramen.

Tsukamoto et al. investigated the N8 (generated from the nerve trunk at the knee), P15 (the greater sciatic foramen), and N21 (the spinal entry zone) components in 12 patients with CIDP, and compared the results with those in 17 with diabetic polyneuropathy. Analyses of latency differences in N8–P15, P15–N21, and N8–P15 showed proximal-dominant conduction slowing in 50% of the CIDP patients and in none of diabetic patients. Furthermore, normal N8 amplitude with decreased P15 or N21 amplitude was found for 83% of the CIDP patients, and 18% of diabetic patients. They conclude that when N8 and P15 components are recorded, tibial SEPs are highly sensitive and specific for the diagnosis of typical CIDP. The similar approach can be performed in median SEPs, analyses of N9 (generated in the brachial plexus) and N13 could provide the similar utility of segmental evaluation in the median sensory axons. However, so far there are few such reports. SEP is expected to be a useful tool to evaluate the proximal segments of the peripheral nerve, and this should be verified in the larger cohort of CIDP patients.

Because in SEP studies, the median nerve is stimulated at the wrist, and tibial nerve at the ankle, the examination entirely skip the distal part of the corresponding nerves, and therefore no information about involvement of the sensory nerve terminal portion

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can be obtained. In this regard, nerve conduction studies and SEPs are complementary; the former can be used to precisely detect demyelination in the motor nerve terminals, and the latter could provide detailed information in the proximal sensory nerve conduction. It is recommended to select the right test in the right place, presumably resulting in a significant increase in the sensitivity for the diagnosis of CIDP.

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