Anti-GQ1b antibody syndrome: anti-ganglioside complex reactivity determines clinical spectrum

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Background and purpose: Anti-GQ1b antibodies have been found in patients with Miller Fisher syndrome as well as its related conditions. Our aim was to identify the mechanism by which autoantibodies produce various clinical presentations in ‘anti-GQ1b antibody syndrome’.

Methods: Immunoglobulin G antibodies to ganglioside complex (GSC) of GQ1b or GT1a with GM1, GD1a, GD1b or GT1b were tested in sera from patients with anti-GQ1b (n = 708) or anti-GT1a (n = 696) IgG antibodies. Optical densities of the single anti-GQ1b or anti-GT1a antibodies were used as reference (100%), and those of anti-GSC antibodies were expressed in percentages to reference. The relationships between anti-GSC antibody reactivity and the corresponding clinical features were assessed by multivariate logistic regression analysis.

Results: Ophthalmoplegia and hypersomnolence were significantly associated with complex-attenuated anti-GQ1b and anti-GT1a antibodies. Ataxia was associated with GD1b- and GT1b-enhanced anti-GQ1b antibodies or GM1-enhanced anti-GT1a antibodies. Bulbar palsy was associated with GT1b-enhanced anti-GQ1b antibodies. Neck weakness was associated with GD1a-enhanced anti-GQ1b antibodies. Arm weakness was associated with GD1b-enhanced anti-GQ1b and GD1a-enhanced anti-GT1a antibodies. Leg weakness was associated with GD1a-enhanced anti-GQ1b and anti-GT1a antibodies.

Conclusions: Differences in fine specificity of anti-GQ1b antibodies are associated with clinical features, possibly due to the different expression of gangliosides in different parts of the nervous system.

Introduction
Anti-GQ1b antibodies, which cross-react with GT1a, are well known as biomarkers of Miller Fisher syndrome (MFS) characterized by ophthalmoplegia and ataxia [1]. The anti-GQ1b autoantibodies are also associated with incomplete forms of MFS [acute ophthalmoparesis without ataxia (AO) and acute ataxic neuropathy without ophthalmoplegia (AAN)] and its central nervous system subtype [Bickerstaff’s brainstem encephalitis (BBE)] and pharyngeal-cervical-brachial weakness (PCB) [2–5]. The identification of common autoantibodies in MFS and its various subtypes supports the notion that a spectrum exists encompassing the different conditions, which can be comprehensively referred to as the ‘anti-GQ1b antibody syndrome’ [6]. However, the mechanism by which the anti-GQ1b antibodies result in the different phenotypes remains unclear.

Some patients with Guillain–Barré syndrome (GBS) are seronegative for autoantibodies to single
glycolipids, but react to heteromeric complexes of two different gangliosides when mixed in a 1:1 molar ratio [7]. In contrast, there are also GBS patients who demonstrate strong immunoglobulin G (IgG) antibody reactivity towards single glycolipids but fail to react to heteromeric complexes [8]. Heteromeric complexes are defined as structurally distinct gangliosides that interact to form new molecular shapes capable of either enhancing or attenuating recognition by anti-ganglioside antibodies (Fig. 1a) [9]. A few studies have shown that the presence of these anti-ganglioside complex antibodies is associated with clinical severity and risk of mechanical ventilation [10,11].

To identify the mechanism by which anti-GQ1b and anti-GT1a antibodies result in different clinical presentations, the relationship between each clinical feature and the various complex-enhanced or complex-attenuated anti-GQ1b and anti-GT1a antibodies was investigated.

Methods

Serum samples

Sera from 10186 patients with various neurological disorders were sent to the neuroimmunology laboratory.

Figure 1 Anti-GQ1b antibodies enhanced, independent and attenuated with other gangliosides. (a) Heteromeric complexes are formed by structurally distinct gangliosides, which interact to form new epitopes capable of either enhancing or attenuating recognition by autoantibodies. Modified from [9] with permission. (b) The reaction of monoclonal anti-GQ1b antibody, FS1, is attenuated when GQ1b is mixed with GM1, GD1a, GD1b or GT1b antigens (blue). The reactivity of monoclonal anti-GQ1b antibody, FS3, remains unchanged when GQ1b is mixed with GM1, GD1a, GD1b and GT1b antigens (green). (c) The reactivity of anti-GQ1b antibodies from Patient 1 is attenuated when GQ1b is mixed with GM1, GD1a, GD1b or GT1b antigens (blue). The reactivity of anti-GQ1b antibodies from Patient 2 is enhanced when GQ1b is mixed with GM1 or GD1a (red); independent when GQ1b is mixed with GD1b (green); and attenuated when GQ1b is mixed with GT1b (blue). These patients did not have reactivity to single gangliosides GM1, GD1a, GD1b or GT1b.
at Dokkyo Medical University between 2001 and 2011. IgG autoantibodies to gangliosides GQ1b, GT1a, GM1, GM1b, GD1a, GD1b and GalNAc-GD1a were tested by an enzyme-linked immunosorbent assay (ELISA) [12], and 915 sera with anti-GQ1b reactivity were stored at −80°C until use. The diagnosis of GBS and its variants was made based on published criteria [13]. The diagnosis of MFS was made in 537 patients, MFS/GBS overlap in 37 patients, GBS in 35 patients, BBE/GBS overlap in 37 patients, GBS in 35 patients, AAN in 20 patients and PCB in four patients. Written informed consent was obtained from all patients. The study was approved by the Ethical Committee of Dokkyo Medical University.

Ganglioside complex antibodies

Serum IgG antibodies against individual gangliosides (GM1, GM1b, GD1a, GalNAc-GD1a, GT1a and GQ1b; 5 pmol/well) were measured by ELISA as described elsewhere. In this study, serum was considered positive for anti-GQ1b (n = 708) or anti-GT1a (n = 696) antibodies when the optical density was 0.5 or more at a dilution of 1:500 [14]. The presence of enhanced or attenuated reactivity of IgG antibodies to complexes of GQ1b or GT1a with GM1, GD1a, GD1b or GT1b (each ganglioside 5 pmol/well) was then investigated. Each sample was done in triplicate. Human sera (diluted to 1:500 or more) or murine monoclonal anti-GQ1b antibodies (FS1 and FS3) [15] were added as the first antibodies. For each serum sample, immunoreactivity to single anti-GQ1b or single anti-GT1a antibodies expressed in optical densities (OD) was used as reference (100%), and the immunoreactivity of antibodies to each GSC was expressed in percentage increase or decrease compared to the reference. Reactivity of anti-GSC that was >100% suggested an enhancement of anti-GQ1b or anti-GT1a antibody affinity, whereas reactivity of anti-GSC of <100% suggested an attenuated affinity of the corresponding anti-GQ1b or anti-GT1a antibodies. The relationship between antibody activity and the relevant clinical features – including ophthalmoplegia, ataxia, hypsarrhythmia, bulbar palsy, neck weakness, arm weakness and leg weakness – was then investigated.

Statistics

The association between each clinical feature and the antibodies positive to a single ganglioside was tested by χ² or Fisher’s exact test using StatView version 5.0 (SAS Institute Inc., Cary, NC, USA). Differences in the antibody reactivity values to GSC with reference to single GQ1b or GT1a were examined by the Mann–Whitney U test and then multivariate logistic regression analysis. A difference was considered significant when the P value was <0.05.

Results

Patients who had autoantibodies not only to GQ1b or GT1a but also to GM1, GM1b, GD1a or GalNAc-GD1a were significantly associated with both neck, arm and leg weakness (anti-GQ1b antibody positive: n = 71, odds ratio 3.0, 2.6 and 2.0; 95% confidence interval 1.5–5.7, 1.6–4.4 and 1.2–3.6; respective P values 0.003, <0.001 and 0.02) (anti-GT1a antibody positive: n = 70, odds ratio 2.9, 2.7 and 2.1; 95% confidence interval 1.5–5.5, 1.6–4.4 and 1.2–3.9; respective P values 0.003, <0.001 and 0.01). However, a significant association was not seen with ophthalmoplegia, ataxia, hypsarrhythmia and oropharyngeal weakness. In contrast, a further 166 patients with limb weakness were seropositive for the anti-ganglioside antibodies tested.

Figure 1a depicts a representative image of the complex-enhanced and complex-attenuated anti-GQ1b antibodies. When seroreactivity of antibody in its single form or complex form remains the same, this is considered as a complex-independent reaction. Monoclonal anti-GQ1b antibody FS1 was attenuated in the presence of other gangliosides, whereas FS3 remained unaffected (Fig. 1b). Patients’ anti-GQ1b antibodies are not monoclonal but oligoclonal [16]. As a result, the anti-GQ1b antibodies may demonstrate an enhanced, independent or attenuated pattern of reactivity (Fig. 1c).

Figure 2 shows the relationship between each neurological sign and the anti-GSC antibody reactivities. Ophthalmoplegia was significantly associated with complex-attenuated anti-GQ1b/GD1a antibodies (median activity 83% vs. 113%, P = 0.037) and anti-GT1a/GD1a and anti-GT1a/GD1b antibodies (GT1a/GD1a, 76% vs. 116%, P = 0.0025; GT1a/GD1b, 93% vs. 152%, P < 0.001). Ataxia was significantly associated with complex-enhanced anti-GQ1b/GD1b and anti-GQ1b/GT1b but attenuation of anti-GQ1b/GD1a antibodies (GQ1b/GD1b, 110% vs. 96%, P = 0.0010; GQ1b/GT1b, 96% vs. 69%, P < 0.001; and GQ1b/GD1a, 82% vs. 97%, P = 0.015). Significant associations were also seen between ataxia and complex-enhanced anti-GT1a/GM1 and complex-attenuated anti-GT1a/GD1a and anti-GT1a/GD1b antibodies (GT1a/GM1, 130% vs. 111%, P = 0.002; GT1a/GD1a, 75% vs. 94%, P < 0.001; GT1a/GT1b, 58% vs. 73%, P = 0.045). Hypsarrhythmia was significantly associated with complex-attenuated anti-GQ1b/GD1b and anti-GQ1b/GT1b antibodies (GQ1b/GD1b, 84% vs. 110%, P = 0.0056; GQ1b/GT1b, 60% vs. 94%,...
As well as anti-GT1a/GM1 and anti-GT1a/GD1b antibodies (GT1a/GM1, 101% vs. 130%, \(P = 0.0012\); GT1a/GD1b, 70% vs. 99%, \(P < 0.001\)). Bulbar palsy was significantly associated with complex-enhanced anti-GQ1b/GT1b antibodies (GQ1b/GT1b, 105% vs. 87%, \(P = 0.013\)). Neck weakness was significantly associated with complex-enhanced anti-GQ1b/GD1a antibodies (GQ1b/GD1a, 116% vs. 81%, \(P < 0.001\)), anti-GT1a/GD1a, anti-GT1a/GD1b and anti-GT1a/GT1b antibodies (GT1a/GD1a, 107% vs. 75%, \(P < 0.001\); GT1a/GD1b, 121% vs. 94%, \(P = 0.0028\); GT1a/GT1b, 84% vs. 58%, \(P = 0.01\)). Arm weakness was significantly associated with complex-enhanced anti-GQ1b/GD1a and anti-GQ1b/GD1b antibodies (GQ1b/GD1a, 92% vs. 81%, \(P = 0.016\); GQ1b/GD1b, 122% vs. 104%, \(P = 0.0097\)) as well as complex-enhanced anti-GT1a/GD1a and anti-GT1a/GD1b antibodies (GT1a/GD1a, 90% vs. 74%, \(P = 0.018\); GT1a/GD1b, 110% vs. 92%, \(P = 0.0037\)). Leg weakness was significantly associated with complex-enhanced anti-GQ1b/GD1a and anti-GT1a/GD1a antibodies (GQ1b/GD1a, 92% vs. 82%,

![Figure 2](image-url)
$P = 0.027$, $\text{GT1a/GD1a, 91\% vs. 75\%, } P = 0.033$) but complex-attenuated anti-GQ1b/GT1b antibodies ($\text{GQ1b/GT1b, 83\% vs. 93\%, } P = 0.021$).

Multivariate logistic regression analysis was also performed and the following significant associations were found. Ophthalmoplegia was significantly associated with anti-GQ1b/GD1a antibodies and anti-GT1a/GD1b antibodies ($\text{P} = \text{0.0031, } < 0.001$, respectively). Ataxia was significantly associated with anti-GQ1b/GD1a, anti-GQ1b/GD1b and anti-GQ1b/GT1b antibodies ($\text{P} = \text{<0.0001, 0.029, 0.0001, respectively}$) and anti-GT1a/GM1 and anti-GT1a/GD1a antibodies ($\text{P} = \text{0.0031, <0.001, respectively}$). Hypersomnolence was significantly associated with anti-GQ1b/GD1b and anti-GT1a/GD1b antibodies ($\text{P} = \text{0.0031, <0.001, respectively}$). Bulbar palsy was significantly associated with anti-GQ1b/GT1b antibodies ($\text{P} = \text{0.0020}$). Neck weakness was significantly associated with anti-GQ1b/GD1a antibodies ($\text{P} = \text{0.0051}$). Arm weakness was significantly associated with anti-GQ1b/GD1b antibodies and anti-GT1a/GD1a antibodies ($\text{P} = \text{0.0050, 0.023, respectively}$). Leg weakness was significantly associated with anti-GQ1b/GD1a and anti-GQ1b/GT1b antibodies ($\text{P} = \text{0.020, 0.0052, respectively}$) and anti-GT1a/GD1a antibodies ($\text{P} = \text{0.030}$).

**Discussion**

In our earlier report of 194 patients with anti-GQ1b antibody syndrome, the distribution of the diagnosis of various subtypes in patients was as follows: 57\% for MFS, 16\% for MFS/GBS, 8\% for AO, 6\% for BBE, 6\% for BBE/GBS and 4\% for GBS [6]. Our present study shows similar results. In all 915 patients, the diagnosis of MFS was made in 59\% of patients, MFS/GBS 17\%, AO 10\%, BBE 4\%, BBE/GBS 4\%, GBS 4\%, AAN 2\% and PCB 0.4\%.

The anti-GQ1b antibodies are likely to have a pathogenic role in the development of MFS based on the following findings. (i) Antecedent infectious agents such as *Campylobacter jejuni* and *Haemophilus influenzae* bear a GQ1b epitope [15,17,18]. (ii) GQ1b is strongly expressed in the oculomotor nerves, large-diameter dorsal root ganglion neurons and muscle spindles [1,19,20]. (iii) Human anti-GQ1b antibodies in the presence of a complement kill rat dorsal root ganglion neurons and muscle spindles [1,19,20]. (iv) The anti-GQ1b antibodies from humans and mice mediate complement-dependent destruction of the murine motor nerve terminals [22]. However, the exact mechanism of why patients with MFS and anti-GQ1b antibodies develop different clinical signs is still unknown.

Our current study confirmed the significant relationship of limb weakness with the antibodies to GM1, GM1b, GD1a or GalNAc-GD1a. In addition, the autoantibodies were also associated with neck weakness, which has not been reported before. Seventy-eight patients who harbored autoantibodies to GM1, GM1b, GD1a or GalNAc-GD1a had limb weakness. A further 166 patients with limb weakness were seronegative for these antigens, raising the possibility

![Figure 3](image-url)
that they may have anti-GQ1b or anti-GT1a complex-enhanced antibodies, which are immunoreactive and responsible for the limb weakness.

Based on our results, a few possibilities were suggested for why the presence of anti-GQ1b antibodies results in different clinical presentations. (i) The expression sites of the target molecule, GQ1b, vary amongst individuals [23]. GQ1b may be expressed on the oculomotor nerves and muscle spindles in some individuals, giving rise to both ophthalmoplegia and ataxia. In others, it may be expressed exclusively on either oculomotor nerves or muscle spindles, causing them to develop ophthalmoplegia or ataxia, respectively. (ii) The accessibility of anti-GQ1b antibodies differs amongst patients. An in vitro study suggested that matrix metalloproteinase 9 disrupted the blood-brain barrier, inducing the development of BBE in some individuals [24]. (iii) Fine specificities of anti-GQ1b antibodies differ amongst conditions. In one study, anti-GQ1b antibodies that cross-reacted with GD1b were associated with deep sense impairment [25]. In other studies, antibodies to GQ1b/GM1 were associated with no sensory impairment [26,27]. However, a large number of autopsy materials are required to prove the hypothesis (i). Other phenotypic differences are not explained by hypotheses (ii) and (iii). Therefore, it is hypothesized that GQ1b or GT1a is expressed alone or in the form of complexes with different gangliosides at different sites, and these complex-enhanced or complex-attenuated anti-GQ1b or anti-GT1a antibodies differ amongst individuals, eventually giving rise to different clinical manifestations.

In the current study, it has been shown that different neurological signs were significantly associated with different complex-enhanced or complex-attenuated antibodies, suggesting that the expression of these complexes might be different in the nervous system (Fig. 3). To validate these hypotheses, further immunohistochemical studies incorporating various monoclonal antibodies are required to demonstrate the presence of the single glycolipid antigens and GSC antigens at these sites. The direct application of these findings to clinical practice may not be possible at this stage, but our study will undoubtedly guide future studies to look into the exact mechanism of the various clinical manifestations among patients with anti-GQ1b antibodies.

In conclusion, the presence of complex-enhanced or complex-attenuated anti-GQ1b or anti-GT1a antibodies may result in different binding affinities to single ganglioside (GQ1b or GT1a) or GSC antigens of GQ1b or GT1a, leading to the clinical spectrum seen in the anti-GQ1b antibody syndrome.

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