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Varied antibody reactivities and clinical relevance in anti-GQ1b antibody–related diseases

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Abstract

Objective

To investigate the relationship between antibody reactivities against glycolipid complexes and clinical features in Miller Fisher syndrome (MFS), Bickerstaff brainstem encephalitis (BBE), and Guillain-Barré syndrome with ophthalmoplegia (GBS-OP).

Methods

Using glycoarray, antibodies against 10 glycolipid antigens (GM1, GM2, GM4, GD1a, GD1b, GQ1b, galactocerebroside, lactosylceramide, GA1, and sulfatide) and 45 glycolipid complexes consisting 2 of the glycolipids were examined in the sera of 63 patients with GBS-OP, 37 patients with MFS, and 27 patients with BBE.

Results

Antibodies to antigens containing GQ1b were identified in 73% of patients with GBS-OP (46/63), 86.5% of patients with MFS (32/37), and 74.1% of patients with BBE (20/27), and GD1b-related antibodies were identified in 49.2% of patients with GBS-OP (31/63), 29.7% of patients with MFS (11/37), and 11.1% of patients with BBE (3/27). Comparing clinical features between patients with GBS-OP with and without both antibodies, the proportion of patients requiring artificial ventilation and presenting moderate or severe muscle weakness was higher in the positive group than in the negative group ($p = 0.017$ and $p = 0.046$, respectively).

Conclusions

Antibodies binding to antigens containing GD1b and to those containing GQ1b may be involved in the development of limb weakness and respiratory failure in anti-GQ1b antibody–related diseases.

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Glossary

BBE = Bickerstaff brainstem encephalitis; **BSA** = bovine serum albumin; **GBS** = Guillain-Barré syndrome; **IgM** = immunoglobulin M; **MFS** = Miller Fisher syndrome; **OP** = ophthalmoplegia; **PBS** = phosphate-buffered saline; **TLC** = thin-layer chromatography.

Antibodies to glycolipids, including gangliosides, are frequently detected in serum samples from patients with immune-mediated neuropathies such as Guillain-Barré syndrome (GBS), IgM (immunoglobulin M) paraproteinemic neuropathy, and multifocal motor neuropathy. In particular, anti-GQ1b antibodies are associated with ophthalmoplegia (OP), ataxia, and areflexia, resulting in the development of GBS with OP, Miller Fisher syndrome (MFS), and Bickerstaff brainstem encephalitis (BBE).

Recently, antibodies against glycolipid complexes were identified in GBS and MFS.[1–3] Glycolipid complexes containing GQ1b can be target antigens in such diseases. However, in these anti-GQ1b antibody–related diseases, the factors that induce clinical differences remain unclear. Here, we investigated the associations between antibody activities to various glycolipid complexes and the clinical features of anti-GQ1b antibody–related diseases, using a combinatorial glycoarray, which can be a useful tool for investigation of the reactivity against multiple glycolipid complexes as reported previously.[4–7]

Methods

Patients and serum samples

Acute-phase serum samples obtained from patients with neuroimmunologic diseases before treatment were sent to our laboratory from various hospitals throughout Japan for testing antiglycolipid antibodies using ELISA. We sent questionnaires to attending physicians of consecutive cases of GBS-OP, MFS, and BBE between 2015 and 2016. Finally, 168 patients, including 63 patients with GBS-OP, 37 with MFS, and 27 with BBE (probable BBE, 14 patients; definite BBE, 13 patients), were enrolled into the present study.

Diagnostic criteria

GBS was diagnosed according to the diagnostic criteria of Asbury and Cornblath,[8] and patients with GBS with weakness of 1 or more extraocular muscles were diagnosed as having GBS-OP. MFS was diagnosed as the presence of the clinical triad (OP, ataxia, and areflexia), without limb weakness, impairment of consciousness, and bulbar palsy. BBE was diagnosed according to the diagnostic criteria presented previously.[9] When a patient fulfilled both the GBS criteria and BBE criteria, the patient was included in the BBE group.

Combinatorial glycoarray

Antibodies against 10 glycolipid antigens (GM1, GM2, GM4, GD1a, GD1b, GQ1b, galactocerebroside, lactosylceramide, GA1, and sulfatide) and 45 glycolipid complexes involving 2 different individual glycolipids were investigated through a combinatorial glycoarray. Each glycolipid was reconstituted in 1:1 chloroform and methanol (1 mg/mL solution). The purity of these glycolipids was confirmed by thin-layer chromatography (TLC). The above glycolipids were diluted to a concentration of 100 μg/mL with methanol. Glycolipid complexes were created by mixing equal volumes of each glycolipid. Spots (0.1 μL of the 100 μg/mL glycolipid solution) were spaced 2 mm apart on a glass slide adhering to a polyvinylidene membrane using a TLC autosampler with winCATS software (Camag, Muttenz, Switzerland). Each sample was introduced in duplicate on 1 slide. After blocking the arrays using 2% (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 hour at room temperature, they were incubated with serum diluted at 1:100 with 1% (w/v) BSA in PBS for 2 hours at 4°C and were then washed with 0.1% (w/v) BSA in PBS for 15, 15, and 30 minutes. They were subsequently incubated with Alexafluor 555 conjugated goat anti-human IgG (H + L) cross-absorbed secondary antibodies (Thermo Fisher Scientific, Eugene, OR) diluted at 1:1,000 with 1% (w/v) BSA in PBS for 1 hour at 4°C and were then washed again. Finally, the glass slides were washed with distilled water for 5 minutes. Fluorescence signals of the arrays were scanned using Typhoon 9200 (GE Healthcare UK Ltd.), and image analysis was performed with Quent TL software (GE Healthcare UK Ltd.). Reactivity to a glycolipid or glycolipid complex was considered positive when the fluorescence intensity was higher than thrice the SD + mean of 41 healthy controls.

Statistical analysis

We compared the positive rates of antibodies against glycolipids and glycolipid complexes. The Bonferroni test was used for three-group comparisons. The χ^2 test or Fisher exact probability was used for 2-group comparisons. A 2-tailed p value <0.05 was considered statistically significant. All analyses were performed using SPSS software (IBM Corp., Armonk, NY).

Study approval and patient consents

This study was approved by the Internal Review Board of Kindai University Faculty of Medicine. All participants provided written informed consent.

Data availability

Anonymized data not published within the article will be shared by request from any qualified investigator.

Results

Patient characteristics

The characteristics of the patients with GBS-OP, MFS, and BBE are presented in table 1. In all 3 patient groups, antecedent infections were frequently observed, and most of them were upper respiratory tract infections. Patients requiring mechanical ventilation were frequent in the GBS-OP (31/63, 49.2%) and BBE (7/27, 25.9%) groups, whereas no patient required mechanical ventilation in the MFS group. Although ataxia was frequently observed in the MFS (37/37, 100%) and BBE (22/24, 91.7%) groups, it was noted in less than half of the patients in the GBS-OP group (39.6%, 25/63). Electrophysiologic data were available for 43 of the 63 patients in the GBS-OP group. A single nerve conduction study was performed at a median of 5 days (interquartile range 3–9 days) following onset. According to the criteria presented previously,[10] the condition in most patients with GBS-OP was

Table 1 Clinical features of patients with Guillain-Barré syndrome with ophthalmoplegia, Miller Fisher syndrome, and Bickerstaff brainstem encephalitis

	GBS-OP (n = 63)	MFS (n = 37)	BBE (n = 27)
Sex (male/female)	32/31	18/19	14/13
Age (y), median (range)	57 (1–87)	47 (22–89)	40 (15–77)
Preceding infection, n (%)	52 (83)	33 (89)	25 (93)
Upper respiratory infectious symptoms	42	29	19
Diarrhea	6	4	6
Other	4	0	0
Neurologic signs, n (%)			
Disturbance of consciousness	0 (0)	0 (0)	27 (100)
External ophthalmoplegia	63 (100)	37 (100)	27 (100)
Bulbar palsy	41 (65)	0 (0)	17[a] (74)
Ataxia	25 (40)	37 (100)	22[a] (92)
Tendon reflexess, n (%)			
Absent or decreased	63 (100)	37 (100)	17 (63)
Normal or brisk	0 (0)	0 (0)	10 (37)
Pathologic reflex	0 (0)	0 (0)	8 (30)
Sensory disturbance	47 (75)	24 (65)	15 (56)
Dysesthesia	40 (63)	17 (46)	12 (44)
Superficial sense impairment	17 (27)	1 (3)	3 (11)
Deep sense impairment	19 (30)	9 (24)	2 (7)
Limb weaknesss, n (%)			
Mild (MRC score = 4)	10 (37)	0 (0)	7[a] (27)
Moderate/severe (MRC score <4)	53 (63)	0 (0)	9[a] (35)
Artificial ventilation, n (%)	31 (49)	0 (0)	7 (26)
CSF, n (%)	n = 59	n = 33	n = 27
Albuminocytologic dissociation	23 (39)	10 (30)	5 (19)
Pleocytosis (≥5/μL)	11 (19)	2 (6)	9 (33)
Serum IgG anti-GQ1b antibodies (ELISA), n (%)	36 (57)	33 (89)	20 (74)

BBE = Bickerstaff brainstem encephalitis, GBS-OP = Guillain-Barré syndrome with ophthalmoplegia, MFS = Miller Fisher syndrome, MRC = Medical Research Council.
[a] Of 27 enrolled patients with BBE, bulbar palsy was evaluated in 23, ataxia in 24, and limb weakness in 26, as it was difficult to evaluate these symptoms owing to consciousness disturbance.

categorized as either unclassified (21/43, 48.8%) or acute inflammatory demyelinating polyneuropathy (18/43, 41.9%), whereas the condition in only 2 patients was categorized as acute motor axonal neuropathy (2/43, 4.7%). Among the patients with BBE, 9 (9/26, 34.6%) had moderate/severe limb weakness (Medical Research Council score < 4) and 8 (8/24, 30.0%) had pathologic reflex or hyperreflexia. Of the 8 presenting pyramidal signs, 2 were patients with definite BBE (2/13, 15.4%) and 6 were patients with probable BBE (6/14, 42.9%). Pleocytosis in CSF was more frequent in patients with BBE (9/27, 33.3%) than in patients with GBS-OP (11/59, 18.6%) and patients with MFS (2/33, 6.0%) (p = 0.336 and p = 0.024, respectively). The 9 patients with BBE with pleocytosis were composed of 5 patients with definite BBE (5/13, 38.5%) and 4 patients with probable BBE (4/14, 28.6%). Brain MRI showed abnormal findings in 1 patient with definite BBE (1/11, 9.1%) and 2 patients with probable BBE (2/13, 15.4%).

Detection of antibodies to glycolipids or glycolipid complexes using a glycoarray

IgG antibodies against isolated GQ1b were detected in 57.1% of patients with GBS-OP (36/63), 89.2% of patients with MFS (33/37), and 74.1% of patients with BBE (20/27) on ELISA. Of the 20 patients with BBE with anti-GQ1b IgG antibodies, 13 were patients with definite BBE (13/13, 100%) and 7 were patients with probable BBE (7/14, 50%). Using the glycoarray, the overall sensitivities of IgG antibodies to GQ1b or glycolipid complexes containing GQ1b were 73.0% in patients with GBS-OP (46/63), 86.5% in patients with MFS (32/37), and 74.1% in patients with BBE (20/27). On using both ELISA and glycoarray assays, the sensitivities of GQ1b-related IgG antibodies increased to 74.6% in patients with GBS-OP (47/63), 91.9% in patients with MFS (34/37), and 81.5% in patients with BBE (22/27).

Among 38 patients without IgG antibodies to GQ1b alone on ELISA, we detected IgG antibodies to glycolipid complexes containing GQ1b in 14 patients using the glycoarray. Most of these patients (11) had GBS-OP. Representative results for antibodies specific to various glycolipid complexes containing GQ1b are shown in figure, A.

Antibodies binding to GD1b-containing antigens (e.g., GM1/GD1b) were more frequently observed in patients with GBS-OP than in patients with MFS and BBE (p = 0.003 and p = 0.012, respectively). There were no differences in reactivities between patients with BBE and MFS. The details are shown in table 2. Patients with GBS-OP more frequently had both antibodies to GQ1b-containing antigens and those to GD1b-containing antigens than in patients with MFS and BBE (GBS-OP, 49.2% [31/63]; MFS, 29.7% [11/37]; BBE, 11.1% [3/27]; p = 0.132 and p < 0.001, respectively) (figure, B).

IgG reactivities to glycolipids or glycolipid complexes are visually shown in figure, C as a heat map.

Antibody binding to both GQ1b and GD1b in patients with GBS-OP

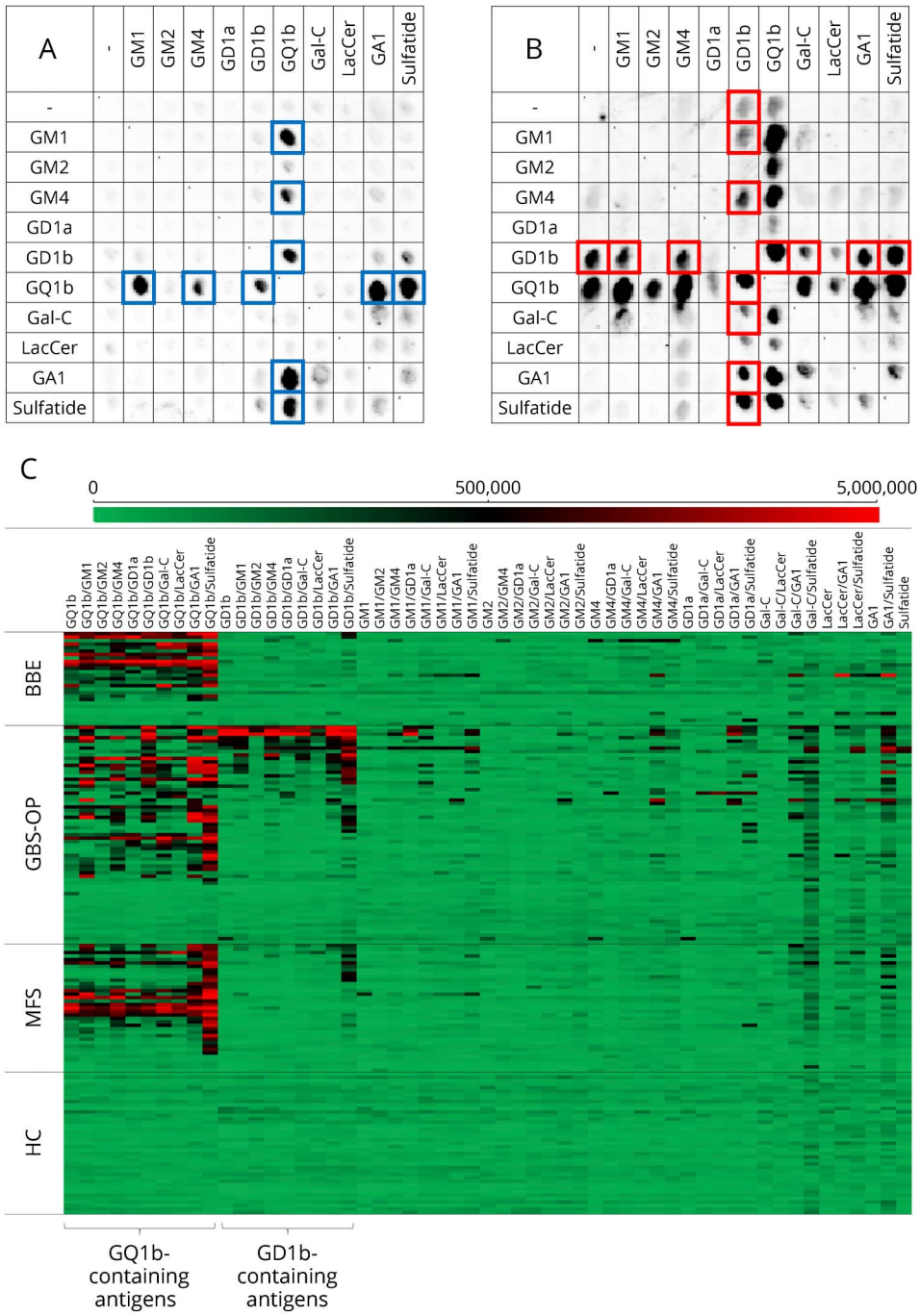
As the proportion of patients with both GQ1b-related antibodies and GD1b-related antibodies was higher in the GBS-OP group than in the MFS and BBE groups, we compared clinical features between patients with GBS-OP with these antibodies and other patients with GBS-OP. The frequency of artificial ventilation was higher in patients with GBS-OP positive for both antibodies (20/31, 64.5%) than in other patients with GBS-OP (11/32, 34.4%) (p = 0.017). In addition, moderate/severe limb weakness was more frequent in patients with GBS-OP positive for both antibodies (24/31, 77.4%) than in other patients with GBS-OP (16/32, 50.0%) (p = 0.046). The immunoabsorption test by ELISA showed that the activities of antibodies binding to both GQ1b and GD1b decreased by absorption using either GQ1b or GD1b antigen in patients with GBS-OP positive for both antibodies (data not shown).

Discussion

Our previous report has shown that IgG antibodies to GQ1b or glycolipid complexes containing GQ1b were more frequently observed in patients with GBS-OP than in patients with GBS without OP.[7] Those results would suggest that GQ1b-related antibodies are involved in OP. In addition, it has been reported that the presence of anti-GQ1b antibodies in patients with GBS may be a predictive factor of artificial ventilation.[11] An in vitro study showed that human and mouse anti-GQ1b antibodies have an alpha-latrotoxin-like blockade effect on neuromuscular transmission.[12] Moreover, an in vivo mouse model generated through intraperitoneal injection of anti-GQ1b antibodies and normal human serum showed respiratory paralysis due to transmission block at diaphragm neuromuscular junctions.[13] However, most patients with MFS having anti-GQ1b antibodies do not need artificial ventilation. It remains to be clarified why anti-GQ1b-positive GBS patients, but not MFS patients, show an association with artificial ventilation requirement. We found that antibodies against glycolipid complexes containing GD1b were more frequent in patients with GBS-OP than in patients with MFS or BBE. Moreover, artificial ventilation was more frequently required, and moderate/severe limb weakness was more common in patients with GBS-OP with both GQ1b-related antibodies and GD1b-related antibodies. Therefore, antibodies binding to not only GQ1b-containing antigens but also GD1b-containing antigens may be significantly associated with both muscle weakness and artificial ventilation requirement in patients with GBS-OP.

In the present study, no significant difference was identified between patients with MFS and BBE. Considering that there was no significant difference in antibody reactivities, we had difficulty in distinguishing patients with MFS from patients with BBE, particularly among patients with BBE who have normal MRI or CSF findings. This result indicates that BBE and MFS are closely related and could form a continuous spectrum.

Figure Representative antibody reactivities and heat map of antibody binding patterns



(A) Glycoarray grids are presented in duplicate with single glycolipids and their 1:1 heteromeric complexes. Specific binding to glycolipid complexes containing GQ1b antigens in a patient with BBE (blue boxes). (B) Antibodies binding to both GQ1b-containing antigens and GD1b-containing antigens are more frequently identified in patients with GBS-OP than in patients with MFS or BBE (red boxes). (C) Heat map of antibody binding patterns to glycolipid antigens in the 3 patient groups and in controls. Each row represents a single patient, and each column represents a single glycolipid or glycolipid complex. BBE serum samples are at the top, followed by GBS-OP, MFS, and HC serum samples. The binding intensity of IgG antibodies for each antigen is shown by a color scale, ranging from green (negative) to red (strongest). BBE = Bickerstaff brainstem encephalitis; GBS-OP = Guillain-Barré syndrome with ophthalmoplegia; HC = healthy control; MFS = Miller Fisher syndrome; ns = no significance.

There are several limitations in the present study. First, because GBS-OP, MFS, and BBE are rarer diseases, clinical information and sera of those patients were collected from various hospitals throughout Japan, so we could not avoid selection bias by attendant physicians. Second, each clinical information was only retrospectively investigated using a questionnaire. Third, the number of antigens investigated in this study was limited despite using a combinatorial glycoarray. Further prospective studies using a larger number of lipid complexes, including phospholipids and cholesterol, should be performed to clarify the causes of phenotypic differences in anti-GQ1b antibody–related diseases.

Author contributions

K. Yoshikawa has contributed to acquisition, has analyzed and interpreted data, and drafted the manuscript. M. Kuwahara has analyzed and interpreted data and participated in drafting the manuscript and revising it. M. Morikawa has contributed to acquisition and analysis of data. Y. Fukumoto, M. Yamana, and Y. Yamagishi have contributed to acquisition of data.

Table 2 Comparison of positive rates among the 3 patient groups

	GBS-OP	MFS	BBE	Two-tailed *p* value		
	(n = 63)	(n = 37)	(n = 27)	GBS-OP vs MFS	GBS-OP vs BBE	MFS vs BBE
Overall GQ1b-containing antigens	46 (73%)	32 (86.5%)	20 (74%)	ns	ns	ns
Overall GD1b-containing antigens	31 (49.2%)	11 (29.7%)	3 (11.1%)	ns	<0.001	ns
GD1b	10 (15.9%)	0 (0%)	0 (0%)	0.021	ns	ns
GM1/GD1b	14 (22.2%)	0 (0%)	0 (0%)	0.003	0.012	ns
GM4/GD1b	13 (20.6%)	0 (0%)	1 (3.7%)	0.003	ns	ns
GD1a/GD1b	10 (15.9%)	0 (0%)	0 (0%)	0.021	ns	ns
Gal-C/GD1b	11 (17.5%)	0 (0%)	0 (0%)	0.012	0.045	ns
GA1/GD1b	16 (25.4%)	2 (5.4%)	0 (0%)	0.027	0.006	ns
Sulfatide/GD1b	25 (39.7%)	11 (29.7%)	2 (7.4%)	ns	0.003	ns
Overall both GQ1b- and GD1b-containing antigens	31 (49.2%)	11 (29.7%)	3 (11.1%)	ns	<0.001	ns

BBE = Bickerstaff brainstem encephalitis, GBS-OP = Guillain-Barré syndrome with ophthalmoplegia, MFS = Miller Fisher syndrome, ns = no significance.
The chi-square test and Fisher exact test were used to compare differences in proportions.
The Bonferroni test was used as a post hoc test in multiple group comparisons.
A two-tailed *p* value <0.05 was considered statistically significant.

S. Kusunoki has made substantial contributions to conception and design of the study and revised the manuscript critically for important intellectual content. S. Kusunoki made final approval of the manuscript.

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