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

Keisuke Yoshikawa, MD, Motoi Kuwahara, MD, PhD, Miyuki Morikawa, MD, PhD, Yuta Fukumoto, MD, Masaki Yamana, MD, Yuko Yamagishi, MD, PhD, and Susumu Kusunoki, MD, PhD

Neurol Neuroimmunol Neuroinflamm 2018;5:e501. doi:10.1212/NXI.0000000000000501

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.
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,4 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 χ² 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,\(^\text{10}\) 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 reflexes, 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 weakness, 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, 33.3%) 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 (33/59, 56.0%) (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%).

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

Overall both GQ1b- and GD1b-containing antigens 31 (49.2%) 11 (29.7%) 3 (11.1%) ns <0.001 ns

GBS-OP = 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.

Acknowledgment
The authors thank Dr. Susan Halstead and Dr. Hugh Willison for their technical coaching regarding glycoarray.

Study funding
Supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid for Scientific Research, 15H04845 and 18H02745), Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED, 16ek0109056h0003), the Ministry of Health, Labour and Welfare of Japan [Health and Labour Sciences Research Grant on Rare and Intractable Diseases (Validation of Evidence-based Diagnosis and Guidelines, and Impact on QOL in Patients with Neuroimmunological Diseases)], and Intramural Research Grant (28–5) for Neurological and Psychiatric Disorders of NCNP.

Disclosure
K. Yoshikawa reports no disclosures. M. Kuwahara received speaker honoraria from Teijin, Nihon, and Japan Blood Products Organization. M. Morikawa, Y. Fukumoto, and M. Yamana report no disclosures. Y. Yamagishi received travel funding and/or speaker honoraria from Sumitomo Dainippon Pharma and was involved with Teijin Limited. S. Kysunoki received speaker honoraria from Teijin Pharma, Nihon, Japan Blood Products Organization, Biogen, Novartis, Dainippon Sumitomo, Kyowa Kirin, Ono Pharma, Pfizer, Alexion, and Chugai; served as an editorial board member of the Journal of Neuroimmunology; served as an associate editor of Neurology and Clinical Neuroscience; and received research support from Novartis, Dai-nippon Sumitomo, Sanofi, Japan Blood Products, Ohtsuka, Kyowa Kirin, Daichii Sanyko, Eisai, Takeda, Nihon, and Ministry of Education, Culture, Sports, Science and Technology of Japan, Japan agency for medical research and development. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NN.

Received June 6, 2018. Accepted in final form July 20, 2018.

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