[6] Yuki N, Odaka M, Hirata K. Acute ophthalmoparesis (without ataxia) associated with anti-GQ1b IgG antibody: clinical features. Ophthalmology 2001;108:196–200.
[7] Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. Brain 2002;125:2591–625.
[8] Nishimoto Y, Odaka M, Hirata K, Yuki N. Usefulness of anti-GQ1b IgG antibody testing in Fisher syndrome compared with cerebrospinal fluid examination. J Neuroimmunol 2004;148:200–5.
[9] Hughes RAC, van Der Meché FGA. Corticosteroids for treating Guillain–Barré syndrome. Cochrane Database Syst Rev 2000;(3): CD001446.
[10] Mori M, Kuwabara S, Fukutake T, Yuki N, Hattori T. Clinical features and prognosis of Miller Fisher syndrome. Neurology 2001;56: 1104–6.

ELSEVIER

Journal of Neuroimmunology 177 (2006) 201 – 208

Journal of
Neuroimmunology

www.elsevier.com/locate/jneuroim

Spectrum of neurological diseases associated with antibodies to minor gangliosides GM1b and GalNAc-GD1a

M. Tatsumoto [a], M. Koga [a], M. Gilbert [b], M. Odaka [a], K. Hirata [a], S. Kuwabara [c], N. Yuki [a,*]

[a] *Department of Neurology, Dokkyo Medical University School of Medicine, Kitakobayashi 880, Mibu, Shimotsuga, Tochigi 321-0293, Japan*
[b] *Institute for Biological Sciences, National Research Council of Canada, Canada*
[c] *Department of Neurology, Chiba University School of Medicine, Japan*

Received 5 January 2006; received in revised form 25 March 2006; accepted 5 April 2006

Abstract

The authors reported the neurological disease spectrum associated with autoantibodies against minor gangliosides GM1b and GalNAc-GD1a. IgG and IgM antibody reactivity against gangliosides GM1, GM2, GM1b, GD1a, GalNAc-GD1a and GQ1b was investigated in sera from 7000 consecutive patients who had various neurological conditions. The clinical diagnoses for 456 anti-GM1b-positive patients were Guillain-Barré syndrome (GBS, 71%), atypical GBS with preserved deep tendon reflexes (12%), Fisher syndrome (10%), Bickerstaff's brainstem encephalitis (2%), ataxic GBS (2%) and acute ophthalmoparesis (1%). For 193 anti-GalNAc-GD1a-positive patients, the diagnoses were GBS (70%), atypical GBS (16%), Fisher syndrome (10%) and Bickerstaff's brainstem encephalitis (3%). Of the patients with GBS or atypical GBS, 28% of 381 anti-GM1b-positive and 31% of 166 anti-GalNAc-GD1a-positive patients had neither anti-GM1 nor anti-GD1a antibodies. Of those patients with Fisher syndrome, Bickerstaff's brainstem encephalitis, ataxic GBS or acute ophthalmoparesis, 33% of 67 anti-GM1b-positive, and 52% of 25 anti-GalNAc-GD1a-positive patients had no anti-GQ1b antibodies. Autoantibodies against GM1b and GalNAc-GD1a are associated with GBS, Fisher syndrome and related conditions. These antibodies should provide useful serological markers for identifying patients who have atypical GBS with preserved deep tendon reflexes, ataxic GBS, Bickerstaff's brainstem encephalitis or acute ophthalmoparesis, especially for those who have no antibodies to GM1, GD1a or GQ1b. A method to prepare GM1b was developed.

Keywords: Anti-ganglioside antibody; Guillain-Barré syndrome; Fisher syndrome; GM1b, GalNAc-GD1a

1. Introduction

Autoantibodies against the major gangliosides GM1, GD1a and GQ1b have been investigated in patients with various neurological conditions, and the spectrum of neurological disease associated with each anti-ganglioside antibody has been determined (Willison and Yuki, 2002). Anti-GM1 IgM antibody is used as a diagnostic marker of multifocal motor neuropathy (MMN). Anti-GM1 and anti-GD1a IgG antibodies are associated with the acute motor axonal neuropathy (AMAN) form of Guillain-Barré syndrome (GBS). Anti-GQ1b IgG antibodies are present not only in Fisher syndrome (FS), but in GBS with ophthalmoplegia, Bickerstaff's brainstem encephalitis (BBE), acute ophthalmoparesis (AO) without ataxia and the ataxic variant of GBS. Clinico-serological observations indicate that BBE can be considered FS with CNS signs, and that AO and ataxic GBS are incomplete forms of typical FS.

GM1b and *N*-acetylgalactosaminyl GD1a (GalNAc-GD1a), minor gangliosides in human peripheral nerves, are target molecules for serum antibodies in GBS (Kusunoki et al., 1994, 1996). In early studies, anti-GM1b antibodies were present in none of 129 patients with other neurological disorders, and anti-GalNAc-GD1a antibody was present in one MMN patient of 119 patients with other neurological disorders. In subsequent studies, high anti-GM1b IgG antibody titer was detected in one FS patient out of 191 patients with other neurological disorders, and no

* Corresponding author. Tel.: +81 282 86 1111x2578; fax: +81 282 86 1776.
E-mail address: yuki@dokkyomed.ac.jp (N. Yuki).

0165-5728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2006.04.005

high anti-GalNAc-GD1a antibody titers were detected in 252 patients with other neurological disorders (Yuki et al., 1996, 1997). The spectrum of neurological disease associated with these anti-ganglioside antibodies, however, has yet to be clarified in a larger study (Ang et al., 1999; Hao et al., 1999; Kaida et al., 2000, 2001; Yuki et al., 2000).

Here, we determined the spectrum by testing sera from a large number of patients who had various neurological conditions. The clinical utility of these anti-ganglioside antibodies is discussed. A new method to synthesize GM1b also was developed because it is unavailable commercially and is difficult to purify from bovine brain.

2. Patients and methods

2.1. Patients

A sequential retrospective study was made of 7000 consecutive patients who had various neurological disorders such as GBS (n=2,155), FS (n=804) and chronic inflammatory demyelinating polyneuropathy (CIDP) (n=735). Serum samples were obtained from those referred to our laboratory for serum anti-ganglioside antibody testing by Japanese university and district general hospitals between July 1999 and August 2004, which period excluded patients tested in our previous studies (Yuki et al., 1996, 1997; Koga et al., 1999; Ikuta et al., 2003).

2.2. Anti-ganglioside antibody testing

GM1b and GalNAc-GD1a were purified from a bovine brain ganglioside mixture (Hirabayashi et al., 1990; Yuki et al., 1996). Serum IgG and IgM antibodies to GM1, GM2, GM1b, GD1a, GalNAc-GD1a and GQ1b (Fig. 1) were measured routinely by enzyme-linked immunosorbent assay as reported elsewhere (Yuki et al., 1997). Absorbance values at 492 nm were calculated by subtracting the optical densities obtained for wells without antigen. Serum was considered positive for anti-ganglioside antibodies when the absorbance value was 0.5 or higher at the dilution of 1:500

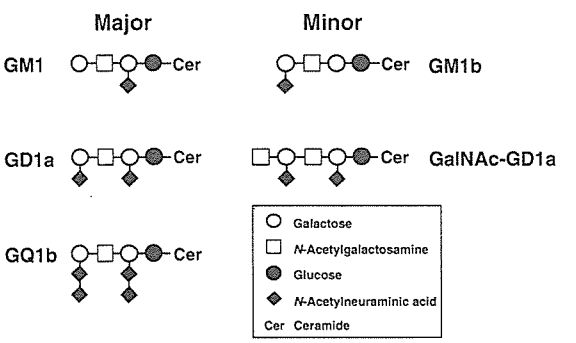


Fig. 1. Structures of major (GM1, GD1a and GQ1b) and minor (GM1b and GalNAc-GD1a) gangliosides.

because this high cut-off level gives high specificity (Tagawa et al., 2002). None of the antibodies tested was detected in 209 Japanese healthy subjects.

2.3. Clinical feature analysis

Information about antecedent illnesses and neurological signs during their illnesses were obtained from those patients in who were positive for anti-GM1b or anti-GalNAc-GD1a antibodies. The neurological signs assessed were consciousness disturbance, ophthalmoplegia, facial palsy, bulbar palsy, nuchal weakness, distribution of limb weakness, deep tendon reflexes (DTR), pathological reflexes, ataxia, sensory impairment and autonomic dysfunction. Patients' clinical features were reviewed by one of the authors (M.T.) by means of their medical records at both admission and discharge obtained from each primary physician. When they did not contain adequate information, questionnaires were sent to the physicians by fax.

2.4. Diagnostic criteria

Diagnoses of GBS and CIDP were based on established criteria (Asbury and Cornblath, 1990; Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force, 1991). Some patients who did not fulfill those criteria because of normal to brisk DTR throughout the illness were assessed as having "atypical GBS with preserved DTR". Diagnoses of FS, BBE, AO and pharyngeal–cervical–brachial weakness (PCB) were based on our published criteria (Odaka et al., 2001; Nagashima et al., 2004).

2.5. Electrophysiology

A subgroup of consecutive patients with GBS (n=111, 87%) and atypical GBS with preserved DTR (n=17, 13%), seen at Dokkyo Medical University Hospital and Chiba University Hospital, were studied. Serial nerve conduction studies were performed as described elsewhere (Kuwabara et al., 2004). Patients were classified as having AMAN or acute inflammatory demyelinating polyneuropathy (AIDP) based on the published electrodiagnostic criteria (Ho et al., 1995).

2.6. Infection serologies

Serum samples taken during the first 3 weeks after neurological onset were tested, as reported elsewhere to detect recent *Campylobacter jejuni* infection (Koga et al., 2005).

2.7. Statistical analysis.

Differences in proportions were examined by the χ^2 or Fisher's exact (two-tailed) test. Differences in medians were examined by the Mann–Whitney U-test. A difference of $p<0.05$ was considered significant.

2.8. Biosynthesis and purification of GM1b

Bovine asialo-GM1 was purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada). The Cst-I α-2,3-sialyltansferase from *C. jejuni* OH4384 (Gilbert et al., 2000) was expressed as MalE fusion (MalE/Cst-I) in *Escherichia coli* AD202 and purified on amylose resin (New England Biolabs, Beverly, MA, USA). Reactions were performed on 1 mg samples. One milligram of asialo-GM1 was dissolved in 6 ml of methanol. The final volume was 20 ml, 30% methanol, 50 mM Hepes pH 7.5, 10 mM $MgCl_2$, 0.5 mM CMP-NeuAc and 1.1 unit of MalE/Cst-I (Gilbert et al., 2000). This mixture was incubated at 37 °C for 2 h, then kept at − 20 °C for at least 12 h. The crude product was pelleted in a microcentrifuge at 16,000×*g* centrifugation for 30 min. Pellets were resuspended in methanol and sonicated (in a sonic bath) for 1 min. Insoluble material was removed by centrifugation (20 min at 16,000×*g*). The crude product (soluble in methanol) was recovered from the supernatant and dried under a nitrogen stream.

The crude product was applied to an Iatrobeads (6RS 8060: Iatron Laboratory, Tokyo, Japan) column, and GM1b eluted from the asialo-GM1 by a gradient of chloroform/methanol/water 55:44:2 to 35:60:5. Its purity was checked by thin-layer chromatography (TLC). One microgram portions of purified and unpurified GM1b were spotted on a precoated Silica Gel 60 TLC plate (Merk, Darmstadt, Germany). The plate was developed with chloroform–methanol–0.2% calcium chloride in water (5:4:1, by volume), then air-dried, dipped for 1 min in *n*-hexane containing 0.4% polyisobutylmethacrylate, and again air-dried. TLC plates were immunostained as described elsewhere (Yuki et al., 1996). In brief, they were incubated at 4 °C overnight with GM1b-specific antiserum (S10862) or GM1-specific antiserum (S10947) at a 1:100 dilution. Both sera were obtained from patients with GBS. After being washed, the plates were overlaid with peroxidase-conjugated anti-human γ-chain-specific antibodies (Dako, Denmark; 1:1000) and kept at 20 °C for 2 h. After another washing of the plates, binding was made visible with 4-chloro-1-naphtol.

3. Results

3.1. Diagnoses

Anti-GM1b antibody was positive in 456, anti-GalNAc-GD1a antibody in 193 (Tables 1 and 2), anti-GM1 antibody in 418 and anti-GD1a antibody in 295. Anti-GM2 IgG ($n=3$, 2%) and IgM ($n=2$, 1%) antibodies were detected in 164 patients with anti-GalNAc-GD1a IgG antibodies, but no anti-GM2 antibodies were in 29 with anti-GalNAc-GD1a IgM. The clinical diagnoses were GBS (anti-GM1b, anti-GalNAc-GD1a antibodies; 71%, 70%) and atypical GBS with preserved DTR (12%, 16%), FS (10%, 10%), BBE (2%, 3%), ataxic GBS (2%, 0%), AO (1%, 0%) and PCB (0.2%, 0%). Others were CIDP (anti-GM1b, anti-GalNAc-GD1a antibodies; $n=4$, $n=1$), meningoencephalitis subsequent to *Mycoplasma* pneumonia ($n=1$, $n=0$), Graves' ophthalmopathy ($n=1$, $n=0$) and trochlear nerve palsy ($n=0$, $n=1$). Patients with typical and atypical GBS often had antibodies to GM1 or GD1a, the predominant isotype being IgG. Interestingly, FS was the most frequent diagnosis for patients who had only anti-GalNAc-GD1a IgM antibodies. Seven of 16 patients with FS and BBE who had only anti-GM1b and anti-GalNAc-GD1a IgM antibodies did not

Table 1
Diagnosis for patients who had antibodies to GM1b

	Total	GBS	Atypical GBS	FS	Ataxic GBS	BBE	AO	PCB	Others
Patients, *n* (%)	456 (100)	325 (71)	56 (12)	45 (10)	10 (2)	7 (2)	5 (1)	2 (0.4)	6 (1)
Sex, M/F	280/176	203/122	37/19	26/19	8/2	2/5	1/4	1/1	2/4
Median age (range)	41 (4–88)	42 (4–87)	33 (12–81)	40 (10–86)	53 (28–88)	29 (21–53)	42 (26–60)	6 and 76	50 (17–87)
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
IgG Ab with and without IgM	438 (96)	315 (97)	51 (91)	44 (98)	10 (100)	6 (86)	5 (100)	2 (100)	5 (83)
IgM Ab alone	18 (4)	10 (3)	5 (9)	1 (2)	0 (0)	1 (14)	0 (0)	0 (0)	1 (17)
Other anti-ganglioside Abs									
IgG Abs to:									
GM1	203 (45)	173 (53)	26 (46)	3 (7)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)
GD1a	201 (44)	171 (53)	22 (39)	3 (7)	1 (10)	1 (14)	0 (0)	1 (50)	2 (33)
GalNAc-GD1a	104 (23)	81 (25)	19 (34)	3 (7)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)
GQ1b	88 (19)	40 (12)	2 (4)	31 (69)	4 (40)	5 (71)	5 (100)	1 (50)	0 (0)
IgM Abs to:									
GM1	27 (6)	21 (6)	6 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GD1a	7 (2)	5 (2)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GalNAc-GD1a	34 (7)	21 (6)	11 (20)	1 (2)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)
GQ1b	5 (1)	3 (1)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

GBS=Guillain-Barré syndrome, FS=Fisher syndrome, BBE=Bickerstaff's brainstem encephalitis, AO=acute ophthalmoparesis.
PCB=pharyngeal–cervical–brachial weakness, Ab=antibody.

Table 2
Diagnoses for patients who had antibodies to GalNAc-GD1a

	Total	GBS	Atypical GBS	FS	BBE	Others
Patients, *n* (%)	193 (100)	135 (70)	31 (16)	20 (10)	5 (3)	2 (1)
Sex, M/F	137/56	94/41	27/4	14/6	2/3	0/2
Median age (range)	36 (5–87)	41 (5–87)	33 (20–70)	28 (7–72)	31 (17–74)	63 and 84
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
IgG Ab with and without IgM	164 (85)	125 (93)	19 (94)	6 (30)	3 (60)	1 (50)
IgM Ab alone	29 (15)	10 (7)	2 (6)	14 (70)	2 (40)	1 (50)
Other anti-ganglioside Abs						
IgG Abs to:						
GM1	91 (47)	72 (53)	17 (55)	2 (10)	0 (0)	0 (0)
GD1a	81 (42)	67 (50)	13 (42)	1 (5)	0 (0)	0 (0)
GM1b	97 (50)	76 (56)	16 (52)	5 (25)	0 (0)	0 (0)
GQ1b	23 (12)	11 (8)	0 (0)	9 (45)	3 (60)	0 (0)
IgM Abs to:						
GM1	13 (7)	8 (6)	4 (13)	1 (5)	0 (0)	0 (0)
GD1a	4 (2)	2 (1)	2 (6)	0 (0)	0 (0)	0 (0)
GM1b	32 (17)	21 (16)	9 (29)	1 (5)	1 (20)	0 (0)
GQ1b	3 (2)	3 (2)	0 (0)	0 (0)	0 (0)	0 (0)

Ab=antibody.

have anti-GQ1b IgG antibodies, whereas patients with FS, BBE, AO and ataxic GBS often had anti-GQ1b IgG antibodies.

3.2. GBS and atypical GBS with preserved DTR

Anti-GM1b and anti-GalNAc-GD1a antibodies respectively were positive in 381 and 166 patients with GBS and atypical GBS with preserved DTR (Table 3). Diagnoses of GBS and atypical GBS were made also in 76% and 11% of the 418 anti-GM1-positive patients, and in 84% and 12% of the 295 anti-GD1a-positive patients. The frequencies of atypical GBS were similar irrespective of the anti-ganglioside antibody reactivity. Twenty-eight percent of those with anti-GM1b antibodies and 31% of those with anti-GalNAc-GD1a antibodies had neither anti-GM1 nor anti-GD1a antibodies. Sex ratio, age distribution, antecedent illness and neurological findings did not differ between anti-GM1b antibody-positive and anti-GalNAc-GD1a antibody-positive patients, with and without antibodies to GM1 or GD1a.

Of 128 consecutive patients seen at Dokkyo Medical University and Chiba University hospitals, 56 who had anti-GM1b antibodies were classified as having AMAN (84%) or AIDP (4%) based on electrodiagnostic criteria and positive *C. jejuni* serology (75%) (Table 4). Thirty patients with anti-GalNAc-GD1a antibodies, classified as having AMAN (83%) or AIDP (3%), had positive *C. jejuni*

Table 3
Patients with typical and atypical Guillain-Barré syndrome who had antibodies to GM1b or GalNAc-GD1a

	Anti-GM1b Ab-positive			Anti-GalNAc-GD1a Ab-positive		
	Total	Abs to GM1 or GD1a		Total	Abs to GM1 or GD1a	
		Negative	Positive		Negative	Positive
Patients, *n* (%)	381 (100)	108 (28)	273 (72)	166 (100)	52 (31)	114 (69)
Sex, M/F	240/141	70/38	170/103	121/45	38/14	83/31
Median age (range)	41 (4–87)	40 (9–87)	42 (4–87)	38 (5–87)	32 (11–80)	41 (5–87)
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Antecedent illness:						
Diarrhea	211 (55)	54 (50)	157 (58)	101 (61)	33 (63)	68 (60)
Upper respiratory tract infection	145 (38)	38 (35)	107 (39)	57 (34)	13 (25)	44 (39)
Neurological findings:						
Ophthalmoplegia	34 (9)	14 (13)	20 (7)	6 (4)	0 (0)	6 (5)
Facial palsy	58 (15)	20 (19)	38 (14)	17 (10)	3 (6)	14 (12)
Bulbar palsy	64 (17)	21 (19)	43 (16)	14 (8)	0 (0)	14 (12)
Distal-dominant weakness	203 (53)	60 (56)	143 (52)	107 (64)	37 (71)	70 (61)
Proximal-dominant weakness	49 (13)	15 (14)	34 (12)	17 (10)	4 (8)	13 (11)
Tendon reflex						
Absent or decreased	325 (85)	84 (78)	241 (88)	135 (81)	42 (81)	93 (82)
Brisk or normal	56 (15)	24 (22)	32 (12)	31 (19)	10 (19)	21 (18)
Sensory disturbance	144 (38)	42 (39)	102 (37)	39 (23)	14 (27)	25 (22)

Ab=antibody.

Table 4
Electrodiagnoses for patients with typical and atypical Guillain-Barré syndrome

	Anti-GM1b Ab-positive (n=56)	Anti-GalNAc-GD1a Ab-positive (n=30)
Sex, M/F	39/17	24/6
Median age (range)	54 (16–86)	35 (17–86)
	n (%)	n (%)
Tendon reflex		
Absent or decreased	46 (82)	26 (87)
Brisk or normal	10 (18)	4 (13)
Electrodiagnosis		
AMAN	47 (84)	25 (83)
AIDP	2 (4)	1 (3)
Unclassified	13 (13)	4 (13)
Campylobactor jejuni serology	42 (75)	22 (73)

Ab=antibody.
AMAN=acute motor axonal neuropathy.
AIDP=acute inflammatory demyelinating polyneuropathy.

serology (73%). Ten patients with typical GBS and six with atypical GBS had antibodies to GM1b or GalNAc-GD1a, but not to GM1 or GD1a.

3.3. FS, BBE, ataxic GBS, AO and PCB

Anti-GM1b and GalNAc-GD1a antibodies were detected respectively in 67 and 25 patients with FS, BBE, ataxic GBS, AO and PCB (Table 5). Thirty-three percent of the former and 52% of the latter had no anti-GQ1b IgG antibodies. Sex ratio, age distribution, antecedent illness and neurological findings for the anti-GM1b antibody-positive patients did not differ from the patients with and without antibodies to GQ1b. For the anti-GalNAc-GD1a antibody-positive patients, sex ratio, age distribution and antecedent illness did not differ between those with and without antibodies to GQ1b, but bulbar palsy was more frequent in the anti-GQ1b antibody-positive than antibody-negative group. Two patients with PCB had anti-GM1b IgG antibodies (Arai et al., 2003): one had additional IgG antibodies to GD1a, GT1a and GQ1b. The other had antibodies to GD1a but not to GT1a.

3.4. Biosynthesis and purification of GM1b

Fusion MalE/Cst-I was used to add a terminal α-2,3-linked sialic acid residue to bovine asialo-GM1 to obtain GM1b. After preliminary trials, the conversion rate was optimal with a starting asialo-GM1 concentration of 50 μg/ml and an incubation time of 2 h at 37 °C. Under those conditions, the yield was approximately 70% GM1b (as estimated from TLC, data not shown) and the other 30% being mostly the starting asialo-GM1 (a sometimes observed faint band that migrated more slowly than GM1b on TLC was presumed to be GD1c). Adding more enzyme and donor to the reaction mix or extending the incubation time did not improve the conversion yield. It therefore was decided to purify GM1b from the starting asialo-GM1 by chromatography on Iatrobeads. The purified sample gave a single band corresponding to GM1b on the orcinol-stained TLC plate, whereas the unpurified sample gave two bands (Fig. 2, Panel A). Immunostain analysis showed that the GM1b-specific antiserum bound to the GM1b-corresponding bands but not to GM1 (Fig. 2, Panel B), whereas GM1-specific antiserum bound neither to the purified nor unpurified GM1b (Fig. 2, Panel C). ELISA analysis also showed that both the purified and unpurified GM1b materials were bound by GM1b-

Table 5
Patients with Fisher syndrome and related conditions who had antibodies to GM1b or GalNAc-GD1a

	Anti-GM1b Ab-positive			Anti-GalNAc-GD1a Ab-positive		
	Total	Ab to GQ1b		Total	Ab to GQ1b	
		Negative	Positive		Negative	Positive
Patients, n (%)	67 (100)	22 (33)	45 (67)	25 (100)	13 (52)	12 (48)
Sex, M/F	37/30	16/6*	21/24*	16/9	8/5	8/4
Median age (range)	38 (10–88)	33 (19–88)	41 (10–86)	29 (7–74)	29 (7–72)	30 (12–74)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Antecedent illness:						
Diarrhea	22 (33)	9 (41)	13 (29)	14 (56)	7 (54)	7 (58)
Upper respiratory tract infection	37 (55)	10 (45)	27 (60)	7 (28)	4 (31)	3 (25)
Neurological findings:						
Ophthalmoplegia	56 (84)	16 (73)	40 (89)	24 (96)	12 (92)	12 (100)
Facial palsy	8 (12)	2 (9)	6 (13)	6 (24)	4 (31)	2 (17)
Bulbar palsy	11 (16)	3 (14)	8 (18)	7 (28)	1 (8)*	6(50)*
Distal-dominant weakness	8 (12)	3 (14)	5 (11)	1 (4)	1 (8)	0 (0)
Proximal-dominant weakness	3 (4)	1 (5)	2 (4)	1 (4)	1 (8)	0 (0)
Tendon reflex						
Absent or decreased	50 (75)	18 (82)	32 (71)	20 (80)	10 (77)	10 (83)
Brisk or normal	17 (25)	4 (18)	13 (29)	5 (20)	3 (23)	2 (17)
Sensory disturbance	32 (48)	13 (59)	19 (42)	10 (40)	6 (46)	4 (33)

Ab=antibody.
* $p<0.05$.

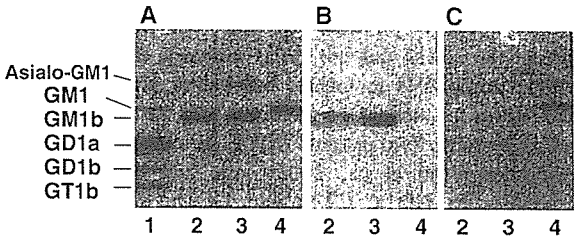


Fig. 2. Thin-layer chromatography of the synthesized GM1b. Stained with orcinol (Panel A)-, GM1b (Panel B)- and GM1 (Panel C)-specific serum IgGs from patients with Guillain-Barré syndrome. Lane 1, bovine brain ganglioside mixture (GM1/GD1a/GD1b/GT1b); lane 2, purified GM1b; lane 3, unpurified GM1b; lane 4, authentic GM1.

specific ($n=2$) but not by GM1-specific ($n=2$) antisera (data not shown). All four sera were from patients with GBS.

4. Discussion

Anti-GM1b and anti-GalNAc-GD1a antibodies originally were reported as diagnostic markers for GBS (Kusunoki et al., 1994, 1996), but our study shows that both antibodies also are present in patients who have various neurological diseases, including atypical GBS with preserved DTR, FS, BBE, ataxic GBS, AO and PCB. Clinical features were similar in patients with anti-GM1b and anti-GalNAc-GD1a antibodies irrespective of any coexisting antibodies against major gangliosides (GM1, GD1a or GQ1b) in typical GBS and FS-related conditions. Of the anti-GalNAc-GD1a antibody-positive patients with FS-related conditions, however, bulbar palsy was more frequent in anti-GQ1b antibody-positive than antibody-negative ones. Why is not clear, but anti-GQ1b IgG cross-reacts with GT1a which is present in human lower cranial nerves (Koga et al., 1998, 2002).

Of note is that anti-GM1b or anti-GalNAc-GD1a antibodies, but not anti-GQ1b antibodies, were detected in some patients with FS, BBE, ataxic GBS and AO, which are regarded as members of the anti-GQ1b IgG syndrome (Odaka et al., 2001). Elsewhere, we reported a patient with BBE who had a diagnosis of a conversion reaction and who had antibodies to GM1b and GalNAc-GD1a but not GQ1b (Matsuo et al., 2004). A patient with ataxic GBS who had IgG antibodies to GM1b and GalNAc-GD1a but not to GQ1b also has been reported (Odaka et al., 2004): No diagnosis was made on admission, but anti-ganglioside antibody testing thereafter supported that diagnosis. Isolated anti-GM1b IgG antibodies have been found in a patient with acute oropharyngeal palsy, a mild form of PCB (Ikuta et al., 2003). These findings suggest that autoantibodies against minor gangliosides are useful serological markers, which support a diagnosis not only of typical GBS, but various of its clinical variants as well, even if testing of anti-GM1, anti-GD1a and anti-GQ1b antibodies gives negative results.

Previously, we reported four patients who had acute progressive motor weakness associated with anti-GM1 antibodies but who did not fulfill GBS criteria (Asbury and Cornblath, 1990) because muscle stretch reflexes persistently were preserved and even brisk throughout their illnesses (Yuki and Hirata, 1998). In the present study, anti-GM1b and anti-GalNAc-GD1a antibodies were detected in atypical GBS with preserved DTR, as well as in typical GBS, further evidence that the two conditions share a common immunopathogenesis. Some patients with DTR-preserved GBS had antibodies to minor gangliosides (GM1b or GalNAc-GD1a) but not to major ones (GM1 or GD1a). These findings suggest that anti-ganglioside antibody testing (including anti-GM1b and anti-GalNAc-GD1a) would be useful for diagnosing atypical GBS with preserved DTR if physicians encounter patients who have acute progressive motor weakness. Actually, such a patient at first was suspected of having post-infectious myelitis, but subsequently atypical GBS with preserved DTR was the diagnosis (Kuwabara et al., 2002). That patient had IgG antibodies to GM1b and GalNAc-GD1a, but not to GM1 and GD1a. Moreover, as in previous GBS studies (Ogawara et al., 2000, 2003), we showed that anti-GM1b or anti-GalNAc-GD1a antibody-positive GBS, irrespective of DTR findings, is associated with AMAN and a preceding *C. jejuni* infection.

We found only a few patients with CIDP who had both antibodies and the titers were very low (data not shown), but there have been several reports of certain chronic neuropathies associated with either antibody. Anti-GalNAc-GD1a IgG antibodies were detected in three patients with chronic motor axonal neuropathy (Kaji et al., 2000). Anti-GM1b IgM antibodies without anti-GM1 reactivity were present in one of 15 Italian patients with MMN (Odaka, Nobile-Orazio and Yuki, unpublished data), whereas anti-GalNAc-GD1a IgM antibodies with anti-GM1 reactivity were present in two of 15 Japanese patients with MMN (Kaji et al., 2000). A patient with chronic motor demyelinating polyneuropathy was reported to carry anti-GalNAc-GD1a IgM M-protein with reactivity against GM2 and GalNAc-GM1b (Ortiz et al., 2001). A similar M-protein has been reported in another patient with polyneuropathy, but the clinical features were not described (Ilyas et al., 1988). Two patients with chronic sensory demyelinating polyneuropathy were reported to have anti-GM2 and anti-GalNAc-GD1a IgM M-protein (Lopate et al., 2002). These reports provide further evidence that patients with anti-GM1b or anti-GalNAc-GD1a antibodies have a variety of neurological conditions.

Isolation of GalNAc-GD1a from bovine brain is not particularly difficult, whereas that of GM1b is. Most laboratories therefore have been unable to use GM1b as an antigen. As discussed above, anti-GM1b, as well as anti-GalNAc-GD1a, antibodies are useful in clinical settings for identifying patients with atypical GBS with preserved DTR, ataxic GBS, BBE and AO, especially those who have no antibodies to GM1, GD1a or GQ1b. Chemical synthesis of GM1b has been reported, but it involves a laborious multi-step protocol (Prabhanjan et al., 1991). We developed a

simple method to prepare GM1b that uses the bacterial enzyme Cst-I. Larger-scale enzymatic synthesis of GM1b would enable the universal use of GM1b as a test antigen.

Acknowledgments

We thank Dr. Yukie Matsumoto and Yukiko Tamura (Department of Neurology, Dokkyo Medical University School of Medicine), Miyuki Masubuchi (Institute of Medical Science, Dokkyo Medical University School of Medicine), and Denis Brochu and Marie-France Karwaski (Institute of Biological Sciences, National Research Council of Canada, Canada) for their technical assistance. Supported in part by a grant-in-aid for Neuroimmunological Disease Research Committe Grant from the Ministry of Health, Labour and Welfare, Japan; a Health Sciences Research Grant (Research on Psychiatric and Neurological Diseases and Mental Health) from the Ministry of Health, Labour and Welfare of Japan; and a grant from the Human Frontier Science Program (RGP-38/2-3-C to M.G. and N.Y.).

References

Ang, C.W., Yuki, N., Jacobs, B.C., Koga, M., van Doorn, P.A., Schmitz, P.I.M., van der Meché, F.G.A., 1999. Rapidly progressive, predominantly motor Guillain-Barré syndrome with anti-GalNAc-GD1a antibodies. Neurology 53, 2122–2127.

Arai, M., Susuki, K., Koga, M., 2003. Axonal pharyngeal–cervical–brachial variant of Guillain-Barré syndrome without anti-GT1a IgG antibody. Muscle Nerve 28, 246–250.

Asbury, A.K., Cornblath, D.R., 1990. Assessment of current diagnostic criteria for Guillain-Barré syndrome. Ann. Neurol. 27, S21–S24.

Gilbert, M., Brisson, J.R., Karwaski, M.F., Michniewicz, J., Cunningham, A.M., Wu, Y., Young, N.M., Wakarchuk, W.W., 2000. Biosynthesis of ganglioside mimics in *Campylobacter jejuni* OH4384: identification of the glycosyltransferase genes, enzymatic synthesis of model compounds, and characterization of nanomole amounts by 600 MHz ^{1}H and ^{13}C NMR analysis. J. Biol. Chem. 275, 3896–3906.

Hao, Q., Saida, T., Yoshino, H., Kuroki, S., Nukina, M., Saida, K., 1999. Anti-GalNAc-GD1a antibody-associated Guillain-Barré syndrome with a predominantly distal weakness without cranial nerve impairment and sensory disturbance. Ann. Neurol. 45, 758–768.

Hirabayashi, Y., Hyogo, A., Nakao, T., Tsuchiya, K., Suzuki, Y., Matsumoto, M., Kon, K., Ando, S., 1990. Isolation and characterization of extremely minor gangliosides, GM1b and GD1α, in adult bovine brains as developmentally regulated antigens. J. Biol. Chem. 265, 8144–8151.

Ho, T.W., Mishu, B., Li, C.Y., Gao, C.Y., Cornblath, D.R., Griffin, J.W., Asbury, A.K., Blaser, M.J., McKhann, G.M., 1995. Guillain-Barré syndrome in northern China: relationship to *Campylobacter jejuni* infection and anti-glycolipid antibodies. Brain 118, 597–605.

Ikuta, N., Fukusako, T., Yuki, N., Morimatsu, M., Koga, M., 2003. Acute oropharyngeal palsy associated with anti-GM1b IgG antibody. J. Neurol. 250, 881–882.

Ilyas, A.A., Li, S.C., Chou, D.K., Li, Y.T., Jungalwala, F.B., Dalakas, M.C., Quarles, R.H., 1988. Gangliosides GM2, IV^{4}GalNAcGM1b, and IV^{4}GalNAcGD1a as antigens for monoclonal immunoglobulin M in neuropathy associated with gammopathy. J. Biol. Chem. 263, 4369–4373.

Kaida, K., Kusunoki, S., Kamakura, K., Motoyoshi, K., Kanazawa, I., 2000. Guillain-Barré syndrome with antibody to a ganglioside, *N*-acetylgalactosaminyl GD1a. Brain 123, 116–124.

Kaida, K., Kusunoki, S., Kamakura, K., Motoyoshi, K., Kanazawa, I., 2001. Guillain-Barré syndrome with IgM antibody to the ganglioside GalNAc-GD1a. J. Neuroimmunol. 113, 260–267.

Kaji, R., Kusunoki, S., Mizutani, K., Oka, N., Kojima, Y., Kohara, N., Kimura, J., 2000. Chronic motor axonal neuropathy associated with antibodies monospecific for *N*-acetylgalactosaminyl GD1a. Muscle Nerve 23, 702–706.

Koga, M., Yuki, N., Ariga, T., Morimatsu, M., Hirata, K., 1998. Is IgG anti-GT1a antibody associated with pharyngeal–cervical–brachial weakness or oropharyngeal palsy in Guillain-Barré syndromé? J. Neuroimmunol. 86, 74–79.

Koga, M., Yuki, N., Hirata, K., 1999. Antiganglioside antibody in patients with Guillain-Barré syndrome who show bulbar palsy as an initial symptom. J. Neurol. Neurosurg. Psychiatry 66, 513–516.

Koga, M., Yoshino, H., Morimatsu, M., Yuki, N., 2002. Anti-GT1a IgG in Guillain-Barré syndrome. J. Neurol. Neurosurg. Psychiatry 72, 767–771.

Koga, M., Gilbert, M., Li, J., Koike, S., Takahashi, M., Furukawa, K., Hirata, K., Yuki, N., 2005. Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. Neurology 64, 1605–1611.

Kusunoki, S., Chiba, A., Kon, K., Ando, S., Arisawa, K., Tate, A., Kanazawa, I., 1994. *N*-Acetylgalactosaminyl GD1a is a target molecule for serum antibody in Guillain-Barré syndrome. Ann. Neurol. 35, 570–576.

Kusunoki, S., Iwamori, M., Chiba, A., Hitoshi, S., Arita, M., Kanazawa, I., 1996. GM1b is a new member of [sic] antigen for serum antibody in Guillain-Barré syndrome. Neurology 47, 237–242.

Kuwabara, S., Nakata, M., Sung, J.-Y., Mori, M., Kato, N., Hattori, T., Koga, M., Yuki, N., 2002. Hyperreflexia axonal Guillain-Barré syndrome subsequent to *Campylobacter jejuni* enteritis. J. Neurol. Sci. 199, 89–92.

Kuwabara, S., Ogawara, K., Misawa, S., Koga, M., Mori, M., Hiraga, A., Kanesaka, T., Hattori, T., Yuki, N., 2004. Does *Campylobacter jejuni* infection elicit "demyelinating" Guillain-Barré syndrome. Neurology 63, 529–533.

Lopate, G., Choksi, R., Pestronk, A., 2002. Severe sensory ataxia and demyelinating polyneuropathy with IgM anti-GM2 and GalNAc-GD1a antibodies. Muscle Nerve 25, 828–836.

Matsuo, M., Odaka, M., Koga, M., Tsuchiya, K., Hamasaki, Y., Yuki, N., 2004. Bickerstaff's brainstem encephalitis associated with IgM antibodies to GM1b and GalNAc-GD1a. J. Neurol. Sci. 217, 225–228.

Nagashima, T., Koga, M., Odaka, M., Hirata, K., Yuki, N., 2004. Clinical correlates of serum anti-GT1a IgG antibodies. J. Neurol. Sci. 219, 139–145.

Odaka, M., Yuki, N., Hirata, K., 2001. Anti-GQ1b IgG antibody syndrome: clinical and immunological range. J. Neurol. Neurosurg. Psychiatry 70, 50–55.

Odaka, M., Yuki, N., Tatsumoto, M., Tateno, M., Hirata, K., 2004. Ataxic Guillain-Barré syndrome associated with anti-GM1b and anti-GalNAc-GD1a antibodies. J. Neurol. 251, 24–29.

Ogawara, K., Kuwabara, S., Mori, M., Hattori, T., Koga, M., Yuki, N., 2000. Axonal Guillain-Barré syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. Ann. Neurol. 48, 624–631.

Ogawara, K., Kuwabara, S., Koga, M., Mori, M., Yuki, N., Hattori, T., 2003. Anti-GM1b IgG antibody is associated with acute motor axonal neuropathy and *Campylobacter jejuni* infection. J. Neurol. Sci. 15, 41–45.

Ortiz, N., Rosa, R., Gallardo, E., Illa, I., Tomas, J., Aubry, J., Santafé, M., 2001. IgM monoclonal antibody against terminal moiety of GM2: GalNAc-GD1a and GalNAc-GM1b from a pure motor chronic demyelinating polyneuropathy patient: effects on neurotransmitter release. J. Neuroimmunol. 119, 114–123.

Prabhanjan, H., Kameyama, A., Ishida, H., Kiso, M., Hasegawa, A., 1991. Regio- and stereo-selective synthesis of ganglioside GM1b and some positional analogs. Carbohydr. Res. 220, 127–143.

Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force, 1991. Research criteria for diagnosis of

chronic inflammatory demyelinating polyneuropathy (CIDP) Neurology 41, 617–618.
Tagawa, Y., Yuki, N., Hirata, K., 2002. High anti-GM1 and anti-GD1a antibody titers are detected in Guillain-Barré syndrome but not in chronic inflammatory demyelinating polyneuropathy. Eur. Neurol. 48, 118–119.
Willison, H.J., Yuki, N., 2002. Peripheral neuropathies and anti-glycolipid antibodies. Brain 125, 2591–2625.
Yuki, N., Taki, T., Handa, S., 1996. Antibody to GalNAc-GD1a and GalNAc-GM1b in Guillain-Barré syndrome subsequent to *Campylobacter jejuni* enteritis. J. Neuroimmunol. 71, 155–161.
Yuki, N., Tagawa, Y., Irie, F., Hirabayashi, Y., Handa, S.. 1997. Close association of Guillain-Barré syndrome with antibodies to minor monosialogangliosides GM1b and GM1α. J. Neuroimmunol. 74, 30–34.
Yuki, N., Hirata, K., 1998. Preserved tendon reflexes in *Campylobacter* neuropathy. Ann. Neurol. 43, 546–547.
Yuki, N., Ang, C.W., Koga, M., Jacobs, B.C., van Doorn, P.A., Hirata, K., van der Meché, F.G.A., 2000. Clinical features and response to treatment in Guillain-Barré syndrome associated with antibodies to GM1b ganglioside. Ann. Neurol. 47, 314–321.

Bacterial infections in Guillain-Barré and Fisher syndromes

Nobuhiro Yuki and Michiaki Koga

Purpose of review

Progress has been made in our understanding of Guillain-Barré syndrome, especially in identifying the *Campylobacter jejuni* genes responsible for the development of clinical features.

Recent findings

C. jejuni is grouped into several classes based on the organization of lipo-oligosaccharide biosynthesis genes. A specific class carrying a sialyltransferase gene (*cst-II*) is associated with the development of Guillain-Barré syndrome, which is essential for the biosynthesis of ganglioside-like lipo-oligosaccharides. The class of *C. jejuni* expressed both GM1-like and GD1a-like lipo-oligosaccharides, which could induce the production of autoantibodies to GM1, to GD1a or to the GM1/GD1a complex, possibly increasing the risk of development. *C. jejuni* sialyltransferase (Cst-II) consists of 291 amino acids, and the 51st amino acid determines its enzymatic activity. Strains with *cst-II* (Thr51) expressed GM1-like or GD1a-like lipo-oligosaccharide whereas strains with *cst-II* (Asn51) expressed GT1a-like or GD1c-like lipo-oligosaccharide. Patients infected with the *cst-II* (Thr51) strains had anti-GM1 or anti-GD1a IgG antibodies, and showed limb weakness. Patients infected with the *cst-II* (Asn51) strains had anti-GQ1b IgG antibodies, and showed ophthalmoplegia and ataxia.

Summary

The *cst-II* gene is responsible for the development of Guillain-Barré and Fisher syndromes, and the polymorphism (Thr/Asn51) determines which syndrome develops after *C. jejuni* enteritis.

Keywords

Campylobacter jejuni, ganglioside, *Haemophilus influenzae*, lipo-oligosaccharide, molecular mimicry

Curr Opin Neurol 19:451–457. © 2006 Lippincott Williams & Wilkins.

Department of Neurology and Research Institute for Neuroimmunological Diseases, Dokkyo Medical University School of Medicine, Tochigi, Japan

Correspondence to Nobuhiro Yuki, MD, PhD, Department of Neurology and Research Institute for Neuroimmunological Diseases, Dokkyo Medical University School of Medicine, Kitakobayashi 880, Mibu, Shimotsuga, Tochigi 321-0293, Japan
Tel: +81 282 86 1111 x2578; fax: +81 282 86 1776;
e-mail: yuki@dokkyomed.ac.jp

Supported in part by a grant for Scientific Research (B) (KAKENHI 14370210 to N.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Health Sciences Research Grant (Research on Psychiatric and Neurological Diseases and Mental Health) to N.Y. from the Ministry of Health, Labour, and Welfare of Japan; and a grant from the Human Frontier Science Program (RGP0038/2003-C to N.Y.).

Current Opinion in Neurology 2006, 19:451–457

Abbreviations

GBS Guillain-Barré syndrome
LOS lipo-oligosaccharide

1350-7540

Introduction

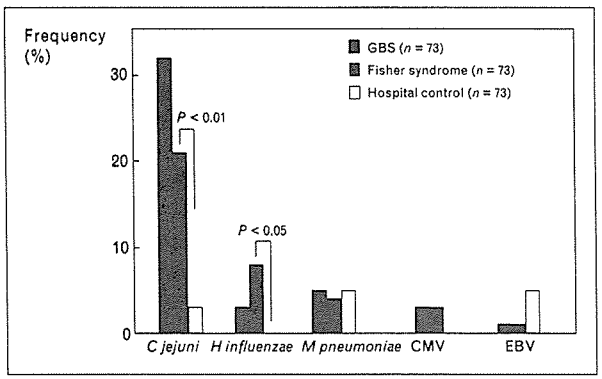
Guillain-Barré syndrome (GBS) is characterized by limb weakness and areflexia, and Fisher syndrome is characterized by ophthalmoplegia, ataxia, and areflexia. Fisher syndrome is positioned as a variant of GBS because some patients who present with Fisher syndrome progress to GBS during the course of the illness [1]. Both syndromes develop after various microbial infections [2]. A Gram-negative bacterium, *Campylobacter jejuni*, a leading cause of acute gastroenteritis in developed countries, is the most common antecedent microorganism in GBS. Molecular mimicry between GM1 ganglioside and *C. jejuni* lipo-oligosaccharide (LOS) is established as one of the causes of GBS for the following reasons: the epidemiological association exists between *C. jejuni* infection and GBS; anti-GM1 IgG antibodies are identified in patients with GBS subsequent to *C. jejuni* enteritis; and the disease model is produced by sensitization of rabbits with GM1 as well as *C. jejuni* LOS [3]. A Gram-negative bacterium, *Haemophilus influenzae*, a major pathogen that can result in respiratory tract infection, was reported as another cause of GBS [4]. Here we review recent studies that have revealed epidemiological evidence of both bacterial infections in Fisher syndrome and identified the *C. jejuni* genes responsible for the development of GBS and Fisher syndrome.

Infections by *C. jejuni* and *H. influenzae*

A prospective case–control study established an epidemiological association between *C. jejuni* infection and GBS [5]. A serological study in The Netherlands showed that *C. jejuni*, cytomegalovirus, Epstein-Barr virus, and *Mycoplasma pneumoniae* were related to GBS [6]. Serological evidence of recent *H. influenzae* infection was reported in 13% of 46 Japanese patients with GBS and in none of 49 healthy controls [4]. In other studies however, this percentage was between 1 and 3% in The Netherlands [6], Japan [7,8••], and the UK [9] and the epidemiological association was not confirmed.

Retrospective studies in Japan [7,10] have suggested that infections with *C. jejuni* and *H. influenzae* are related to

Figure 1 Frequency of positive infectious serology



Hospital controls were sex and age-matched (±5 years) with each patient with Fisher syndrome. CMV, cytomegalovirus; EBV, Epstein-Barr virus.

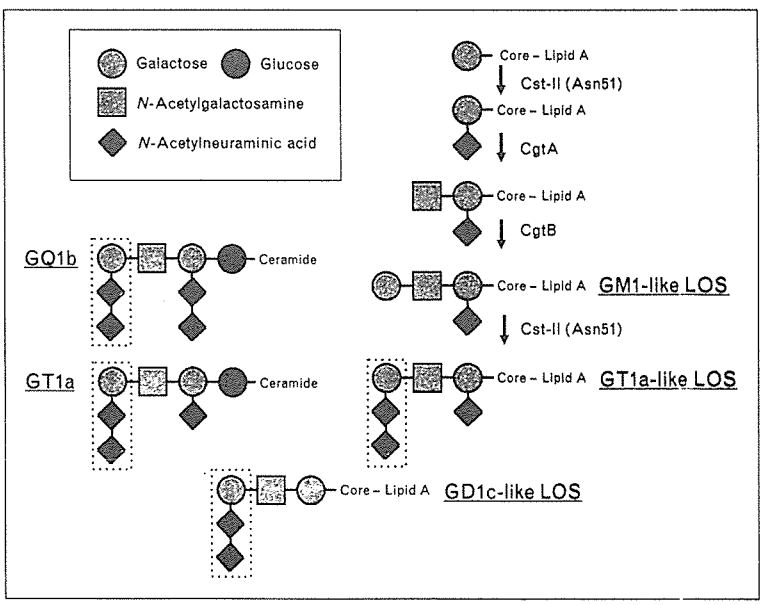
Fisher syndrome. A prospective case–control serologic study of five microbial infections (*C. jejuni*, cytomegalovirus, Epstein-Barr virus, *M. pneumoniae*, and *H. influenzae*) was conducted in 73 Japanese patients with Fisher syndrome and 73 sex and age-matched hospital controls [8••]. Serologic evidence in Fisher syndrome patients of *C. jejuni* (21%) and *H. influenzae* (8%) infections was present significantly more often than in the hospital controls (Fig. 1). None of the five pathogens examined was found in 67% of the Fisher syndrome patients. Epidemiological associations have been established between both bacterial infections and Fisher syndrome, although the causative agents remain unclear in the majority of patients with Fisher syndrome.

Anti-GQ1b immunoglobulin G (IgG) antibodies, which cross-react with GT1a [11], were detected in most Fisher syndrome patients infected with *C. jejuni* or *H. influenzae* [8••]. Mass spectrometry analysis identified a *C. jejuni* strain (CF93-6) carrying a GT1a-like LOS mimicking GQ1b that had been isolated from a patient with Fisher syndrome (Fig. 2). GD1c-like LOS, which mimics GQ1b, was identified in *C. jejuni* isolates from Fisher syndrome [12] (M. Gilbert and N. Yuki, unpublished data). GM2/GD2 synthase knockout mice, which lack GQ1b and GT1a, are immune-naïve hosts that can be used to obtain high antiganglioside antibody responses. Immunization of the mice with the *C. jejuni* LOS generated monoclonal IgG antibodies that reacted with GQ1b and GT1a [8••]. The anti-GQ1b antibodies bound to the Fisher-syndrome related *C. jejuni* LOS more commonly than in the GBS-related or enteritis-related strains. The GQ1b epitope was detected in some Fisher syndrome-related *H. influenzae* strains but was absent in strains from patients with GBS and uncomplicated respiratory infections. Infections by both bacteria bearing GT1a-like or GD1c-like LOS could induce the production of anti-GQ1b IgG antibodies.

C. jejuni isolation is the standard diagnosis for this bacterial infection, and an epidemiological study of more than 100 *C. jejuni*-isolated patients with GBS and Fisher

Figure 2 Carbohydrate mimicry of GQ1b/GT1a, *Campylobacter jejuni* lipo-oligosaccharides (LOSs) and enzymatic synthesis of the GT1a-like LOSs

The structure of the terminal trisaccharides of GQ1b is identical to that of GT1a, GT1a-like LOS and GD1c-like LOS (shown by the dotted line). GT1a-like LOS is synthesized by sialyltransferase Cst-II (Asn51), *N*-acetylgalactosaminyl-transferase (CgtA) and galactosyltransferase (CgtB). Reproduced with permission from [3].



syndrome was reported [13•]. The isolation rates were 22% for patients with a history of diarrhea and 2% for the others. There was no noticeable seasonal distribution in the onset of *C. jejuni* isolated from patients with GBS or Fisher syndrome. The male/female ratios were 1.7 : 1 for GBS and 2.2 : 1 for Fisher syndrome. The patient age range showed a peak in patients aged 10–30 years who had GBS and in patients aged 10–20 years who had Fisher syndrome. The predominance of young adults and male patients who had *C. jejuni*-isolated GBS or Fisher syndrome may be related to the preponderance of young adults and male patients who had *C. jejuni* enteritis. The median interval from diarrhea onset to neurological symptom onset was 10 days for GBS or Fisher syndrome. Penner's *C. jejuni* serotype HS:19 was more frequently present in GBS than in enteritis patients. HS:2 was more frequent in Fisher syndrome than in enteritis patients. The findings suggested that certain *C. jejuni* strains specifically trigger GBS and that others specifically trigger Fisher syndrome.

As mentioned above, *H. influenzae* is a possible cause of GBS and Fisher syndrome. Some patients in whom *H. influenzae* was isolated were also seropositive for *C. jejuni* [14•]. Antiganglioside IgG antibodies in these four patients did not cross-react with their *H. influenzae* LOS, whereas antiganglioside antibodies in the three patients with positive serology for *H. influenzae* did. The findings suggested that *H. influenzae* isolation is not always indicative of the causative pathogen in these syndromes. *Campylobacter coli* was proposed as a cause of GBS [15], but *C. coli* isolates from two GBS patients had neither ganglioside mimic nor cross-reactivity with antiganglioside autoantibodies from the patients [16•]. Some GBS patients had high titers of anti-*C. coli* antibody, as well as those of anti-*C. jejuni* antibody. These findings suggested that *C. coli* is not a causative agent of GBS. Although *C. curvus*, *C. upsaliensis*, and *Streptococcus pyogenes* were isolated from patients with GBS or Fisher syndrome, serological studies could not show epidemiological relationships between each bacterial infection and GBS or Fisher syndrome [17–19]. The determination of causative microorganisms should be carried out carefully, leading to a decrease in the numerous case reports of sham causal infection in these syndromes.

Here we propose the postulates to prove that a bacterium is a causative microorganism. Four criteria should be satisfied to draw a definite conclusion that a certain bacterium is a causative microorganism of GBS or Fisher syndrome associated with antiganglioside IgG antibodies:

(1) An epidemiological association is established between the microbial infection and GBS or Fisher syndrome;
(2) the microorganism is isolated from GBS or Fisher syndrome patients associated with antiganglioside IgG antibodies at the progressive phase of the illness, and is not isolated from the patients at the recovery phase;
(3) the microbial mimic of ganglioside is identified; and
(4) the GBS or Fisher syndrome model associated with antiganglioside IgG antibodies is produced by sensitization with the microbial itself or the component as well as with the ganglioside.

C. jejuni and GBS fulfill all four criteria, and the bacterium is a definitive cause of GBS [3]. The latter three criteria should be satisfied to draw a probable conclusion that a bacterium is a causative microorganism of GBS or Fisher syndrome.

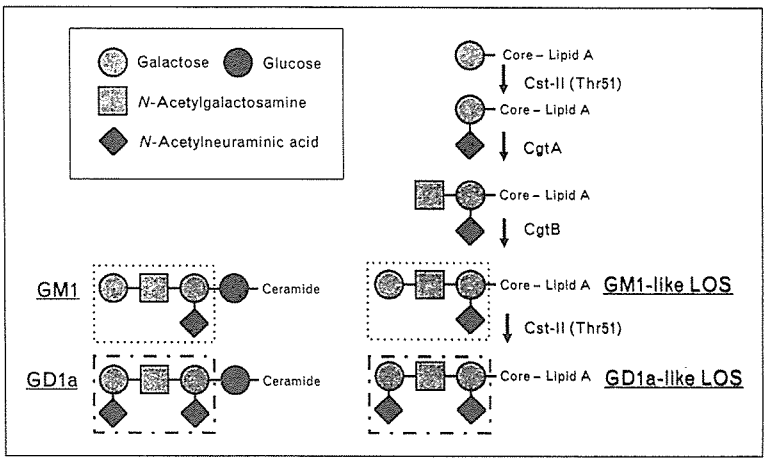
C. jejuni genes as the determinant of development and clinical features

C. jejuni genes, which are involved in the biosynthesis of ganglioside-like LOS, were cloned [20]. A gene encoding *Campylobacter* sialyltransferase, *cst-II*, was present in all eight strains with LOS bearing GQ1b epitope, but *cst-II* frequency did not differ between the GBS or Fisher syndrome and uncomplicated enteritis strains studied [21]. In contrast, another study [22] showed that the *cst-II* gene was more often present in 28 GBS isolates than in enteritis isolates. The *cst-II* gene encodes an enzyme that transfers sialic acid to the LOS, and *neuA1* encodes an enzyme that synthesizes the donor (CMP-sialic acid) used by the Cst-II sialyltransferase [20]. Since both genes are involved in LOS sialylation, they are essential for ganglioside-like LOS synthesis. Mutants of *C. jejuni* that lack these genes were made and analysed [23]. Whereas a mixture of GM1 and GD1a-like structures was identified in wild-type *C. jejuni* strains isolated from GBS patients, neither structure was found in the mutants. The *cst-II* and *neuA1* knockout mutants, unlike the wild types, had decreased reactivity to the sera of GBS patients. Immunization of GM2/GD2 synthase knockout mice lacking GM1 and GD1a with the wild-type strain induced an anti-GD1a IgG antibody response in these mice, whereas immunization with the mutant strains did not. This shows that the genes involved in LOS sialylation are essential for the induction of antiganglioside antibodies.

The Cst-II sialyltransferase consists of 291 amino acids, and the 51st amino acid determines its enzymatic activity [20]. Cst-II (Thr51) only has α-2,3-sialyltransferase activity (monofunctional), and can make GM1-like and GD1a-like LOS (Fig. 3). In contrast, Cst-II (Asn51) has both α-2,3 and α-2,8-sialyltransferase activities (bifunctional), and can make GT1a-like and GD1c-like LOSs mimicking GQ1b (Fig. 2). *C. jejuni* isolates were collected from 105 patients with GBS (including its

Figure 3 Carbohydrate mimicry of GM1/GD1a, *Campylobacter jejuni* lipo-oligosaccharides (LOSs), and enzymatic synthesis of the GM1 and GD1a-like LOSs

The structure of the terminal tetrasaccharides of GM1-like LOS is identical to that of GM1 (shown by the dotted lines). The structure of the terminal pentasaccharides of GD1a-like LOS is identical to that of GD1a (shown by the dashed lines). GM1-like and GD1a-like LOSs are synthesized by sialyltransferase Cst-II (Thr51), *N*-acetylgalactosaminyl-transferase (CgtA) and galactosyltransferase (CgtB). Reproduced with permission from [3].



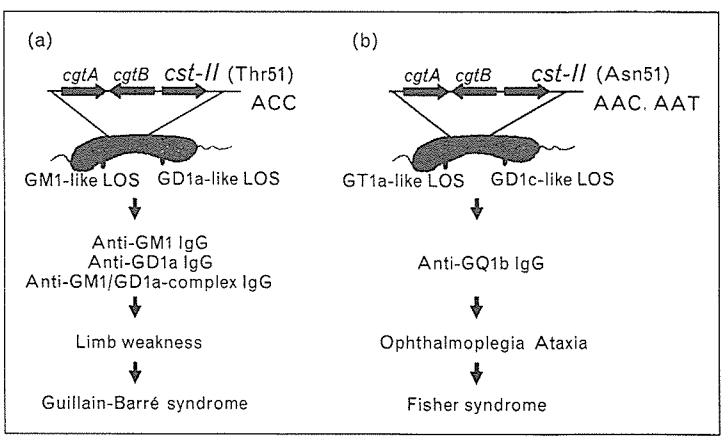
variants) and 65 patients with uncomplicated enteritis [24••]. Neuropathic strains more frequently had *cst-II* than the enteritic strains. Strains with *cst-II* (Thr51) had the GM1 or GD1a epitope, whereas those with *cst-II* (Asn51) regularly expressed the GQ1b epitope. Patients who had *C. jejuni* (Thr51) were more frequently positive for anti-GM1 or anti-GD1a IgG and had limb weakness, and GBS was diagnosed for the patients. Patients infected with *C. jejuni* (Asn51) were more often positive for anti-GQ1b IgG and had ophthalmoparesis and ataxia, and Fisher syndrome and the related conditions were diagnosed for most of the patients.

GM1 and GD1a are expressed on motor nerve axons, and GQ1b is in the oculomotor nerves and some primary sensory neurons [25]. Here we summarize the molecular pathogenesis of GBS and Fisher syndrome subsequent to *C. jejuni* enteritis (Fig. 4). *C. jejuni* (Thr51) strains can make GM1-like and GD1a-like LOSs, and induce the production of anti-GM1 or anti-GD1a IgG antibodies. Then the patients develop limb weakness. *C. jejuni* (Asn51) strains can make GT1a-like or GD1c-like LOS, and induce the production of anti-GQ1b IgG antibodies. Then the patients develop ophthalmoplegia and ataxia. In other words, the polymorphism (Thr/Asn51) determines whether GBS or Fisher syndrome develops after *C. jejuni* enteritis. This is a new paradigm that microbial genetic polymorphism can determine the clinical presentation of human autoimmune disease.

Previous studies have failed to find a specific *C. jejuni* genotype for GBS and Fisher syndrome, but a recent study using the 'genome-wide' and 'nucleotide sequence variation' screening found that the potential markers for

Figure 4 *Campylobacter jejuni* gene polymorphism as a determinant of clinical neuropathies after infection by the bacterium

(a) *C. jejuni* carrying *cst-II* (Thr51) can express GM1-like or GD1a-like lipo-oligosaccharide (LOS) on its cell surfaces. Infection by such *C. jejuni* strains can induce anti-GM1, anti-GD1a or anti-GM1/GD1a-complex immunoglobulin G (IgG) production in certain patients. These IgG antibodies bind to GM1, GD1a, or clustered epitope of GM1 and GD1a gangliosides; these are expressed on motor nerves in the four limbs. This binding induces the development of Guillain-Barré syndrome. (b) By contrast, *C. jejuni* carrying *cst-II* (Asn51) can express GT1a-like or GD1c-like LOS on its cell surfaces. Infection by such *C. jejuni* strains can induce anti-GQ1b IgG production in certain patients. Anti-GQ1b IgG antibodies bind to GQ1b, which is expressed on the oculomotor nerves and primary sensory neurons. This induces the development of Fisher syndrome.



GBS development were limited to LOS biosynthesis locus [26•]. LOS biosynthesis proteins are encoded by a large gene cluster [27], and many glycosyltransferase genes have been cloned, which are necessary for synthesizing the ganglioside-like structure on the LOS [28••]. Contents of the LOS biosynthesis genes differ among the strains, making the variation in LOS structure, and the LOS biosynthesis locus can be classified into at least eight classes (from classes A to H) based on the gene organization [28••,29•] (Fig. 5). Classes A, B, and C carry sialyltransferase genes, and the strains can make ganglioside-like LOS. A study using clinical isolates from The Netherlands and Belgium reported that the class A locus was overpresented in GBS-associated as compared with enteritis strains (9/17 (53%) versus 3/21 (14%)), whereas all Fisher syndrome-related strains belonged to class B (4/4 (100%) versus enteritis, 7/21 (33%)) [23]. Japanese GBS isolates were more frequently grouped in the LOS biosynthesis locus class A than were enteritis isolates (72/106 (68%) versus 17/103 (17%)) [30•]. Another study using 16 GBS strains from various countries confirmed the association [29•]. Accordingly, the class A LOS biosynthesis locus has been recognized as a worldwide risk factor for the development of GBS.

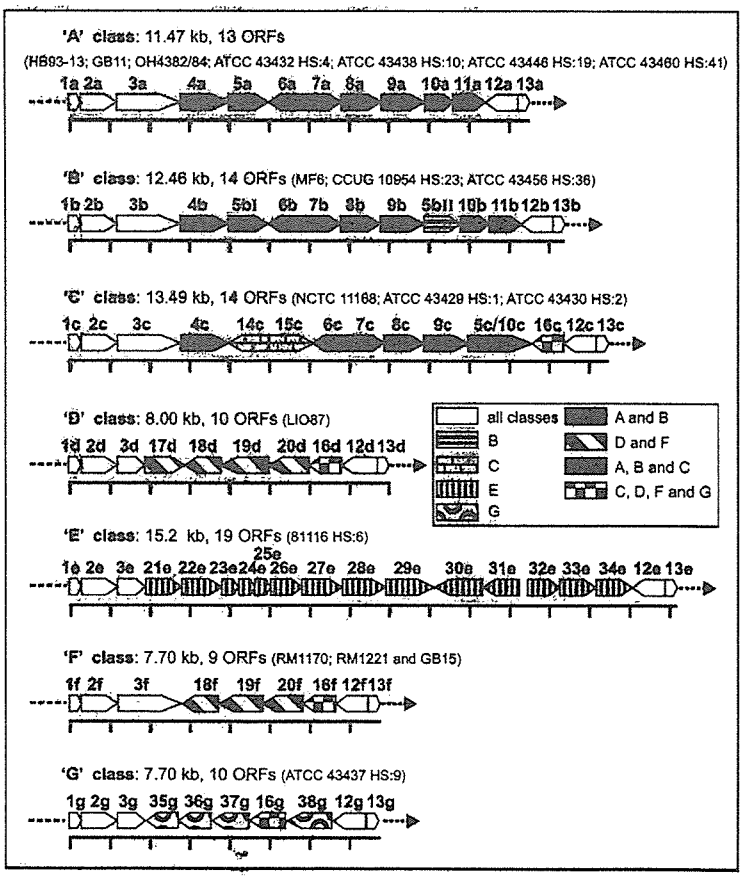
Class A strains predominantly had the serotype HS:19 and genotype *cst-II* (Thr51), the latter being responsible for biosynthesis of GM1-like and GD1a-like LOSs [30•]. Both anti-GM1 and anti-GD1a monoclonal antibodies regularly bound to class A strain LOSs, whereas only one or neither antibody bound to other class LOSs. Mass spectrometry analysis showed that a class A strain (CF90-26) carried GD1a-like as well as GM1-like LOSs. Logistic regression analysis showed that HS:19 and a class A locus were predictive of GBS development. Class A locus and serotype HS:19 seem to be linked to *cst-II* polymorphism, resulting in promotion of both GM1-like and GD1a-like structure synthesis on LOS, consequently increasing the risk of producing antiganglioside antibodies and developing GBS.

Autoantibodies to ganglioside complex

Autoantibodies specific for a complex of gangliosides GD1a and GD1b were found in sera from eight of 100 GBS patients [31]. Those sera had little or no reactivity to

Figure 5 Genetic organization of lipo-oligosaccharide (LOS) biosynthesis loci of *Campylobacter jejuni*

Contents of the LOS biosynthesis genes differ among the strains, making the variation in the LOS structure, and the LOS biosynthesis locus can be classified into at least eight groups (from classes A to H) based on the gene organization (only class A to G loci are shown). Reproduced with permission from [28••].



each ganglioside alone, indicating that complex formation of GD1a and GD1b makes new epitope which could be bound by the antibody. Autoantibody reactivity to complexes GQ1b/GM1 or GQ1b/GD1a was higher than that to isolated GQ1b in about a half of Fisher syndrome patients [32•]. Since a high proportion of gangliosides is clustered in cholesterol-rich plasma membrane microdomains termed lipid rafts, ganglioside complexes could actually be formed in plasma membrane in the nervous system and be targeted by the autoantibodies [33].

Detection of antibodies to the ganglioside complex gives us some suggestions regarding the mechanism of antiganglioside antibody production. As described above, *C. jejuini* strains from GBS patients are characterized by having the class A LOS biosynthesis locus, which is related to the biosynthesis of GM1-like and GD1a-like LOSs. The class C locus, however, which is rarer in *C. jejuni* strains from GBS patients than those from uncomplicated enteritis patients [23,30•], also contains all the glycosyltransferase genes required for biosynthesis of GM1-like LOS [20]. We recently found that IgG antibody to a mixture of GM1 and GD1a was detected more frequently in patients with isolation of *C. jejuni* bearing both GM1-like and GD1a-like LOSs than those with isolation of *C. jejuni* bearing only GM1-like LOS (M. Koga and N. Yuki, unpublished data). This suggests that complex formation of GM1 and GD1a epitopes on bacterial LOS makes a new epitope, resulting in the generation of an autoantibody which has different reactivity from antibodies specific for each ganglioside. Complex formation of GM1-like and GD1a-like LOSs appears to be one of the characteristics of *C. jejuni* strains with class A LOS biosynthesis locus and could account for the association of class A locus with GBS development. This is the first example demonstrating that complex formation of bacterial structures is another way to make molecular mimicry in autoantibody production.

Host genetic factors

An epidemiological study showed that about one of 3000 *C. jejuni* enteritis patients developed GBS [34]. The *C. jejuni* genes responsible for the development of GBS or Fisher syndrome alone do not sufficiently explain why an autoimmune response is triggered only in a minority of individuals with *C. jejuni* enteritis. Occurrence of GBS within families suggests that host susceptibility is also important [35]. Previous attempts to find common host immunogenetic factors among *C. jejuni*-related GBS patients, however, have been negative or diverse for HLA typing [36–38,39•], T-cell receptor genotyping [40], and for polymorphism analysis of CD14 and Toll-like receptor 4 [41]. A genome-wide search of single nucleotide polymorphisms could identify the host susceptibility genes in GBS and Fisher syndrome subsequent to *C. jejuni* enteritis.

Conclusion

C. jejuni is a definitive microorganism causing GBS, and it may also cause Fisher syndrome. Production of a Fisher syndrome model by sensitization with GQ1b and *C. jejuni* LOS should prove this hypothesis conclusively. *H. influenzae* is a possible cause of GBS and Fisher syndrome, but the chemical structure of ganglioside-like LOS should be determined. Whether the microbial genetic polymorphism similar to *cst-II* (Thr/Asn51) determines the ganglioside-like structure would be of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 490–491).

1 Mori M, Kuwabara S, Fukutake T, *et al.* Clinical features and prognosis of Miller Fisher syndrome. Neurology 2001; 56:1104–1106.

2 Yuki N. Infectious origins of, and molecular mimicry in Guillain-Barré and Fisher syndromes. Lancet Infect Dis 2001; 1:29–37.

3 Yuki N. Carbohydrate mimicry: a new paradigm of autoimmune diseases. Curr Opin Immunol 2005; 17:577–582.

4 Mori M, Kuwabara S, Miyake M, *et al. Haemophilus influenzae* infection and Guillain-Barré syndrome. Brain 2000; 123:2171–2178.

5 Rees JH, Soudain SE, Gregson NA, Hughes RAC. *Campylobacter jejuni* infection and Guillain-Barré syndrome. N Engl J Med 1995; 333: 1374–1379.

6 Jacobs BC, Rothbarth PH, van der Meché FGA, *et al.* The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. Neurology 1998; 51:1110–1155.

7 Koga M, Yuki N, Tai T, Hirata K. Miller Fisher syndrome and *Haemophilus influenzae* infection. Neurology 2001; 57:686–691.

8 •• Koga M, Gilbert M, Li J, *et al.* Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. Neurology 2005; 64:1605–1611.

This prospective, case–control, serological study showed that *H. influenzae* as well as *C. jejuni* infections are associated with Fisher syndrome.

9 Ju YY, Womersley H, Pritchard J, *et al. Haemophilus influenzae* as a possible cause of Guillain-Barré syndrome. J Neuroimmunol 2004; 149: 160–166.

10 Koga M, Yuki N, Takahashi M, *et al.* Close association of IgA antiganglioside antibodies with antecedent *Campylobacter jejuni* infection in Guillain-Barré and Fisher's syndromes. J Neuroimmunol 1998; 81:138–143.

11 Chiba A, Kusunoki S, Obata H, *et al.* Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. Neurology 1993; 43: 1911–1917.

12 Nam Shin JE, Ackloo S, Mainkar AS, *et al.* Lipo-oligosaccharides of *Campylobacter jejuni* serotype O:10: structures of core oligosaccharide regions from a bacterial isolate from a patient with the Miller-Fisher [sic] syndrome and from the serotype reference strain. Carbohydr Res 1997; 305:223–232.

13 • Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of *Campylobacter jejuni*-isolated Guillain-Barré and Fisher syndromes in Japan. J Clin Microbiol 2005; 43:335–339.

The authors reported the epidemiological features of *C. jejuni*-isolated GBS and Fisher syndrome using a large number of patients/isolates.

14 • Koga M, Koike S, Hirata K, Yuki N. Ambiguous value of *Haemophilus influenzae* isolation in Guillain-Barré and Fisher syndromes. J Neurol Neurosurg Psychiatry 2005; 76:1736–1738.

This study showed that *Haemophilus influenzae* isolation is not always indicative of the causative agent in GBS and that tests for other infections should be made, even in cases of positive culture.

15 Bersudsky M, Rosenberg P, Rudensky B, Wirguin I. Lipopolysaccharides of a *Campylobacter coli* isolate from a patient with Guillain-Barré syndrome display ganglioside mimicry. Neuromuscul Disord 2000; 10:182–186.

16 • Funakoshi K, Koga M, Takahashi M, *et al. Campylobacter coli* enteritis and Guillain-Barré syndrome: no evidence of molecular mimicry and serological relationship. J Neurol Sci 2006; 246:163–168.

Although *C. coli* had been isolated from two axonal GBS patients with antiganglioside antibodies, the authors failed to find any evidence of molecular mimicry and a serological relationship.

17 Ho TW, Hsieh ST, Nachamkin I, *et al.* Motor nerve terminal degeneration provides a potential mechanism for rapid recovery in acute motor axona neuropathy after *Campylobacter* infection. Neurology 1997; 48:717–724.

18 Yuki N, Hirata K. Fisher's syndrome and group A streptococcal infection. J Neurol Sci 1998; 160:64–66.

19 Koga M, Yuki N, Takahashi M, *et al.* Are *Campylobacter curvus* and *Campylobacter upsaliensis* antecedent infectious agent in Guillain-Barré and Fisher's syndromes? J Neurol Sci 1999; 163:53–57.

20 Gilbert M, Karwaski M-F, Bernatchez S, *et al.* The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, *Campylobacter jejuni*: biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. J Biol Chem 2002; 277:327–337.

21 van Belkum A, van den Braak N, Godschalk P, *et al. Campylobacter jejuni* gene associated with immune-mediated neuropathy. Nat Med 2001; 7:752–753.

22 Nachamkin I, Liu J, Li M, *et al. Campylobacter jejuni* from patients with Guillain-Barré syndrome preferentially expresses a GD1a-like epitope. Infect Immun 2002; 70:5299–5303.

23 Godschalk PCR, Heikema AP, Gilbert M, *et al.* The crucial role of *Campylobacter jejuni* genes in autoimmune antibody induction. J Clin Invest 2004; 114:1659–1665.

24 •• Koga M, Takahashi M, Masuda M, *et al. Campylobacter* gene polymorphism as a determinant of clinical features of Guillain-Barré syndrome. Neurology 2005; 65:1376–1381.

This study showed that polymorphism of a *Campylobacter jejuni* gene, *cst-II*, as a determinant of clinical features of GBS. The *cst-II* (Asn51) strains carried GQ1b epitope and were prone to cause Fisher syndrome associated with anti-GQ1b IgG antibody.

25 Willison HJ, Yuki N. Peripheral neuropathies and antiglycolipid antibodies. Brain 2002; 125:2591–2625.

26 • Godschalk PCR, Bergman MP, Gorkink RFJ, *et al.* Identification of DNA sequence variation in *Campylobacter jejuni* strains associated with the Guillain-Barré syndrome by high-throughput AFLP analysis. BMC Microbiol 2006; 6:32.

The authors developed a new approach to find a possible GBS-specific marker at 'genome-wide' and 'nucleotide sequence variation' screening, but failed to detect it.

27 Parkhill J, Wren BW, Mungall K, *et al.* The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 2000; 403:665–668.

28 •• Gilbert M, Godschalk PCR, Parker CT, *et al.* Genetic bases for the variation in the lipooligosaccharide outer core of *Campylobacter jejuni* and possible association of glycosyltransferase genes with postinfectious neuropathies. In: Ketley J, Konkel M, editors. Campylobacter: molecular and cellular biology. Norwich, UK: Horizon Scientific Press; 2005. pp. 219–248.

An excellent and comprehensive review of the mechanisms in which *C. jejuni* expresses various types of ganglioside-like LOSs.

29 • Parker CT, Horn ST, Gilbert M, *et al.* Comparison of *Campylobacter jejuni* LOS biosynthesis loci from a variety of sources. J Clin Microbiol 2005; 43:2771–2781.

The authors confirmed the previous results that specific organization of *C. jejuni* genes ('class A' LOS biosynthesis locus) is associated with GBS using 16 isolates from various countries.

30 • Koga M, Gilbert M, Takahashi M, *et al.* Comprehensive analysis of bacterial risk factors for developing Guillain-Barré syndrome after *Campylobacter jejuni* enteritis. J Infect Dis 2006; 193:547–555.

This study showed that *C. jejuni* strains with 'class A' LOS biosynthesis locus carry GM1 and GD1a epitopes, both of which are important for the development of GBS.

31 Kaida K, Morita D, Kanzaki M, *et al.* Ganglioside complexes as new target antigens in Guillain-Barré syndrome. Ann Neurol 2004; 56:567–571.

32 • Kaida K, Kanzaki M, Morita D, *et al.* Antiganglioside complex antibodies in Miller Fisher syndrome. J Neurol Neurosurg Psychiatry 2006; 13 April [Epub ahead of print].

Antibodies against GQ1b/GM1 or GQ1b/GD1a complex were detected in some patients with Fisher syndrome, suggesting that clustered epitopes of gangliosides are candidates for prime target antigens for serum antibodies.

33 Willison HJ. Ganglioside complexes: new autoantibody targets in Guillain-Barré syndrome. Nat Clin Prac Neurol 2005; 1:2–3.

34 McCarthy N, Giesecke J. Incidence of Guillain-Barré syndrome following infection with *Campylobacter jejuni*. Am J Epidemiol 2001; 153: 610–614.

35 Geleijns K, Brouwer BA, Jacobs BC, *et al.* The occurrence of Guillain-Barré syndrome within families. Neurology 2004; 63:1747–1750.

36 Rees JH, Vaughan RW, Kondeatis E, Hughes RAC. HLA-class II alleles in Guillain-Barré syndrome and Miller Fisher syndrome and their association with preceding *Campylobacter jejuni* infection. J Neuroimmunol 1995; 62:53–57.

37 Koga M, Yuki N, Kashiwase K, *et al.* Guillain-Barré and Fisher's syndromes subsequent to *Campylobacter jejuni* enteritis are associated with HLA-B54 and Cw1 independent of antiganglioside antibodies. J Neuroimmunol 1998; 88:62–66.

38 Magira EE, Papaioakim M, Nachamkin I, *et al.* Differential distribution of HLA-DQβ/DRβ epitopes in the two forms of Guillain-Barré syndrome, acute motor axonal neuropathy and acute inflammatory demyelinating polyneuropathy (AIDP): identification of DQβ epitopes associated with susceptibility to and protection from AIDP. J Immunol 2003; 170:3074–3080.

39 • Geleijns K, Schreuder GMTh, Jacobs BC, *et al.* HLA class II alleles are not a general susceptibility factor in Guillain-Barré syndrome. Neurology 2005; 64:44–49.

This study showed that HLA-DRB1/DQB1 allele frequencies did not differ between GBS patients and controls, but that the HLA-DRB1*01 allele was frequent in severely affected patients.

40 Ma JJ, Nishimura M, Mine H, *et al.* HLA and T-cell receptor gene polymorphisms in Guillain-Barré syndrome. Neurology 1998; 51:379–384.

41 Geleijns K, Jacobs BC, van Rijs W, *et al.* Functional polymorphisms in LPS receptors CD14 and TLR4 are not associated with disease susceptibility or *Campylobacter jejuni* infection in Guillain-Barré patients. J Neuroimmunol 2004; 150:132–138.

balt4/znl-neurol/znl-neurol/znl02206/znl3688-06a | eideg | S=5 | 10/20/06 | 10:24 | Art: WNL178591 | Input-nlm

Relationship of bacterial strains to clinical syndromes of *Campylobacter*-associated neuropathies

K. Kimoto, MD*; M. Koga, MD, PhD*; M. Odaka, MD, PhD; K. Hirata, MD, PhD; M. Takahashi, DVM, PhD; J. Li, PhD; M. Gilbert, PhD; and N. Yuki, MD, PhD

Abstract—*Background:* Clinical and serologic studies suggest that Guillain–Barré syndrome (GBS) and atypical GBS with preserved muscle stretch reflexes (MSRs) form a continuous spectrum as well as do Fisher syndrome (FS), FS/GBS overlap, Bickerstaff brainstem encephalitis (BBE), BBE/GBS overlap, acute ophthalmoparesis (AO), ataxic GBS, and acute oropharyngeal palsy. *Objective:* To clarify the spectrum of neurologic disorders that occur subsequent to *Campylobacter jejuni* enteritis. *Methods:* We recruited patients with various neurologic conditions and from whom *C jejuni* was isolated. Bacterial features were investigated. *Results:* Diagnoses for the patients from whom *C jejuni* was isolated were GBS (n = 90), FS (n = 22), MSR-preserved GBS (n = 10), FS/GBS (n = 6), BBE (n = 1), BBE/GBS (n = 2), AO (n = 3), ataxic GBS (n = 1), and acute oropharyngeal palsy (n = 3). Isolates from MSR-preserved GBS were similar to those of GBS in serotype (HS:19), genotype (lipo-oligosaccharide [LOS] locus class A or B, *cst-II* genotype [Thr51]), and GM1 or GD1a epitope expression on LOS. FS/GBS overlap, BBE, BBE/GBS overlap, AO, ataxic GBS, and acute oropharyngeal palsy isolates were similar to those of FS in serotype (HS:2 or HS:4-complex), genotype (LOS locus class A or B, *cst-II* genotype [Asn51]), and GQ1b epitope expression on LOS. *Conclusions:* The bacterial findings support the proposal that Guillain–Barré syndrome (GBS) and muscle stretch reflex–preserved GBS comprise a continuous spectrum as well as do Fisher syndrome (FS), FS/GBS overlap, Bickerstaff brainstem encephalitis (BBE), BBE/GBS overlap, acute ophthalmoparesis, ataxic GBS, and acute oropharyngeal palsy.

NEUROLOGY 2006;67:1837–1843

nl *Campylobacter jejuni* is isolated from many patients with Guillain–Barré syndrome (GBS) and Fisher syndrome (FS),[1] but comprehensive studies of other neurologic disorders have yet to be made. To clarify the spectrum of neurologic disorders that may occur after *C jejuni* enteritis, we recruited patients who had various neurologic conditions and from which *C jejuni* was isolated, and analyzed their clinical features.

Patients with GBS subsequent to *C jejuni* enteritis often have anti-GM1 and anti-GD1a immunoglobulin G (IgG) antibodies,[2,3] whereas those with FS have anti-GQ1b IgG antibodies, which cross-react with GT1a (anti-GQ1b/GT1a IgG antibodies).[4] Penner serotyping showed that in Japan, GBS is associated with HS:19, and FS is associated with HS:2 and the HS:4-complex.[1] Lipo-oligosaccharide (LOS) is a major cell-surface structure expressed by *C jejuni*, which is divided into several LOS locus classes based on organization of the LOS biosynthesis genes. GBS and FS are associated with classes A and B.[5-7] *C jejuni* sialyltransferase, Cst-II, present in classes A and B, functions in the biosynthesis of ganglioside-like LOS, and variation in the nucleotide sequence of the *cst-II* gene affects its activity.[8] Cst-II (Thr51) produces GM1-like and GD1a-like LOSs,[8] and *cst-II* (Thr51) strains are associated with GBS.[9] In contrast, Cst-II (Asn51) produces GT1a-like, GD1c-like, and GD3-like LOSs, in which each terminal trisaccharide is identical to that of GQ1b (figure E-1 on the *Neurology* Web site at www.neurology.org),[8,10-12] and *cst-II* (Asn51) strains are associated with FS.[9] To clarify the pathogenesis and identify the nosologic

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the November 28 issue to find the title link for this article.

*These authors contributed equally to this work.

From the Department of Neurology and Research Institute for Neuroimmunological Diseases (K.K., M.K., M.O., K.H., N.Y.), Dokkyo Medical University School of Medicine, Tochigi, Japan; Department of Microbiology (M.T.), Tokyo Metropolitan Institute of Public Health, Tokyo, Japan; and Institute for Biological Sciences (J.L., M.G.), National Research Council Canada, Ottawa, Ontario, Canada.

Supported in part by grants from the Uehara Memorial Foundation to M.K.; the Naito Foundation to M.K.; Dokkyo Medical University School of Medicine to M.K. (no. 2005-01-2); the Mizutani Foundation for Glycoscience to N.Y.; a grant for Scientific Research (B) (KAKENHI 16390254 to N.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Research Grants on Measures for Intractable Diseases (1724360 and 17243601 to N.Y.) from the Ministry of Health, Labor and Welfare of Japan; and a grant from the Human Frontier Science Program (RGP0038/2003-C to M.G. and N.Y.).

Disclosure: The authors report no conflicts of interest.

Received May 10, 2006. Accepted in final form August 9, 2006.

Address correspondence and reprint requests to Dr. N. Yuki, Department of Neurology and Research Institute for Neuroimmunological Diseases, Dokkyo Medical University School of Medicine, Kitakobayashi 880, Mibu, Shimotsuga, Tochigi 321-0293, Japan; e-mail: yuki@dokkyomed.ac.jp

<article article-type="research-article"> •
<article-id pub-id-type="doi">10.1212/01.wnl.0000244468.22377.6b</article-id>

position of each neurologic disorder, we examined the bacterial features of isolates obtained from patients with various neurologic conditions.

Methods. *Patients and* C jejuni *strains.* From May 1996 to April 2005, we received approximately 9,900 requests from Japanese physicians to test serum antiganglioside antibodies from patients who had GBS (n = 3,358), FS (n = 1,114), chronic inflammatory demyelinating polyneuropathy (CIDP) (n = 1,070), acute disseminated encephalomyelitis (n = 34), encephalitis (n = 115), and encephalopathy (n = 22). On receipt of the serum samples, we requested the primary physicians to obtain stool cultures and send them to the Tokyo Metropolitan Institute of Public Health. When *C jejuni* was isolated at the hospitals, we asked that they be sent to the institute. Patients' clinical features were reviewed in an application form, as were their medical records both at admission and at discharge (obtained from each primary physician) to recruit *C jejuni*–isolate cases as well as to ascertain diagnoses. Information was obtained on age, sex, onset month, antecedent infectious symptoms, initial symptoms, neurologic signs during the illness, and CSF findings. When medical records did not contain adequate information, questionnaires were faxed to the physicians concerned.

Diagnoses of GBS and CIDP were based on established criteria.[13,14] GBS-like patients who did not fulfill the criteria because of normal to brisk muscle stretch reflexes (MSRs) throughout the illness were considered to have "MSR-preserved GBS." Patients seen at Dokkyo Medical University Hospital were classified as having acute motor axonal neuropathy (AMAN) or acute inflammatory demyelinating polyneuropathy if they fulfilled the published electrodiagnostic criteria.[15] Diagnoses of FS, BBE, and acute ophthalmoparesis (AO) without ataxia were based on our published criteria.[16] Patients with ophthalmoplegia and severe limb weakness (3 or less on the Medical Research Council scale) were considered to have overlapping FS and GBS (FS/GBS), and those with consciousness disturbance, in addition to ophthalmoplegia and severe limb weakness, were considered to have overlapping BBE and GBS (BBE/GBS). FS-like patients who did not fulfill the criteria because they had no external ophthalmoplegia throughout the illness were classified as having "ataxic GBS."[17] Pharyngeal–cervical–brachial weakness-like patients who did not fulfill the criteria because they had no cervical–brachial weakness throughout the illness were classified as having "acute oropharyngeal palsy."[18,19]

Penner serotyping was performed as described previously.[1] The *C jejuni* LOS locus class (A to F) was determined in a PCR performed with the specific primers for each locus.[5] The *cst-II* gene genotype (Asn/Thr51) was determined by direct sequencing of the PCR fragment.[9]

ELISA and thin-layer chromatography with immunostaining. Serum IgG and IgM antibodies to GM1, GD1a, GT1a, and GQ1b (figure E-1) were measured by ELISAs as described elsewhere.[10] In brief, serum samples diluted 1:500 were placed in separate microtiter plate wells. The mean value for triplicate reference wells without antigen was subtracted from the mean value for triplicate wells of each sample, and the optical density (OD_{492}) was assessed. An OD_{492} of less than 0.1 was judged to be negative. An OD_{492} of 0.1 to 0.5 was categorized as 1+; 0.5 to 1.0, 2+; 1.0 to 1.5, 3+; 1.5 to 2.0, 4+; 2.0 to 2.5, 5+; and 2.5 or more, 6+.

Crude LOS was prepared as described elsewhere.[10] Whether ganglioside epitopes (GM1, GD1a, and GQ1b) were present on the *C jejuni* LOS was determined by thin-layer chromatography with immunostaining of the sera from patients with GBS (S6960 [anti-GM1], S5174 [anti-GD1a]) and FS (S7577 [anti-GQ1b/GT1a]). To determine the reactivity of patients' sera with each *C jejuni* LOS, thin-layer chromatography plates with serum diluted 1:100 were incubated, then incubated again with peroxidase-conjugated anti-human γ-chain–specific antibodies (Dako, Denmark; 1:250).

Statistical analysis. Differences in group frequencies were compared by the χ^2 or Fisher exact test (two-tailed) with SPSS 12.0J software (SPSS Inc., Chicago, IL). Differences in medians were examined by the Mann–Whitney *U* test. A *p* value of less than 0.05 was considered significant.

Analysis of strains GC033, GC057, and GC124. Overnight growth (*C jejuni* isolates GC033, GC057, and GC124) on each agar plate was treated as described elsewhere.[10] *O*-deacylated LOS samples were analyzed by capillary electrophoresis-electrospray ionization mass spectrometry. Isolation of genomic DNA from the isolates was done with a DNeasy Tissue kit (Qiagen Inc., CA). Genes involved in the biosynthesis of the LOS outer core were amplified using an Advantage 2 PCR enzyme system (BD Biosciences Clontech, Palo Alto, CA). The 7.2-kb region (from *waaC* to *cst-II*) was sequenced by means of custom-made primers used previously to sequence this locus in multiple *C jejuni* strains.[8] DNA sequencing was performed with a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Quebec, Canada). Products were analyzed in an ABI 3100 Genetic Analyzer (Applied Biosystems).

Results. *Clinical and serologic features.* We found 141 *C jejuni*–isolate patients, two of whom were excluded because of inadequate information. Diagnoses for the other 139 patients were GBS (n = 90, 65%), FS (n = 22, 16%), MSR-preserved GBS (n = 10, 7%), FS/GBS (n = 6, 4%), BBE (n = 1, 0.7%), BBE/GBS (n = 2, 1.4%), AO (n = 3, 2.1%), ataxic GBS (n = 1, 0.7%), acute oropharyngeal palsy (n = 3, 2.1%), and CIDP (n = 1, 0.7%).

Because the clinical and serologic features of *C jejuni*–isolate patients with GBS or FS have already been reported,[1] we focused on the other patients. Table 1 shows the clinical and serologic features of patients with MSR-preserved GBS, FS/GBS, BBE, BBE/GBS, AO, ataxic GBS, and acute oropharyngeal palsy. We have tentatively called last six "FS-related conditions." The sex ratio, frequency of antecedent diarrhea, interval from onset of antecedent diarrhea to neurologic symptom onset, and frequency of CSF albuminocytologic dissociation, as well as seasonal distribution, did not differ significantly between GBS and MSR-preserved GBS or between FS and FS-related conditions, whereas the age distribution did differ significantly between FS and FS-related conditions. None of the patients with GBS and MSR-preserved GBS showed sensory involvement. Those factors did not differ between FS and FS/GBS or between FS/GBS and GBS. Based on electrodiagnostic criteria, of the patients seen at our hospital, two with MSR-preserved GBS and one with GBS were classified as having AMAN. T1

There were no significant differences in the positive frequencies of anti-GM1 or anti-GD1a IgG antibodies between GBS (89%) and MSR-preserved GBS (90%) or in those of anti-GQ1b/GT1a IgG antibodies between FS (91%) and FS-related conditions (93%) (table 2). Anti-GM1 or anti-GD1a IgG antibodies were positive in FS (18%), FS/GBS (50%), and GBS (89%), and anti-GQ1b/GT1a IgG antibodies in FS (91%), FS/GBS (83%), and GBS (6%) (tables 1 and 2). None of the antiganglioside antibodies were detected in the CIDP patient. T2

Bacterial features. Because the bacterial features of *C jejuni* isolated from GBS and FS have been reported,[5] we focused on isolates from the other conditions. Eight strains obtained from the 10 *C jejuni*–isolate patients with MSR-preserved GBS and 11 from the 25 patients with FS-related disorders underwent bacterial analyses. Seven of the 8 isolates from the MSR-preserved GBS patients had bacterial features in common with those of GBS; serotypes of HS:19, LOS locus class A or B, a *cst-II* (Thr51) content, and GM1 or GD1a epitope on LOS (table 3).[1,5,9,10] In contrast, 9 of the 11 isolates from FS-related condition patients had bacterial features in common with those of FS isolates; serotypes of HS:2 or the HS:4-complex, LOS locus class A or B, *cst-II* (Asn51) content, and GQ1b epitope expression on LOS. Serum IgGs from these patients re- T3

Table 1 Campylobacter jejuni*–isolate patients of whose diagnoses were neither Guillain–Barré nor Fisher syndromes*

Patient	Age, y/sex	Diagnosis	Antecedent symptom		Interval,* days	IgG antibodies to			
			Diarrhea	Fever		GM1	GD1a	GT1a	GQ1b
1	31/M	MSR-preserved GBS	+		8	3+	2+		
2	26/F	MSR-preserved GBS	+	+	14	1+			
3	14/M	MSR-preserved GBS	+	+	7	5+			
4	29/F	MSR-preserved GBS	+		9	6+			
5	53/M	MSR-preserved GBS	+	+	7	6+	2+		
6	24/M	MSR-preserved GBS		+	9	4+			
7	31/F	MSR-preserved GBS	+	+	7		2+		
8	33/M	MSR-preserved GBS	+	+	9	6+	2+		
9	12/M	MSR-preserved GBS	+	+	6	6+			
10	27/M	MSR-preserved GBS	+	+	5				
11	40/M	FS/GBS	+		2				3+
12	69/F	FS/GBS	+	+	7	2+	2+		
13	26/M	FS/GBS		+	28	1+	1+	3+	5+
14	56/M	FS/GBS	+	+	6			2+	3+
15	19/M	FS/GBS	+		10	2+	1+	6+	6+
16	69/F	FS/GBS	+	+	9			3+	6+
17	14/M	BBE		+	8				1+
18	17/M	BBE/GBS	+	+	12	1+		1+	1+
19	18/M	BBE/GBS	+	+	12			2+	1+
20	13/M	AO	+	+	14				1+
21	22/M	AO	+	+	7			1+	1+
22	22/F	AO	+	+	7			2+	2+
23	51/M	Ataxic GBS	+		5			3+	4+
24	46/M	Acute oropharyngeal palsy			18	1+	3+	5+	6+
25	15/F	Acute oropharyngeal palsy		+	10			6+	5+

* From antecedent infectious symptoms to neurologic onset.

IgG = immunoglobulin G; MSR = muscle stretch reflex; GBS = Guillain–Barré syndrome; FS = Fisher syndrome; FS/GBS = FS overlapping with GBS; BBE = Bickerstaff brainstem encephalitis; BBE/GBS = BBE overlapping with GBS; AO = acute ophthalmoparesis.

acted with each LOS, whereas serum IgG from the CIDP patient did not (figure 1).

Mass spectrometric analysis of the *O*-deacylated LOS from *C jejuni* GC033 (FS-related), GC124 (BBE/GBS-related), and GC057 (AO-related) gave rise to spectra that were consistent with the presence of multiple mass species. The differences in observed masses within each strain were the result of variation in lipid A composition and, in the case of GC057, to the presence of one or two terminal sialic acids (tables E-1, E-2, and E-3).

Table 2 *Clinical profiles of* Campylobacter jejuni*–isolate patients*

	GBS, n = 90	MSR-preserved GBS, n = 10	FS, n = 22	FS-related conditions, n = 15
Age distribution, median (range), years	26 (1–83)	28 (12–53)	13 (6–65)*	22 (13–69)
Sex ratio, M/F	49/41	7/3	15/7	11/4
Antecedent diarrhea, %	87	100	91	80
Interval from the antecedent diarrhea onset to the neurologic symptom onset, median (range), days	10 (1–30)	8 (5–14)	10 (1–21)	9 (2–28)
CSF albuminocytologic dissociation, %	57	38	39	57
IgG antibodies to GM1 or GD1a, %	89	90	18	27
GQ1b or GT1a, %	6	10	91	93

* $p < 0.05$, compared with patients with Fisher syndrome (FS)–related conditions.

GBS = Guillain–Barré syndrome; MSR = muscle stretch reflex; IgG = immunoglobulin G.

Table 3 *Characteristics of* Campylobacter jejuni *isolates*

Isolate	Patient	Patient's diagnosis	Serotype	LOS locus class	*cst-II* genotype	Ganglioside mimic on LOS		
						GM1	GD1a	GQ1b
GC072	1	MSR-preserved GBS	HS:19	A	Thr51	+		
GC102	2	MSR-preserved GBS	HS:19	A	Thr51	+	+	
GC120	4	MSR-preserved GBS	HS:19	A	Thr51	+	+	
GC161	5	MSR-preserved GBS	HS:1/44	C		+		
GC176	6	MSR-preserved GBS	HS:19	A	Thr51	+	+	
GC191	7	MSR-preserved GBS	HS:19	A	Thr51			
GC204	8	MSR-preserved GBS	HS:19	A	Thr51	+	+	
GC227	10	MSR-preserved GBS	HS:19	A	Thr51	+	+	
GC112	12	FS/GBS	HS:19	A	Thr51	+	+	
GC177	14	FS/GBS	HS:2	B	Asn51			+
GC181	15	FS/GBS	HS:4-complex	B	Asn51			+
GC233	16	FS/GBS	HS:4-complex	B	Asn51			+
GC159	17	BBE	HS:4-complex	B	Asn51			+
GC078	18	BBE/GBS	HS:4-complex	B	Asn51			+
GC124	19	BBE/GBS	HS:2	A	Asn51			+
GC057	22	AO	HS:2	B	Asn51			+
GC216	23	Ataxic GBS	Untypable	A	Asn51			+
GC183	24	Acute oropharyngeal palsy	HS:4-complex	A	Asn51			+
GC229	25	Acute oropharyngeal palsy	HS:2	B	Thr51			
		GBS*	HS:19 (67%)	A or B (85%)	Thr51 (64%)	70%	60%	16%
		FS*	HS:2 or HS:4-complex (78%)	A or B (84%)	Asn51 (70%)	20%	10%	70%

* Serotype data from reference 1, *cst-II* genotype data from reference 9, ganglioside mimicking lipo-oligosaccharide (LOS) data from reference 10, and LOS locus classification data from reference 5.

MSR = muscle stretch reflex; GBS = Guillain–Barré syndrome; FS = Fisher syndrome; FS/GBS = FS overlapping with GBS; BBE = Bickerstaff brainstem encephalitis; BBE/GBS = BBE overlapping with GBS; AO = acute ophthalmoparesis.

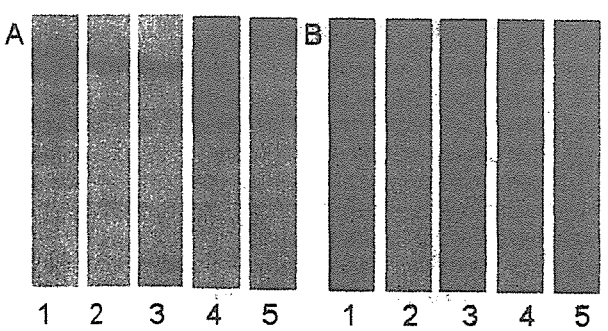


Figure 1. Serum reactivity with Campylobacter jejuni *lipo-oligosaccharide isolates. A, resorcinol staining; B, immunostaining of patients' sera with* C jejuni *lipo-oligosaccharides. Lane 1, GC159 (Bickerstaff brainstem encephalitis); lane 2, GC057 (acute ophthalmoparesis); lane 3, GC216 (ataxic Guillain–Barré syndrome); lane 4, GC183 (acute oropharyngeal palsy); lane 5, GC111 (chronic inflammatory demyelinating polyneuropathy).*

GC033 and GC124 had the same mass species; therefore, we propose that both express a GD1c mimic (figure 2A). The presence of two sialic acids forming a chain was confirmed by tandem mass spectrometry and a search for the precursor ion at *m/z* 581 (data not shown). We speculate that two sialic acids are substituted on the terminal galactose because these strains have a Cj1135 allele (putative glucosyltransferase, GenBank accession numbers DQ536321 and DQ536322) that can transfer a glucose to heptose II, the heptose substituted with the inner galactose. The presence of glucose on heptose II prevents sialylation of the inner galactose, indicative that the two sialic acids are on the terminal galactose.[20]

Mass spectrometric analysis of the *O*-deacylated LOS sample from GC057 showed mass species with either one or two sialic acids (table E-2). The presence of two sialic acids forming a chain was confirmed by tandem mass spectrometry and a search for the precursor ion at *m/z* 581 (data not shown). We speculate that the mass species with one sialic acid corresponds to a GM1b mimic and species with two sialic acids to a GD1c mimic (figure 2B) and that