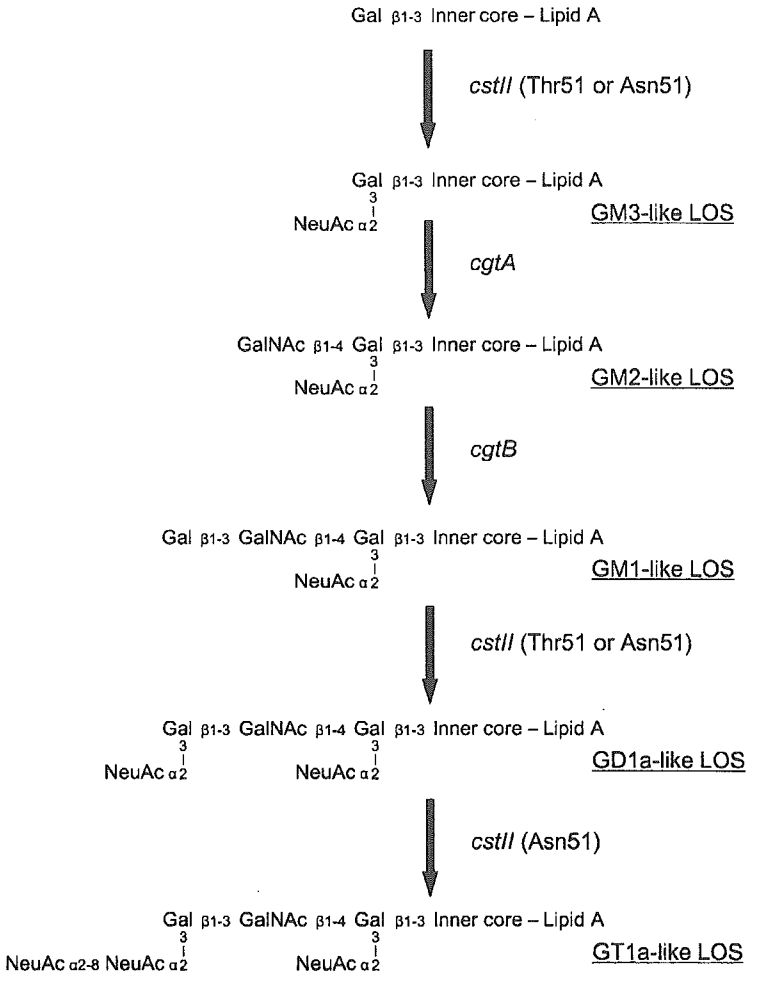

Figure 1. Proposed biosynthesis pathway of *Campylobacter jejuni* lipo-oligosaccharides (LOSs) mimicking gangliosides with a single sialic acid on the inner galactosyl residue [27, 28]. Gal, galactose; GalNAc, *N*-acetylgalactosamine; NeuAc, *N*-acetylneuraminic acid.

polymerase (TaKaRa Ex Taq; Takara Bio), 4 nmol of dNTPs, and buffer (2 mmol/L Mg^{2+}). After the first denaturation step of 5 min at 95°C, the amplification mixture was subjected to 30 cycles of amplification (table 1). Variation at codon 51 of *cstII* was investigated by direct sequencing of the PCR fragment [29].

Ganglioside-like LOS. Crude LOS fractions were prepared from the strains as described elsewhere [33]. The presence of ganglioside epitopes (GM1, GD1a, and GQ1b) on the *C. jejuni* LOS was determined using an ELISA [34]. The reagents used were monoclonal antibodies (mAbs; GB2 [anti-GM1], GB1 [anti-GD1a], and FS3 [anti-GQ1b/GT1a]) [14, 34].

Analysis of O-deacylated LOS. *C. jejuni* was grown overnight on a single agar plate, and the cells were treated as described elsewhere [35], with minor modification [34]. The *O*-deacylated LOS sample was analyzed by capillary electrophoresis–electrospray ionization mass spectrometry (CE-ESI-MS) [36].

Sequencing of LOS biosynthesis genes. Isolation of genomic DNA from *C. jejuni* strain CF90-26 was performed with a DNeasy Tissue kit (Qiagen). A 6.1-kb PCR product bearing genes encoding the LOS outer core glycosyltransferases was amplified with an Advantage 2 PCR kit (Clontech Laboratories) and the primers CJ-99 (5′-ATTAAAAAAAGACCTTGGGAATAC-3′) and CJ-147 (5′-AAGGTGTGCTAAGATAAACAAGAC-3′). The 6.1-kb PCR

Table 1. Polymerase chain reactions for the lipo-oligosaccharide gene loci of *Campylobacter jejuni* strains.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

Table 2. Lipo-oligosaccharide (LOS) locus classes of *Campylobacter jejuni* strains.

LOS locus class	Guillain-Barré syndrome–associated strains (n = 106)		Fisher syndrome–associated strains (n = 32)		Enteritis-associated strains, no. (%) (n = 103)
	No. (%)	2-tailed P[a]	No. (%)	2-tailed P[a]	
A	72 (68)	<.001	12 (38)	.02	17 (17)
B	18 (17)	.02	15 (47)	.14	33 (32)
C	12 (11)	.13	1 (3)	.03	20 (19)
D	0	.12	0	1.0	3 (3)
E	2 (2)	.001	2 (6)	.36	14 (14)
F	0	.01	0	.34	6 (6)
A/C[b]	0	.49	0	1.0	1 (1)
B/C[c]	0	.49	0	1.0	1 (1)
Unclassified	2 (2)	.06	2 (6)	1.0	8 (8)
A, B, or C	102 (96)	<.001	28 (88)	.06	72 (70)

[a] Compared with enteritis-associated strains (Fisher's exact test).
[b] Overlapping class A and C loci.
[c] Overlapping class B and C loci.

product was sequenced by means of custom-made primers that were used previously to sequence this locus in multiple *C. jejuni* strains [27]. DNA sequencing was performed with a BigDye Terminator mix (Applied Biosystems). Products were analyzed in an ABI 3100 Genetic Analyzer (Applied Biosystems).

Antiganglioside autoantibodies. Serum samples obtained during acute phases of GBS and FS were available from 126 patients (95 patients with GBS and 31 patients with FS). IgG autoantibodies to GM1, GD1a, and GQ1b were measured by ELISA [37]. Serum was considered to be positive for antibody when the titer was ≥500.

Statistical analysis. Frequency differences between groups were compared using Fisher's exact test. Differences in medians were examined using the Mann-Whitney U test, and Scheffé's test was used in the case of multiple comparisons. The association between the LOS locus class and either GBS or FS was first investigated by univariate analysis, without adjustment for confounding variables. A multiple logistic regression model was then used to determine the relative weighting of each variable. Statistical calculations were made with SPSS (version 12.0J; SPSS). A difference was considered to be statistically significant when $P < .05$.

RESULTS

LOS locus classification. Preliminary analysis of control strains of each LOS locus class confirmed that PCR-based LOS locus classification works well (data not shown). The class A locus was predominant in the GBS-associated strains, and its frequency was significantly higher in GBS-associated strains than in enteritis-associated strains (table 2). The other LOS locus classes were rarer in GBS-associated strains than in enteritis-associated strains. These findings agree with those of Godschalk et al. [15]. In contrast, FS-associated strains most frequently had the class B locus, but, compared with the enteritis-associated strains, the difference did not reach statistical significance, because it also was the most common class found in enteritis-associated strains. In the study by Godschalk et al. [15], all 4 FS-associated strains had the class B locus, whereas, in the present study, a significant number of FS-associated strains had the class A locus. In 12 strains (2 GBS associated, 2 FS associated, and 8 enteritis associated), there was no amplification of any class-specific genes. Two enteritis-associated strains were grouped as having overlapping class A and C or B and C loci.

Sialyltransferase-encoding genes (*cstII* or *cstIII*) are present in class A, B, and C loci [27], and this enables strains with these LOS locus classes to be characterized as a single group. Our data showed that 96% of GBS-associated strains had sialyltransferase-carrying LOS locus classes (A, B, or C), and this percentage was significantly higher than that of enteritis-associated strains (70%) (table 2). FS-associated strains also regularly had these LOS locus classes (88%).

Serotype. Table 3 shows the associations between the LOS locus class and the Penner serotype in GBS-associated, FS-associated, and enteritis-associated strains. LOS locus classes were closely—but not absolutely—associated with the Penner serotype, because strains with each LOS locus class were grouped into several serotypes, as was reported by Parker et al. [26]. Most class A strains were serotype HS:19, whereas the serotypes of class B strains varied. Conversely, most of the HS:19 strains had the class A locus, whereas most of the HS:2 and HS:4-complex strains had the class B or A locus.

cstII *polymorphism.* Class A and B loci are reported to carry the *cstII* gene [27]. Therefore, the association between *cstII* polymorphism and the class A or B locus was examined. Most of the class A strains had the *cstII* (Thr51) genotype (78/

Table 3. Associations between lipo-oligosaccharide (LOS) locus class and Penner serogroup in *Campylobacter jejuni* strains.

LOS locus class	Strains, no.	Serogroup (serotype)						
		A (HS:1/44)	B (HS:2)	D (HS:4/13/16/43/50)	G (HS:8)	O (HS:19)	Y (HS:37)	Other
A[a]	101	0	7 (7)	15 (15)	0	73 (72)	1 (1)	6 (6)
B	66	1 (2)	21 (32)	21 (32)	6 (9)	6 (9)	0	17 (26)
C[b]	33	14 (42)	7 (21)	0	5 (15)	0	0	8 (24)
D	3	0	0	0	0	0	0	3 (100)
E	18	0	0	0	0	0	8 (44)	10 (56)
F	6	0	1 (17)	0	0	0	0	5 (83)
A/C	1	0	1 (100)	0	0	0	0	0
B/C	1	0	0	0	0	0	0	1 (100)
Unclassified	12	2 (17)	2 (17)	0	0	2 (17)	0	6 (50)

NOTE. Data are no. (%) of strains, unless otherwise indicated.

[a] One strain was serotyped as the O/Y serogroup.

[b] One strain was serotyped as the A/G serogroup.

101; 77%), whereas most of the class B strains had the *cstII* (Asn51) genotype (49/66; 74%). FS-associated strains, however, were closely associated with the *cstII* (Asn51) genotype, irrespective of whether the LOS locus class was A or B; 8 (80%) of 10 class A strains and all 12 (100%) class B strains had the *cstII* (Asn51) genotype. This suggests that *cstII* polymorphism, and not LOS locus class, is critical for the development of FS.

Ganglioside-like LOS and antiganglioside autoantibodies. On the whole, reactivity to anti-GM1 mAb was increased in class A and C strains, and that of anti-GD1a mAb was increased only in class A strains (figure 2); this is indicative of a difference in sialyltransferase substrate specificity between classes A *(cstII)* and C *(cstIII)* [25]. Some class B strains had high reactivity to anti-GM1 and anti-GD1a mAbs, but the median optical density was low. Reactivity to anti-GQ1b/GT1a mAb was high overall in class B strains and in some class A strains, but it was not high in strains with loci of other classes.

We defined ganglioside epitopes as being present on LOSs when the OD of mAb in the ELISA was $\geq$0.2. There was an obvious difference in ganglioside epitopes between strains with the class A, B, or C locus and strains with the class D, E, or F locus, with epitopes being frequent in the former group and absent in the latter group. For example, the GM1 epitope was judged to be present in 80% of class A strains, 26% of class B strains, and 64% of class C strains but in none of the strains with the class D, E, or F locus. Furthermore, class A strains regularly expressed both the GM1 and has the GD1a epitope, whereas class C strains expressed only the GM1 epitope; the GD1a epitope was detected in 76% of class A strains but in only 3% of class C strains. The GQ1b/GT1a epitope was present in 37% and 50% of class A or B strains, respectively; no strain with the class C, D, E, or F locus had this epitope. Notably, several unclassified strains also had ganglioside-mimicking LOS (figure 2), and this is indicative of an unknown sialyltransferase gene being present at an unclassified locus.

Patients with class A or C strains often were positive for IgG autoantibodies against GM1 (72% and 75%, respectively). Interestingly, the frequency of anti-GD1a IgG autoantibodies was higher in patients with class A strains (51%) than in patients with class C strains (33%). These data agree with the finding that anti-GD1a mAb bound to class A LOS but not to class C LOS. In contrast, patients with class B strains more commonly had anti-GQ1b IgG autoantibodies (44%) than anti-GM1 IgG autoantibodies (25%) or anti-GD1a IgG autoantibodies (25%). Anti-GQ1b IgG autoantibodies were rarely detected in patients with class A (14%) or class C (0%) strains. These data agree with the finding that anti-GQ1b/GT1a mAb regularly bound to class B LOS.

LOS structure and glycosyltransferase genes of strain CF90-26. Because the above data suggested the importance of the GM1 and GD1a epitopes on class A strains, we investigated in detail the LOS structure and gene sequences of the *cstII*, *cgtA*, and *cgtB* glycosyltransferase genes. Elsewhere, we showed that *C. jejuni* strain CF90-26 (a serotype HS:19 class A strain from a patient with GBS who had high anti–GM1 IgG autoantibody titers), which was used in the present study, has a GM1-like structure, on the basis of nuclear magnetic resonance analysis [16], and has the GD1a epitope, on the basis of thin-layer chromatography with immunostaining [38]. CE-ESI-MS analysis of an *O*-deacylated LOS sample from *C. jejuni* strain CF90-26 yielded various masses, and the predominant species was $[M\text{-}4H]^{4-}$ (3645 Da). The differences in observed masses (table 4) were due to lipid A variation, as well as to the presence or absence of a terminal sialic acid (in addition to the sialic acid that is present on the inner galactosyl residue). CE-ESI-MS analysis showed that the absence of the terminal sialic acid resulted in a GM1 mimic, and its presence in a GD1a mimic (figure 3) provided evidence that CF90-26 has both GM1-like and GD1a-like LOSs. The LOS biosynthesis gene sequence in strain CF90-26 (GenBank accession number AY661458) was

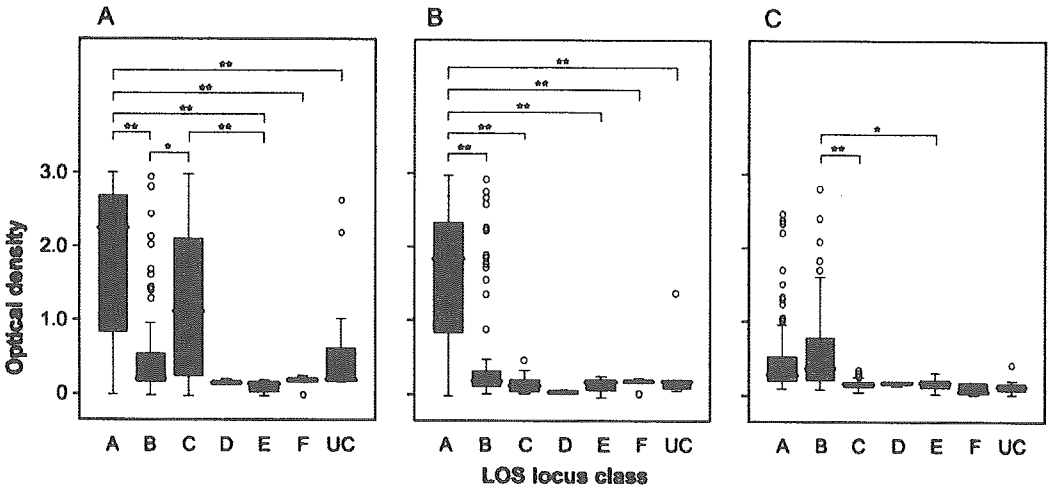

Figure 2. Box and whisker plot of *Campylobacter jejuni* lipo-oligosaccharide reactivity with anti-GM1 (GB2; *A*), anti-GD1a (GB1; *B*), and anti-GQ1b/GT1a (FS3; *C*) monoclonal antibodies in an ELISA. Strains are grouped by lipo-oligosaccharide (LOS) locus class (A–F and unclassified [UC]). Center lines denote medians, boxes denote 25%–75% percentiles, whiskers denote 10% and 90% percentiles, and white circles denote outliers. $^{*}P < .05$; $^{**}P < .01$ (Scheffé's test).

100% identical to the corresponding region in the *C. jejuni* HS:19 type strain (GenBank accession number AF167344), which also expresses a mixture of GM1 and GD1a mimics in its LOS outer core [17].

Risk factors for development of GBS. Because univariate analysis showed that class A strains were associated with GBS, we compared the features of GBS-associated and enteritis-associated class A strains. Differences remained significant between GBS-associated and enteritis-associated strains in the frequency of the HS:19 serotype, the frequency of *cstII* (Thr51), and LOS binding of anti-GM1 and anti-GD1a IgG autoantibodies (table 5). All HS:19 strains with the class A locus had the *cstII* (Thr51) genotype, except for the 2 GBS-associated strains (OH4382 and OH4384) that were obtained from siblings with GBS and external ophthalmoplegia [30]; these 2 strains were known to carry GD3-like or GT1a-like LOSs [17], as well as the GM1 epitope [39], all of which are present with the *cstII* (Asn51) genotype. Multiple logistic regression modeling was used to adjust the comparisons between GBS-associated and enteritis-associated strains for the class A locus, the HS:19 serotype, the *cstII* (Thr51) genotype, and GM1-like and GD1a-like LOSs. In that analysis, the difference remained significant for the HS:19 serotype (odds ratio [OR], 16.5 [95% confidence interval {CI}, 4.0–68.8]; $P < .001$) and the class A locus (OR, 5.6 [95% CI, 2.1–15.1]; $P = .001$).

DISCUSSION

We confirmed the finding of Godschalk et al. [15] that GBS is associated with the class A locus of *C. jejuni* and provided evidence of the first GBS-related *C. jejuni* characteristic that is common to strains from Asia and Europe. Moreover, we found that strains with the class A locus regularly express both the GM1 and the GD1a epitope on their LOSs; this unique LOS profile among *C. jejuni* strains results in an increased risk of producing anti-GM1 and anti-GD1a IgG autoantibodies and, therefore, developing GBS. Expression of the GM1 and GD1a epitopes in class A strains was enhanced in strains that were also serotype HS:19, and this expression was possibly dependent on the predominance of the *cstII* (Thr51) genotype in HS:19 strains. Of course, microbial properties alone do not sufficiently explain why an autoimmune response is triggered in only a minority of individuals with *C. jejuni* enteritis. Host susceptibility must be much more important. Previous attempts to find common host immunogenetic factors in patients with *C. jejuni* GBS, however, have had negative or conflicting results [40–44].

The class A locus is 11.5 kb and has 13 genes. A and B class loci have the same gene profile, except that the class B locus has *orf5II (cgtAII)*, which may be the result of duplication of *orf5I (cgtAI)* [27]. This raises the question as to why GBS-associated strains primarily have the class A locus. Our findings suggest that nucleotide sequence variation within genes is the answer. In fact, strains with the same LOS biosynthesis

Table 4. Lipid A variants and variable terminal sialic acids of an *O*-deacylated sample from *Campylobacter jejuni* strain CF90-26.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

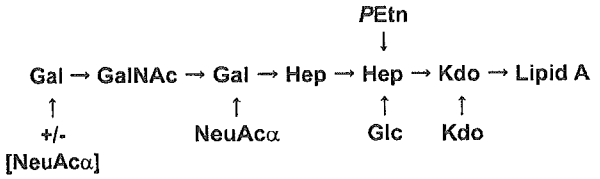

Figure 3. Proposed lipo-oligosaccharide outer core structures as determined on the basis of capillary electrophoresis–electrospray ionization mass spectrometric analysis of *O*-deacylated lipo-oligosaccharide samples from *Campylobacter jejuni* strain CF90-26. Gal, galactose; GalNAc, *N*-acetylgalactosamine; Hep, L-glycero-D-manno-heptose; Glc, glucose; Kdo, 3-deoxy-D-manno-2-octulosonic acid; NeuAc, *N*-acetylneuraminic acid; PEtn, phosphoethanolamine.

gene content have been shown to express diverse ganglioside mimics because of phase variation, a single nucleotide deletion, or single or multiple nucleotide mutations of LOS biosynthesis genes [27]. As we reported in the present study, class A strains regularly had *cstII* (Thr51), which is indicative that this genotype is a feature of class A strains and is related to the onset of GBS, because *cstII* (Thr51) encodes an enzyme with α-2,3-sialyltransferase activity that transfers a single α-2,3-sialic acid to both the inner and the terminal galactose residues, resulting in GM1 and GD1a mimics (figure 1) [27].

Candidate enzyme functions of the class A locus have been proposed, and most of them seem to be essential for the biosynthesis of ganglioside-like LOS [28, 45–47]. This suggests that the content of a single gene of *C. jejuni* is insufficient for the development of GBS; the entire gene content of the class A locus is essential. Nachamkin et al. [19] reported that the 3 glycosyltransferase genes that are necessary for ganglioside-like LOS biosynthesis—*cstII/cstIII* (sialyltransferase), *cgtA* (β-1,4-*N*-acetylgalactosaminyltransferase), and *cgtB* (β-1,3-galactosyltransferase)—[28] are more often present in GBS-associated strains than in enteritis-associated strains. These 3 genes are present in class A, B, and C loci [27], which agrees with our finding that 96% of GBS-associated strains had the class A, B, or C locus. This strongly suggests that the class A, B, or C locus is required to induce the development of GBS, although the class C locus is a much weaker risk factor than is the class A locus.

Interestingly, class A strains regularly express GM1-like and GD1a-like LOSs, whereas class C strains express GM1-like LOS only. GM1 and GD1a are candidate target antigens for the circulating autoantibody [10]. Our results suggest that multiple ganglioside mimicry is more effective for developing GBS than is single ganglioside mimicry. This disagrees with the findings of Nachamkin et al. [19] that the expression of the GD1a epitope alone is associated with GBS. The reason for this discrepancy is not known. We have shown in the present study, however, that patients from whom class A strains were isolated often had both anti-GM1 and anti-GD1a IgG autoantibodies; in addition, the inoculation of rabbits with CF90-26 LOS (which has GM1 and GD1a mimics) caused acute motor axonal neuropathy that was accompanied by anti-GM1 antibodies, not by anti-GD1a antibodies [14]. The assumption that GM1 mimicry, in addition to GD1a mimicry, is responsible for the development of GBS, therefore, is reasonable.

On the basis of chemical analysis, the coexistence of the GM1 and GD1a epitopes on the outer core of the LOS of the HS:19 serotype reference strain has been reported [17]. Elsewhere, we showed by use of mAb immunostaining that both epitopes were present in a GBS-associated HS:19 strain (CF90-26) [38], and we confirmed that finding in the present study by use of mass-spectrometric analysis. Furthermore, the nucleotide sequence of the 6.1-kb PCR product that included *cstII*, *cgtA*, and *cgtB* (as well as downstream and upstream sequences) was identical to the corresponding region in the *C. jejuni* HS:19 type strain that also expresses mixed GM1 and GD1a mimics [17]. This finding confirms that the DNA sequence, as well as

Table 5. Comparison of Guillain-Barré syndrome–associated and enteritis-associated *Campylobacter jejuni* strains with the class A lipo-oligosaccharide (LOS) locus.

Characteristic	Guillain-Barré syndrome–associated strains (n = 72)	Enteritis-associated strains (n = 17)	2-tailed P[a]	OR (95% CI)
Serotype HS:19	66 (92)	6 (35)	<.001	20.2 (5.5–73.9)
cstII polymorphism				
Thr51	66 (92)	10 (58)	.002	7.7 (2.1–27.6)
Asn51	6 (8)	7 (41)	.002	0.13 (0.036–0.47)
Median OD ± SD of mAb to LOS[b]				
GM1-like LOS	2.402 ± 0.897	1.106 ± 1.107	.002	
GD1a-like LOS	1.976 ± 0.830	0.558 ± 1.215	.03	
GQ1b/GT1a-like LOS	0.086 ± 0.431	0.126 ± 0.500	.11	

NOTE. Data are no. (%) of strains, unless otherwise indicated. CI, confidence interval; mAb, monoclonal antibody; OR, odds ratio.

[a] Fisher's exact test or Mann-Whitney *U* test.

[b] ODs were measured by an ELISA with mAbs GB2 (GM1), GB1 (GD1a), and FS3 (GQ1b/GT1a).

the makeup of glycosyltransferase genes, is responsible for determining the type of ganglioside mimic that is formed on LOSs.

In the present study, we found that most of the FS-associated strains had the class A or B locus, which supports the finding of van Belkum et al. [48] that *cstII* was present in all 8 strains with GQ1b-like LOS that they tested. Godschalk et al. [15] found that all 4 of the FS-associated strains that they tested had the class B locus, whereas a significant number of FS-associated strains that we tested in the present study had the class A locus. Furthermore, the differences between the class A and the class B locus were not important in our FS-associated strains, whereas the *cstII* (Asn51) genotype was critical. *cstII* (Asn51) has both α-2,3- and α-2,8-sialyltransferase activities [27], which are essential for transferring the disialyl moiety to the outer core of LOS, thereby mimicking GQ1b and GT1a gangliosides. Our findings suggest that the ganglioside-like LOS synthesis gene contents of *cstII, cgtA,* and *cgtB,* which are common to the class A and B loci, are important for triggering an autoimmune response and that *cstII* polymorphism is the determinant of autoantibody reactivity and neurological presentations in GBS and FS.

Acknowledgments

We thank Maki Okazaki, for her support during the polymerase chain reaction experiment; Denis Brochu, Scott Houliston, Frank St. Michael, and Evgeny Vinogradov, for the lipo-oligosaccharide analyses; Marie-France Karwaski and Sonia Leclerc, for help with the DNA sequencing; and Yukihiro Nishimoto (Department of Pediatrics, Kinan General Hospital, Wakayama, Japan), Takayuki Masaki (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan), Shigeru Matsushita (Tama Branch Laboratory, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan), and Maiko Murai (Department of Neurology, Saiseikai Central Hospital, Tokyo, Japan), for providing the *Campylobacter jejuni* strains from patients with enteritis.

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Campylobacter gene polymorphism as a determinant of clinical features of Guillain–Barré syndrome

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Abstract—*Background:* Ganglioside epitopes on *Campylobacter jejuni* are hypothesized as the key to the development and characterization of Guillain–Barré syndrome (GBS), but a comprehensive theory has yet to be established. A *C jejuni* gene, *cst-II*, involved in the biosynthesis of ganglioside-like lipo-oligosaccharide, shows a polymorphism (Asn/Thr51) that affects ganglioside epitopes. *Objective:* To examine the hypothesis that this polymorphism determines autoantibody reactivity, and thereby neurologic presentations in GBS. *Methods: C jejuni* isolates were collected from 105 GBS (including its variants) and 65 uncomplicated enteritis patients. The authors examined the frequency of *cst-II* and polymorphism (Asn/Thr51) in connection with the bacterial ganglioside epitopes, autoantibody reactivities against GM1, GD1a, and GQ1b, and patients' neurologic findings. *Results:* Neuropathic strains more frequently had *cst-II*, in particular *cst-II* (Thr51), than did enteritic ones (85% vs 52%; $p < 0.001$). Strains with *cst-II* (Asn51) regularly expressed the GQ1b epitope (83%), whereas those with *cst-II* (Thr51) had the GM1 (92%) and GD1a (91%) epitopes. The presence of these bacterial epitopes in neuropathy patients corresponded to autoantibody reactivity. Patients infected with *C jejuni* (Asn51) more often were positive for anti-GQ1b IgG (56% vs 8%; $p < 0.001$) and had ophthalmoparesis (64% vs 13%; $p < 0.001$) and ataxia (42% vs 11%; $p = 0.001$). Patients who had *C jejuni* (Thr51) more frequently were positive for anti-GM1 (88% vs 35%; $p < 0.001$) and anti-GD1a IgG (52% vs 24%; $p = 0.006$) and had limb weakness (98% vs 71%; $p < 0.001$). *Conclusions:* The genetic polymorphism of *C jejuni* determines autoantibody reactivity as well as the clinical presentation of Guillain–Barré syndrome (GBS), possibly through modification of the host-mimicking molecule. The GBS paradigm is the first to explain the detailed pathogenesis of a postinfectious, autoimmune-mediated, molecular mimicry-triggering disorder.

NEUROLOGY 2005;65:1376–1381

Guillain–Barré syndrome (GBS) is characterized by limb weakness and loss of tendon reflexes, but has a variety of other neurologic presentations.[1] Anti-GM1 and anti-GD1a IgG antibodies are associated with axonal GBS,[2] whereas anti-GQ1b IgG antibody is specific to patients with Fisher syndrome (FS) or GBS with ophthalmoplegia.[3] *Campylobacter jejuni* is the most frequent antecedent infectious agent in GBS.[4] The critical factor that causes the development of neuropathy after *C jejuni* infection is unknown, but the bacterial lipo-oligosaccharide (LOS) is a candidate because its terminal sugar regions mimic the sugar residues of the gangliosides GM1, GD1a, and GQ1b.[5-7] The development of axonal GBS model after inoculation of rabbits with the GM1-like LOS has provided conclusive evidence for the hypothesis that the ganglioside-mimicry of *C jejuni* LOS is a cause of GBS.[8] However, determinant factor of anti-ganglioside antibody specificity and neurologic presentation in GBS remains unclear.

Determination of the complete genome sequence of *C jejuni* NCTC 11168 showed that many LOS biosynthesis genes are encoded in a large cluster.[9] A subsequent study identified the genes involved in the

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This article was previously published in electronic format as an Expedited E-Pub on September 14, 2005, at www.neurology.org.

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Supported in part by grants from the Ichiro Kanehara Foundation to M.K.; the Kanae Foundation for Life & Socio-Medical Science to M.K.; the Japan Intractable Diseases Research Foundation to M.K.; Mizutani Foundation for Glycoscience to N.Y.; a grant for Scientific Research (B) (KAKENHI 14370210 to N.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Research Grant for Neuroimmunological Diseases to N.Y. from the Ministry of Health, Labour and Welfare 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.).

Disclosure: The authors report no conflicts of interest.

Received April 4, 2005. Accepted in final form June 20, 2005.

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transfer of galactose, *N*-acetylgalactosamine, and sialic acid to the LOS outer core.[10] Because ganglioside classification is based on the sialylation type (see figure E-1 on the *Neurology* Web site at www.neurology.org),[11] sialyltransferase-encoding genes may be associated with the variation in the ganglioside epitope on LOS. It was reported that *cst-II*, a gene encoding sialyltransferase,[10,12] was present in all eight strains with GQ1b-like LOS and proposed that its presence is associated with various ganglioside-like LOSs, although *cst-II* frequency did not differ between the GBS/FS and uncomplicated enteritis strains studied.[13] In contrast, based on tests of 28 GBS isolates, it was reported that the *cst-II* gene is more often present in GBS isolates than in enteritis isolates.[14] Whether the presence of this gene is a risk factor for developing neuropathy after *C jejuni* enteritis has yet to be proved.

The ganglioside-like structure of the *C jejuni* LOS is, in part, determined by the multiple mechanisms the bacterium uses to turn on or off a gene or to modulate the substrate specificities of its glycosyltransferases, as well as by different gene contents.[15] Interestingly, variation in the nucleotide sequence of *cst-II* might affect enzymatic activity; Cst-II (Thr51) has only α-2,3-sialyltransferase activity (monofunctional), whereas Cst-II (Asn51) has both α-2,3- and α-2,8-sialyltransferase activities (bifunctional).[15] Because both α-2,3- and α-2,8-sialyltransferase activities are required for the biosynthesis of GQ1b mimics such as GT1a- or GD1c-like LOS,[6,7] *cst-II* polymorphism is assumed to affect autoantibody reactivities through change in the ganglioside epitope on the LOS outer core, resulting in the diverse neurologic features shown by patients with GBS. In this study we used 105 *C jejuni* isolates from patients with GBS or a clinical variant and compared *cst-II* gene frequency in the neuropathic and enteritic strains. We also examined the hypothesis that the genetic polymorphism of the bacterium produces the differences in the clinical manifestation of GBS.

Methods. *Bacterial strains and patients.* Since 1990, we have received more than 8,000 requests from Japanese physicians to test serum anti-ganglioside antibodies from patients presenting with various neurologic disorders. On receipt of serum samples from patients with GBS or a clinical variant, we request the primary physicians to do a stool culture and to send the patient's stool specimen to the Tokyo Metropolitan Institute of Public Health for *C jejuni* isolation. From 1990 to 2002, 81 *C jejuni* strains were isolated successfully by one of the authors (M.T.), and 24 strains were obtained from hospitals. These were used in this study (n = 105: GBS, 80; FS, 20; Bickerstaff brainstem encephalitis,[16] 3; acute ophthalmoparesis,[17] 1; acute oropharyngeal palsy,[18] 1). Most of the strains used were included in our previous study.[19] Two, OH4384 and OH4382, were obtained from sibling GBS patients[6,20]; the others were from sporadic cases with geographically equal distributions. A questionnaire was filled in by the primary physician as to whether diagnostic criteria for GBS or a clinical variant were fulfilled. We also reviewed the patients' medical records to ascertain diagnoses and neurologic findings. GBS, FS, Bickerstaff brainstem encephalitis, and acute ophthalmoparesis diagnoses were all based on clinical criteria.[21,22] ELISAs were used to test for the presence of IgG antibodies to GM1, GD1a, and GQ1b, as reported elsewhere.[23] Sixty-five strains that had been isolated from patients with uncomplicated enteritis and collected throughout Japan were the controls. Anti-ganglioside antibodies were not tested in the enteritis patients because previous studies showed that such patients did not have the autoantibodies.[24,25]

Detection of ganglioside epitopes on LOS. Crude LOS fractions were prepared from the isolates, as described elsewhere,[26] with minor modifications.[27] We first performed sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and silver staining to ensure LOS had been extracted then used western blotting with the cholera toxin B-subunit (a ligand for GM1-oligosaccharide structure) to examine whether the GM1 epitope was present on it. A 5 μL portion of the bacterial lysate was added to each well, after which the samples were separated on 15% tricine-SDS-polyacrylamide gels (SPU-15S series, Atto Corporation, Tokyo, Japan).[28] After electrophoresis, the LOS samples were blotted on polyvinylidene difluoride membranes (Atto Corporation), and the membranes incubated at 4 °C for 2 hours with the peroxidase-conjugated cholera toxin B-subunit (List Biologic Laboratories, Campbell, CA) diluted 1:2,000 in phosphate-buffered saline containing 0.5% casein. Binding was made visible with 4-chloro-1-naphtol (Konica Immunostaining HRP-1000, Konica, Tokyo, Japan).

Ganglioside-like LOS also was investigated by thin-layer chromatography (TLC) with immunostaining because this method decreases the volume of reagent needed in the immunostaining step. The reagents used are the monoclonal antibodies (GB1 [anti-GD1a], GB2 [anti-GM1], and FS1 [anti-GQ1b]),[8,27] and sera from patients with GBS (S6960 [anti-GM1] and S5174 [anti-GD1a]) or FS (S7577 [anti-GQ1b]). A 10 μL portion of each bacterial lysate was spotted on a precoated Silica Gel 60 TLC plate (Merck, Darmstadt, Germany), developed with an *n*-propanol-water-25% ammonia solution (6:5:1, by volume), after which the plates were immunostained as reported elsewhere.[23] The TLC analysis was done by one of the authors (M.K.).

PCR and DNA sequencing. A single bacterial colony was suspended in 300 μL of sterile deionized distilled water and boiled for 10 minutes. After centrifugation at 15,000 rpm for 1 minute, the supernatant was used as the template in the PCR amplification. Oligonucleotide primers were designed based on the reported *C jejuni cst-II* sequence (GenBank accession no. AF400048): forward directional primer, 17F (5′-TTTCTGGAAATGGACCAAGTTT-3′), and reverse, 220R (5′-CGGTCTCATATTCTTGATTTTGG-3′). These primers amplify the 204 bp fragment of the *cst-II* gene carrying the 51st codon. Amplification reactions were run in a total volume of 50 μL with 20 pmol of each primer, 10 μL of template, and 2.5 units of *Taq* DNA polymerase (TaKaRa Ex Taq, Takara Bio Inc., Shiga, Japan), 40 nmol dNTPs, and buffer (2 mM Mg^{2+}), as recommended by the manufacturer. After a denaturation step of 5 minutes at 95 °C, the amplification mixture underwent 30 cycles of amplification, each cycle consisting of 10 seconds at 94 °C, 10 seconds at 55 °C, and 30 seconds at 72 °C, in a GeneAmp PCR System 9700 (PE Biosystems, Foster City, CA). PCR products were purified with a QIAquick PCR Purification Kit (Qiagen K.K., Tokyo, Japan). Nucleotide sequences of PCR products were determined with an ABI BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Statistical analysis. Frequency differences between the groups were compared by means of Fisher's exact test using SPSS 12.0J software (SPSS Inc., Chicago, IL). A difference was considered significant when the two-sided p value was less than 0.05.

Results. cst-II *Gene frequency and polymorphism (Thr/Asn51).* PCR and direct nucleotide sequence analyses showed that the *cst-II* gene more often was present in neuropathic than enteritic strains (table 1). Although the 51st codon of *cst-II* is reported to be AAT (corresponding to Asn) or ACC (Thr),[15] some strains in our study had AAC (Asn). This indicates that a single base change (A to C or C to A) which may occur during infection can alter the substrate specificity of Cst-II (monofunctional or bifunctional). *cst-II* (Thr51) was more common in the neuropathic (50%) than enteritic (25%) strains ($p = 0.001$), whereas *cst-II* (Asn51) did not differ (34% vs 26%; $p = 0.31$). Also, 9 of 16

Table 1 *Comparison of genetic and phenotypic properties of neuropathic and enteritic* Campylobacter jejuni *strains*

	Neuropathic strains, n = 105	Enteritic strains, n = 65	*p* Value
Gene			
cst-II	89 (85)	33 (51)	<0.001
cst-III	9 (9)	15 (23)	0.012
GM1 epitope*			
Cholera toxin	62 (59)	25 (38)	0.01
GB2	63 (60)	25 (38)	0.007
S6960	60 (57)	22 (34)	0.004
GD1a epitope*			
GB1	51 (49)	13 (20)	<0.001
S5174	49 (47)	12 (18)	<0.001
GQ1b epitope*			
FS1	22 (21)	13 (20)	1.0
S7577	32 (30)	17 (26)	0.60

Values are n (%).

* The reagents used are the monoclonal antibodies (GB1 [anti-GD1a], GB2 [anti-GM1], and FS1 [anti-GQ1b]), and sera from patients with Guillain-Barré syndrome (S6960 [anti-GM1] and S5174 [anti-GD1a]) or Fisher syndrome (S7577 [anti-GQ1b]). Cholera toxin (B-subunit) is a ligand for GM1-oligosaccharide structure.

neuropathic strains without *cst-II* had the *cst-III* gene,[12] and this gene was significantly less frequent in the neuropathic strains compared to the enteritic ones (see table 1).

Ganglioside-like LOS. Although we used two or three reagents in the detection of each ganglioside epitope, the overall results were identical, except for some discrepancies probably due to differences in the sensitivities of the reagents (see table 1). Figure E-2 shows the ganglioside epitope detection in representative strains. Neuropathic strains more commonly expressed GM1 and GD1a epitopes than did enteritic strains. In contrast, the frequency of the GQ1b epitope did not differ between them. Immunostaining results for the patients' sera showed that the GM1 and GD1a epitopes commonly were present in the same neuropathic strains (n = 48; 46%), whereas the GQ1b epitope was present isolatedly (n = 27; 26%). GQ1b and GM1

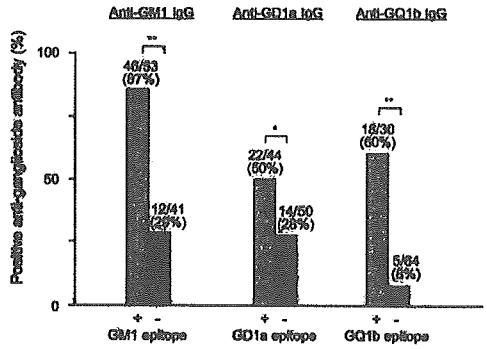

Figure. The ganglioside-like lipo-oligosaccharide of Campylobacter jejuni *and anti-ganglioside autoantibody reactivity in patients with Guillain–Barré syndrome or a variant. Frequencies of positive IgG antibodies against GM1 (left), GD1a (middle), and GQ1b (right) are compared between patients whose isolates carry ganglioside epitope on lipo-oligosaccharide (LOS) (pale bars) or not (dark bars). *p = 0.04; **p < 0.001.*

epitopes coexisted in five strains. Seventeen (16%) of the strains had none of the ganglioside epitopes examined.

cst-II Polymorphism (Thr/Asn51) was closely related to the ganglioside epitopes on LOS in both the neuropathic and enteritic strains. Asn51 strains more often expressed GQ1b epitope than did the others (table 2). In contrast, most Thr51 strains carried GM1- and GD1a-like LOS. Because the 53rd codon of *cst-II* is reported to affect α-2,3- and α-2,8-sialyltransferase activities in an Asn51 strain,[15] we analyzed its association with ganglioside-like LOS. Of the seven strains with *cst-II* (Asn51/Ile53) thought to have relatively low α-2,3- and α-2,8-sialyltransferase activities,[15] four had both GQ1b and GM1 epitopes, three had none. In contrast, all 22 strains (Asn51/Ser53 and Asn51/Gly53) thought to have high α-2,8-sialyltransferase activity had only the GQ1b epitope. Four of seven strains (Asn51/Tyr53) also had only the GQ1b epitope. The other three had neither epitope.

Serologic and neurologic features. Detailed neurologic information was available for 97 neuropathy patients, and pretreatment serum samples were obtained within 4 weeks of neuropathic onset for 94. Serum anti-ganglioside autoantibody reactivities overall corresponded to the ganglioside epitopes on the LOSs of the isolates (figure): GM1 epitope on LOS and anti-GM1 IgG (p value < 0.001);

Table 2 *Association of bacterial properties with* cst-II *content and polymorphism in neuropathic* Campylobacter jejuni *strains*

	cst-II			*cst-II* (Asn51)			*cst-II* (Thr51)		
	Present	Absent	*p* Value	Present	Absent	*p* Value	Present	Absent	*p* Value
Lipo-oligosaccharide	n = 89	n = 16		n = 36	n = 69		n = 53	n = 52	
GM1 epitope*	53 (60)	7 (44)	0.28	4 (11)	56 (81)	<0.001	49 (92)	11 (21)	<0.001
GD1a epitope*	48 (54)	1 (6)	<0.001	0	49 (71)	<0.001	48 (91)	1 (2)	<0.001
GQ1b epitope*	31 (35)	1 (6)	0.04	30 (83)	2 (3)	<0.001	1 (2)	31 (60)	<0.001

Values are n (%).

* Judged by results of thin-layer chromatography with immunostaining of patients' sera (S6960 [anti-GM1], S5174 [anti-GD1a], and S7577 [anti-GQ1b]) having high anti-ganglioside antibody titer.

Table 3 Association of patients' clinical features with cst-II *content and polymorphism of their* Campylobacter jejuni *isolates*

	cst-II			*cst-II* (Asn51)			*cst-II* (Thr51)		
	Present	Absent	*p* Value	Present	Absent	*p* Value	Present	Absent	*p* Value
IgG antibody to	n = 80	n = 14		n = 32	n = 62)		n = 48	n = 46	
GM1	49 (61)	9 (64)	1.0	7 (22)	51 (82)	<0.001	42 (88)	16 (35)	<0.001
GD1a	32 (40)	4 (29)	0.56	7 (22)	29 (47)	0.03	25 (52)	11 (24)	0.006
GQ1b	20 (25)	3 (21)	1.0	18 (56)	5 (8)	<0.001	2 (4)	21 (46)	<0.001
Neurological sign	n = 81	n = 16		n = 33	n = 64		n = 48	n = 49	
Ophthalmoparesis	25 (31)	4 (25)	0.77	21 (64)	8 (13)	<0.001	4 (8)	25 (51)	<0.001
Facial palsy	14 (17)	1 (6)	0.45	11 (33)	4 (6)	0.001	3 (6)	12 (24)	0.02
Bulbar palsy	17 (21)	0	0.07	13 (39)	4 (6)	<0.001	4 (8)	13 (27)	0.03
Limb weakness	69 (85)	13 (81)	0.71	22 (67)	60 (94)	0.001	47 (98)	35 (71)	<0.001
Ataxia	17 (21)	4 (25)	0.74	14 (42)	7 (11)	0.001	3 (6)	18 (37)	<0.001
Sensory disturbance	26 (32)	3 (19)	0.38	9 (27)	20 (31)	0.82	17 (35)	12 (24)	0.27
Diagnosis	n = 89	n = 16		n = 36	n = 69		n = 53	n = 52)	
Guillain–Barré syndrome	68 (76)	12 (75)	1.0	17 (47)	63 (91)	<0.001	51 (96)	29 (56)	<0.001
Fisher syndrome	16 (18)	4 (25)	0.50	14 (39)	6 (9)	<0.001	2 (4)	18 (35)	<0.001

Values are n (%).

GD1a epitope and anti-GD1a IgG (p value = 0.04); and GQ1b epitope and anti-GQ1b IgG ($p < 0.001$).

As expected, *cst-II* polymorphism was closely related to serum anti-ganglioside autoantibody reactivities, and therefore to neurologic features, whereas gene content was not (table 3). Patients from whom *C jejuni* (Asn51) had been isolated more often had serum anti-GQ1b IgG autoantibody, cranial nerve palsies, and ataxia. In contrast, *C jejuni* (Thr51) was associated with anti-GM1 and anti-GD1a IgG antibodies and limb weakness. The diagnosis was GBS for most patients with *C jejuni* (Thr51), whereas it was GBS for 47% and FS for 39% of patients with *C jejuni* (Asn51). Conversely, compared to the enteritis patients, FS patients more often had been infected by *C jejuni* (Asn51) (70% vs 26%; p value = 0.001), whereas GBS patients had been by *C jejuni* (Thr51) (64% vs 25%; p value < 0.001). Variation in the 53rd codon was not associated with any type of autoantibody or neurologic feature in spite of its association with ganglioside-like LOS (data not shown).

Discussion. The pathogenesis of many post-infectious disorders is still unknown, no autoantigens or virulence factors having been identified. For example, why some group A streptococci can cause acute rheumatic fever and others acute glomerulonephritis is unclear.[29] In contrast, we are the first to show the detailed molecular mechanism of GBS after *C jejuni* enteritis, based on our and others findings that the genetic polymorphism of the bacterium alters the substrate specificity of the LOS biosynthesis enzyme[15] and that autoantibody reactivity determines the clinical presentation of GBS.[30] These findings suggest that the genetic polymorphism of antecedent agents determines autoantibody reactivities and clinical manifestations through change to the host-mimicking molecule in some post-infectious disorders. We believe that GBS is the first paradigm to explain the detailed pathogenesis of a post-infectious, autoimmune-mediated, molecular mimicry-triggering disorder.

We confirmed results of a previous report that *cst-II* more frequently exists in GBS than in enteritis isolates, indicative that its presence is a risk factor for developing GBS.[14] However, considerable numbers of strains from patients with uncomplicated enteritis also had *cst-II* gene, indicating that *cst-II* gene is necessary but not adequate for initiating autoimmune response, although *cst-II* genotype is important in determining antibody reactivity when autoimmune response is triggered. Certain other genes (e.g., *cst-III*[12] or an unidentified gene) might produce the enzyme protein instead, subsequently sialylating LOS. Three genes, *cst-I*, *-II*, and *-III*, are reported to encode sialyltransferase protein,[12,15] but *cst-I* was lacking in some strains with sialylated LOS and therefore is unlikely to be responsible for LOS sialylation.[10] Because Cst-III appears to have only α-2,3-sialyltransferase activity (monofunction),[15] *cst-II* content must be essential for α-2,8-sialyltransferase activity and thereby biosynthesis of the GQ1b epitope.[13,15]

Most of the previous studies have failed to find a specific *C jejuni* genotype for GBS and FS.[31-35] It was recently reported that the class A LOS biosynthesis locus was over-represented in GBS-associated as compared to enteritis strains, whereas all four of the FS-related strains belonged to class B.[36] The authors suspected that the frequent expression of a GM1 epitope in class A and a GQ1b epitope in class B strains is responsible for the development of GBS and FS. Their findings, however, do not provide the answers as to which difference leads to diverse

ganglioside-mimics (GM1 and GQ1b) in spite of there being almost the same class A and B gene profiles.[15] In contrast, our data clearly indicate that both presence and polymorphism of bacterial *cst-II* have a major role in the type of ganglioside-like structure on LOS, thereby determining autoantibody reactivity and the pattern of neurologic presentation. Phase variation owing to a homopolymeric G-tract in *cst-II* or other LOS synthesis genes also may be related to what ganglioside epitopes are present.[10] Along with those of a previous study,[15] however, our findings suggest that mainly it is variation in the *cst-II* 51st codon and supplemental variation in the 53rd codon that determine which ganglioside epitopes are present on LOS, but only the former was related to the autoantibody reactivity in and neurologic features of neuropathy patients. Interestingly, *Haemophilus influenzae*, a pathogen recently suggested to cause GBS and FS,[27,37] also has sialylated LOS.[38] Three genes (*lic3A*, *siaA*, *lsgB*) have been cloned for that sialylation enzyme.[39,40] Whether the polymorphism of *H influenzae* genes also is related to autoantibody reactivity and consequently to the neurologic features of GBS and FS requires investigation.

C jejuni strains which had been isolated from anti-GQ1b antibody-positive patients often carried GQ1b epitope on the LOS.[41] The specificity of anti-ganglioside antibody induced by immunization of *C jejuni* LOS overall corresponds to ganglioside epitopes on the LOS in rabbits.[42] In this study, we showed that the target ganglioside (GM1, GD1a, or GQ1b) for serum autoantibody corresponds to the LOS-mimicking gangliosides of isolates from individual patients. However, other gangliosides such as GM1b and GalNAc-GD1a could be target antigens for autoantibodies in GBS[30] and further investigation is necessary to explain the variety of the clinical manifestation of GBS in more detail.

Acknowledgment

The authors thank Dr. Michel Gilbert (Institute for Biologic Sciences, National Research Council of Canada, Ottawa, Canada) for critical reading of the paper; Saiko Koike and Takashi Namatame (Institute for Medical Science, Dokkyo University School of Medicine, Tochigi, Japan) for help with the bacterial and sequence analyses; Maki Okazaki (Department of Neurology, Dokkyo University School of Medicine, Tochigi, Japan) for technical assistance; and Dr. Yukihiro Nishimoto (Department of Pediatrics, Kinan General Hospital, Wakayama, Japan) and Dr. Shigeru Matsushita (Tama Branch Laboratory, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan) for providing the *C jejuni* strains from enteritis patients.

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ELSEVIER

Carbohydrate mimicry: a new paradigm of autoimmune diseases

Nobuhiro Yuki

Molecular mimicry of microbial components by self components is thought to be the mechanism that accounts for the antigen and tissue specificity of immune responses in post-infectious autoimmune diseases. Little direct evidence exists, and research in this area has focused principally on T cell mediated anti-peptide responses, rather than on humoral responses to carbohydrate structures. Guillain–Barré syndrome, the most frequent cause of acute neuromuscular paralysis, sometimes occurs after *Campylobacter jejuni* enteritis. Recent studies have revealed that carbohydrate mimicry of the bacterial lipo-oligosaccharide by the human ganglioside is an important cause of the syndrome. This new concept that carbohydrate mimicry can cause an autoimmune disease provides a clue to inducing the resolution of pathogenesis of other immune-mediated diseases.

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Current Opinion in Immunology 2005, 17:577–582

This review comes from a themed issue on
Autoimmunity
Edited by Jean-François Bach and Shimon Sakaguchi

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2005.09.004

Introduction

Molecular mimicry has been proposed as a pathogenic mechanism for autoimmune disease. The hypothesis is based on the epidemiological, clinical and experimental evidence of an association of infectious agents with autoimmune disease and observed cross-reactivity of immune agents with host 'self' antigens and microbial determinants. Many studies are consistent with the mimicry hypothesis, but none have convincingly demonstrated that mimicry is an important mechanism in the development of autoimmune disease in humans [1]. No replicas of human autoimmune diseases have been obtained by immunization of animals with a mimic of component of an infectious agent associated with epidemiological evidence between the disease and the agent.

Guillain–Barré syndrome (GBS), characterized by limb weakness and areflexia is a typical post-infectious autoimmune disease [2]. Since the near-elimination of poliomyelitis in the world, GBS has become the most frequent cause of acute flaccid paralysis. Most GBS patients have had gastrointestinal or upper respiratory symptoms one to two weeks before the onset of neurological symptoms. The Gram-negative bacterium *Campylobacter jejuni*, a leading cause of acute gastroenteritis in humans, is the most frequent antecedent pathogen. This review shows that carbohydrate mimicry is a cause of GBS, and that the bacterial gene polymorphism is a determinant of the clinical features. Both are new paradigms in the molecular pathogenesis of autoimmune diseases.

A true case of molecular mimicry

Four criteria must be satisfied to conclude that a disease is triggered by molecular mimicry [3•]: first, an epidemiological association between the infectious agent and the immune-mediated disease; second, the identification of T cells or antibodies directed against the patient's target antigens; third, the identification of microbial mimics of the target antigen; and forth, reproduction of the disease in an animal model. As reviewed here, GBS subsequent to *C. jejuni* enteritis fulfils all four criteria and provides the first verification that molecular mimicry is a cause of human autoimmune diseases.

Epidemiological association of *Campylobacter jejuni* with Guillain–Barré syndrome

A case-control study detected evidence of recent *C. jejuni* infection in 26% of patients with GBS in comparison to 2% of the household controls (a member of the patient's household) and 1% of the age-matched hospital controls [4]. This study established an epidemiological association between *C. jejuni* infection and GBS. *C. jejuni*-isolated GBS was most common in 10–30 year-old individuals, and the male:female ratio was 1.7:1 [5]. The median latent period between antecedent symptoms and the onset of neuropathy was 10 days.

GBS was originally considered to be a demyelinating disease of peripheral nerves. This view of GBS was shaped largely by what is known of its laboratory analogue, the experimental allergic neuritis induced by immunization with P2 protein (a component of peripheral nerve myelin). Now the presence of a primary axonal GBS, acute motor axonal neuropathy (AMAN), is widely recognized through findings of autopsy studies [6,7]. *C. jejuni* infection is associated with AMAN, but not with demyelinating GBS [8].

Autoantibodies against gangliosides in Guillain–Barré syndrome

Autoantibodies against the surface components of peripheral nerves were originally considered to be pathogenic

substances that induce GBS because plasma exchange facilitates the rate of recovery [2]. Gangliosides constitute a large family predominantly made up of cell-surface glycosphingolipids bearing a ceramide moiety anchored in the external leaflet of the lipid bilayer and a sialylated oligosaccharide core exposed extracellularly.

In AMAN, IgG is deposited on the axolemma of the spinal anterior roots [7]. This indicates that IgG, which binds effectively with complement components, is an important factor in the development of AMAN. Patients who developed AMAN subsequent to *C. jejuni* enteritis had IgG antibody against GM1, and their autoantibody titers decreased with the clinical course [9]. By contrast, patients who had had *C. jejuni* enteritis but no neurological disorder did not have the autoantibodies. GD1a as well as GM1 is an autoantigen for IgG antibodies in patients with AMAN subsequent to *C. jejuni* enteritis [10].

Campylobacter jejuni mimic of gangliosides

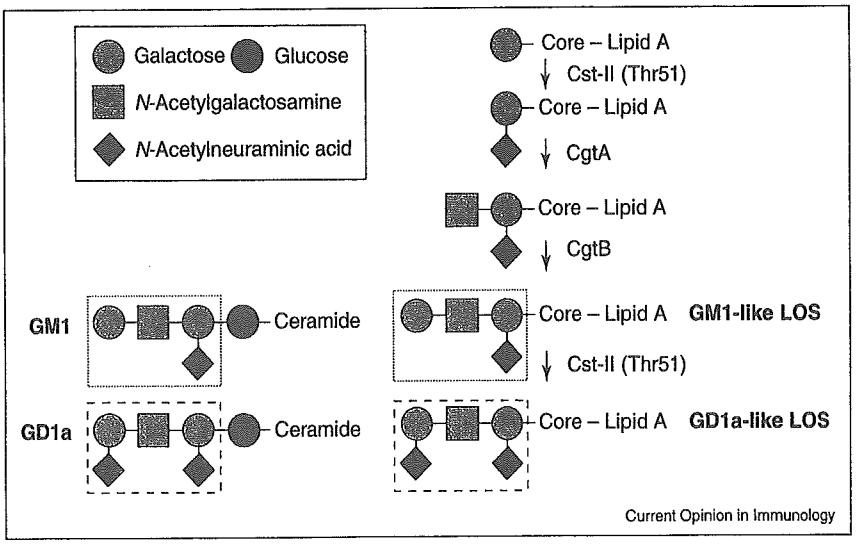
Lipo-oligosaccharide (LOS) is one of the most important cell-surface structures expressed by *C. jejuni*. One *C. jejuni* strain (CF90-26), isolated from an AMAN patient carrying anti-GM1 IgG antibodies, expresses an oligosaccharide structure (Gal β1–3 GalNAc β1–4 [NeuAc α2–3] Galβ), which protrudes from the LOS core (Figure 1) [11]. This terminal structure is identical to the terminal tetrasaccharide of the GM1 ganglioside. This was the first definitive evidence of molecular mimicry between human nerve tissue and *C. jejuni*. This strain also carries a GD1a-like LOS (Figure 1) [12]. Another *C. jejuni* strain (16971.94GSH), isolated from a patient with GBS, carried a GM1-like LOS [13]. One *C. jejuni* strain (ATCC 43446) from an enteritis patient also had GM1-like and GD1a-like LOSs [14].

Animal models of Guillain–Barré syndrome

Gangliosides extracted from bovine brain tissue have been widely used in Western Europe and South America as therapeutic agents for various neurological disorders. After receiving bovine brain ganglioside or an isolated GM1, some patients developed AMAN, and anti-GM1 IgG antibody was detected in these patients [15]. An AMAN model was established by sensitization of Japanese white rabbits with a bovine brain ganglioside mixture or an isolated GM1 [16]. The rabbits developed high anti-GM1 IgG antibody titres, then flaccid limb weakness of acute onset with a monophasic course. Pathological findings in their peripheral nerves showed predominant Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination. IgG was deposited on the axons of the ventral roots, internodal axolemmas and nodes of Ranvier. Cauda equina and spinal nerve root specimens from the paralyzed rabbits showed macrophage infiltration in the periaxonal space [17]. Surrounding myelin sheaths were almost intact. These findings correspond well with pathological findings for human AMAN [6,7]. This AMAN rabbit model was also reproducible in New Zealand white rabbits [18].

The most straightforward way to verify whether molecular mimicry between microbes and autoantigens causes GBS is to establish a GBS model by immunizing animals with components of antecedent infectious agents. An

Figure 1



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

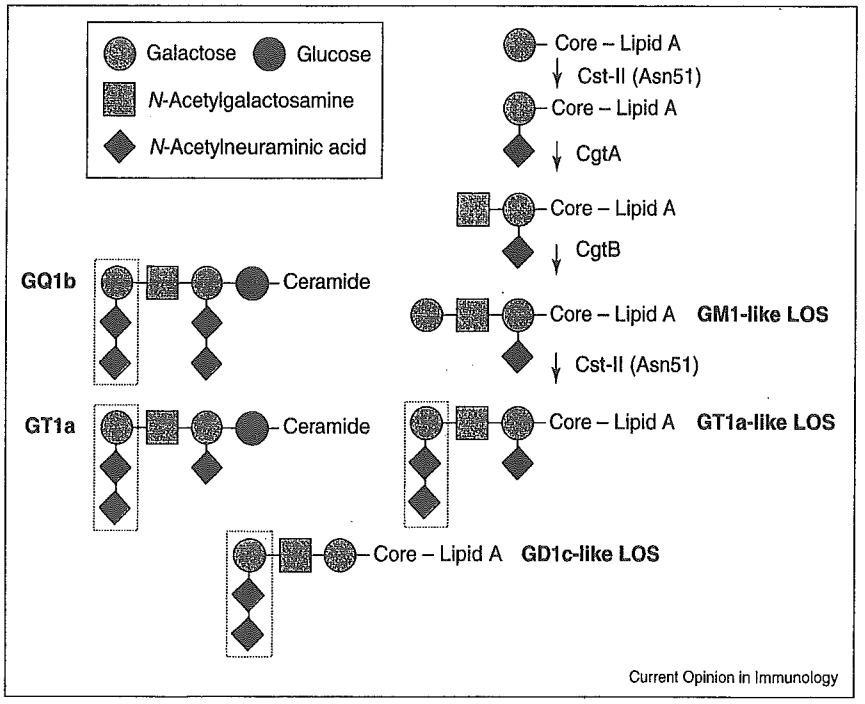
AMAN model was established by the immunization of Japanese white rabbits with *C. jejuni* LOS bearing a GM1-like structure [19••]. Following sensitization with this GM1-like LOS, rabbits developed high anti-GM1 IgG antibody titres and subsequent flaccid limb weakness. Their nerve roots had occasional macrophages in the periaxonal spaces surrounded by almost intact myelin sheaths. Axons of these nerve fibres showed various degrees of degeneration. Demyelination and remyelination were rare. These findings, which are compatible with the features of human AMAN, are evidence that rabbits inoculated with *C. jejuni* LOS constitute a valid AMAN model. This is the first definitive replica of a human autoimmune disease produced by immunization with the mimic of an infectious agent associated with epidemiological evidence of microbial infection.

Campylobacter jejuni genes associated with Guillain–Barré syndrome

As stated, ganglioside mimicry of *C. jejuni* LOS is a cause of GBS. Ganglioside-like LOS is synthesized by sialyltransferase Cst-II, *N*-acetylgalactosaminyl-transferase CgtA, and galactosyltransferase CgtB (Figures 1 and 2) [20]. Compared with gastroenteritis-related isolates, GBS-related *C. jejuni* isolates have a strong association with the expression of GD1a mimicry [21]. Some genes (*cst-II*, *cgtA* and *cgtB*) that are involved in ganglioside mimicry are also associated with GBS-related bacterial strains. These LOS biosynthesis genes cluster at the LOS biosynthesis gene locus [22]. A specific type of gene locus (called a class A gene locus), which includes these three genes, is associated both with GBS and with the GM1-like LOS [23••]. We have shown that isolates from GBS patients have specific LOS biosynthesis gene loci, which contain *cst-II* or *cst-III* more frequently than loci from enteritis patients (102/106 [96%] versus 72/103 [70%]; the difference is statistically significant), and that the GBS isolates expressed the GM1 or GD1a epitope, or both (M Koga and N Yuki, unpublished).

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]. Because both genes are involved in LOS sialylation, they are essential for ganglioside-like LOS synthesis. Mutants of *C. jejuni* that lack these genes have been made and analysed [23••]. Whereas a mixture of GM1-like and GD1a-like structures were identified in wild-type *C. jejuni* strains isolated from GBS patients, neither structure was present in the mutants. Both the *cst-II* and *neuA1* knockout mutants, unlike the wild types, had decreased reactivity to the sera of GBS patients. GM2/GD2 synthase knockout mice, which lack GM1 and GD1a, are immune-naïve hosts that

Figure 2



Carbohydrate mimicry of GQ1b/GT1a gangliosides, *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).

can be used to obtain high-titre anti-ganglioside antibody responses. Immunization 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 anti-ganglioside antibodies.

A bacterial gene (*cst-II*) polymorphism as a determinant of neurological features

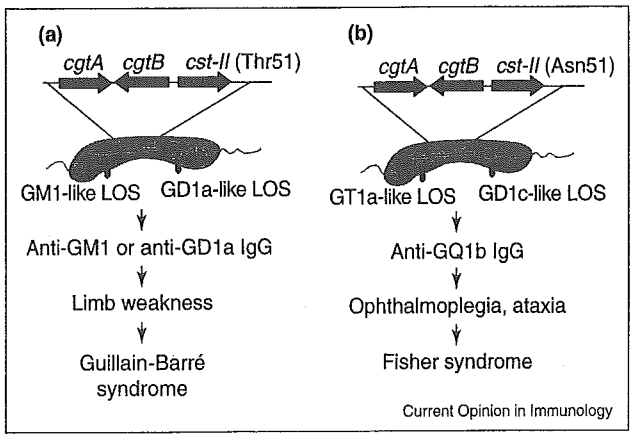
The reason why a certain microbial infection can induce the development of different autoimmune diseases has yet to be clarified. For example, group A streptococcal infections can induce the development of acute rheumatic fever in some patients and acute glomerulonephritis in others; however, the molecular pathogenesis is unknown [24]. The mechanism by which *C. jejuni* infection induces the development of GBS in some patients and Fisher syndrome (FS) in others has recently been clarified [25••].

Whereas GBS is characterized by limb weakness and areflexia, FS is characterized by ophthalmoplegia, ataxia and areflexia [2]. FS is regarded as a clinical variant of GBS because they sometimes overlap (some FS patients develop GBS during the illness). Although the presence of anti-GM1 and anti-GD1a IgG antibodies is closely related to AMAN, the presence of IgG antibodies to GQ1b, which is expressed in the oculomotor nerves and primary sensory neurons, are highly specific to patients with FS [26,27]. The anti-GQ1b IgG antibody cross-reacts with GT1a, indicating that the autoantibody recognizes the terminal trisaccharides common to GQ1b and GT1a (Figure 2). A case-control study showed serologic evidence of *C. jejuni* infections in FS patients was present significantly more often than in the sex- and age-matched hospital controls (21% versus 3%; [28•]). One *C. jejuni* strain (CF93-6) carried a GT1a-like LOS that had been isolated from a patient with FS. Another *C. jejuni* strain (PG836) isolated from an FS patient carried a GD1c-like LOS [29]. Both GT1a-like and GD1c-like LOS have a trisaccharide residue that is also common to GQ1b ganglioside (Figure 2).

Variation in the nucleotide sequence of *cst-II* might affect enzymatic activity; Cst-II (Thr51) has only α-2,3-sialyltransferase activity (monofunctional) and can make GM1-like and GD1a-like LOSs, whereas Cst-II (Asn51) has both α-2,3- and α-2,8-sialyltransferase activities (bifunctional) and can make GT1a-like and GD1c-like LOSs (Figure 2) [22]. Neuropathic strains more frequently have *cst-II* than enteritic strains [25••]. Strains with *cst-II* (Thr51) have the GM1 and GD1a epitopes, whereas those with *cst-II* (Asn51) regularly express the GQ1b epitope. Patients infected with *C. jejuni* (Thr51) more frequently than *C. jejuni* (Asn51) were positive for anti-GM1 and anti-GD1a IgG and had limb weakness, and GBS was diagnosed. Patients infected with *C. jejuni* (Asn51) were more often positive for anti-GQ1b IgG and had ophthalmoplegia and ataxia, and FS and the related conditions were diagnosed.

These results have presented a new paradigm that bacterial genetic polymorphism determines the clinical presentation of autoimmune diseases (Figure 3).

Figure 3



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 LOS on its cell surfaces. Infection by such *C. jejuni* strains can induce anti-GM1 or anti-GD1a IgG production in certain patients. Anti-GM1 or anti-GD1a IgG antibodies bind to GM1 or GD1a, respectively; 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 induces anti-GQ1b IgG production in certain patients. Anti-GQ1b IgG antibody binds to GQ1b, which is expressed on the oculomotor nerves and primary sensory neurons. This induces the development of Fisher syndrome.

Conclusions

Convincing evidence has shown that carbohydrate mimicry is a cause of GBS/FS subsequent to *C. jejuni* enteritis. Moreover, the bacterial gene responsible for this has been determined (*cst-II*). Only a minority of those who have *C. jejuni* enteritis develop GBS [30], and no host susceptibility genes associated with the development of GBS have yet been identified. The lipopolysaccharide receptors CD14 and Toll-like receptor 4 (TLR4) are important in antigen presentation and intracellular signalling, but the functional polymorphisms in *CD14* and *TLR4* are not associated with susceptibility to *C. jejuni*-associated GBS [31]. A genome-wide search of single nucleotide polymorphisms could identify the host susceptibility genes.

Acknowledgements

I thank M Gilbert for critical reading of the manuscript. The work that contributed to this review is supported in part by a grant-in-aid for Scientific Research (B) (KAKENHI 14370210) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Health Science Research Grant (Research on Brain Science) from the Ministry of Health and Welfare of Japan; and, a grant from the Human Frontier Science Program (RGP0038/2003-C).

References and recommended reading

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- • of special interest
- •• of outstanding interest

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Ganglioside mimicry as a cause of Guillain–Barré syndrome

Nobuhiro Yuki and Masaaki Odaka

Purpose of review

Campylobacter jejuni is the most frequent agent of antecedent infection in an axonal variant of Guillain–Barré syndrome, acute motor axonal neuropathy, and anti-GM1 or anti-GD1a IgG antibody is also associated with acute motor axonal neuropathy. Molecular mimicry has been found between human GM1 ganglioside and the lipo-oligosaccharide of *C. jejuni* isolated from an acute motor axonal neuropathy patient. Progress has been made in Guillain–Barré syndrome research, especially on acute motor axonal neuropathy subsequent to *C. jejuni* infection.

Recent findings

Sensitization of rabbits with *C. jejuni* lipo-oligosaccharide as well as GM1 induced the production of anti-GM1 IgG antibody and the subsequent development of acute flaccid paralysis. Pathological changes in rabbit peripheral nerves were identical to those seen in human acute motor axonal neuropathy. These findings provide conclusive evidence that molecular mimicry is a cause of human autoimmune disease. Ganglioside-like lipo-oligosaccharide is synthesized by sialyltransferase CgtB, *N*-acetylgalactosaminyltransferase CgtA, and galactosyltransferase CgtB. There is a strong association between the simultaneous presence of these genes and Guillain–Barré syndrome associated *C. jejuni* infection. Knockout mutants of *C. jejuni* genes involved in lipo-oligosaccharide sialylation had reduced reactivity with anti-GM1 sera from Guillain–Barré syndrome patients and did not induce an anti-GD1a IgG antibody response in mice. Lipo-oligosaccharide biosynthesis genes appear to be essential for the induction of anti-GM1 or anti-GD1a IgG antibody and the subsequent development of acute motor axonal neuropathy.

Summary

The concept that carbohydrate mimicry causes autoimmune disease provides a clue to the resolution of the pathogenesis of other immune-mediated diseases.

Keywords

acute motor axonal neuropathy, *Campylobacter jejuni*, ganglioside, Guillain–Barré syndrome, lipo-oligosaccharide, molecular mimicry

Curr Opin Neurol 18:557–561. © 2005 Lippincott Williams & Wilkins.

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Current Opinion in Neurology 2005, 18:557–561

Abbreviations

AIDP	acute inflammatory demyelinating polyneuropathy
AMAN	acute motor axonal neuropathy
GBS	Guillain–Barré syndrome

1350-7540

Introduction

Guillain–Barré syndrome (GBS), characterized by acute flaccid paralysis and areflexia, is the prototype of post-infectious autoimmune diseases. Most GBS patients have had gastrointestinal or upper respiratory symptoms one to two weeks before the onset of limb weakness. The relatively short period between the antecedent illness and neuropathy makes possible both the determination of the antecedent pathogen and its isolation from GBS patients. The Gram-negative bacterium, *Campylobacter jejuni*, a leading cause of acute gastroenteritis in humans, is the most frequent antecedent pathogen in GBS [1]. This review provides an update on molecular mimicry as a cause of GBS after *C. jejuni* infection.

A true case of molecular mimicry

Molecular mimicry is one mechanism by which infectious agents may trigger an immune response against autoantigens. Many study findings are consistent with the mimicry hypothesis, but none has convincingly demonstrated that mimicry is an important mechanism in the development of autoimmune disease in humans. Several examples of molecular mimicry between microbial and self components are known, but in most cases no epidemiological relationship between autoimmune disease and microbial infection has been established. Moreover, in some studies, no replica of human autoimmune disease has been obtained by immunization with the mimic of an infectious agent. Replicas associated with definite, epidemiological evidence of microbial infection are required to test the molecular mimicry theory of the development of autoimmune diseases.

Four criteria must be satisfied to conclude that a disease is triggered by molecular mimicry [2•]: (1) the establishment of an epidemiological association between the infectious agent and the immune-mediated disease; (2) the identification of T cells or antibodies directed against the patient's target antigens; (3) the identification