Lipopolysaccharides from Campylobacter jejuni O:41 Strains Associated with Guillain-Barré Syndrome Exhibit Mimicry of GM₁ Ganglioside

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Three Campylobacter jejuni, biotype 2, serotype O:41 strains that were isolated from patients who developed Guillain-Barré syndrome (GBS) and one C. jejuni isolate from a patient who developed enteritis only were examined. The aim of the study was to determine the structure of the core oligosaccharide (OS) of the lipopolysaccharide (LPS) of C. jejuni serotype O:41, a serotype rarely associated with the development of GBS, and to determine if the LPS shares similar epitopes with any of the major human gangliosides. Electroblotting with silver staining or immunoblotting demonstrated that the strains had LPS profiles characteristic of low-molecular-weight LPS. Colorimetric analysis detected N-acetylneuraminic (sialic) acid in the core OSs of all the strains. Thin-layer chromatography with immunostaining showed that antisera raised against the GBS strains reacted with the GM₁ ganglioside, suggesting that C. jejuni serotype O:41 LPSs and the GM₁ ganglioside have similar epitopes. Furthermore, polyclonal anti-GM₁ and anti-asialoGM₁ antibodies cross-reacted with each C. jejuni O:41 LPS tested, suggesting that the serotype O:41 core OS has a GM₁- and asialoGM₁-like structure. LPSs extracted from C. jejuni serostrains O:2, O:3, and O:19 were also used in the study. Cholera toxin (a GM₁ ligand) and peanut agglutinin (a Galα1-3GalNAc ligand) recognized all serotype O:41 LPSs and the serostrain O:2 LPS. Immunoaffinity sorption results confirmed GM₁ relatedness. Moreover, the core OS was isolated from a GBS-associated C. jejuni O:41 LPS by gel permeation chromatography. An analysis by gas-liquid chromatography (GLC), GLC-mass spectrometry, and nuclear magnetic resonance showed the core OS of one of the C. jejuni O:41 GBS isolates to have a tetrasaccharide structure consistent with GM₁ mimicry.

Guillain-Barré syndrome (GBS) is characterized as an acute, inflammatory polyneuropathy (48), and approximately two-thirds of GBS patients develop the syndrome following various infections of the respiratory or gastrointestinal tract (27). GBS is clinically very heterogeneous, and several variants of the disorder occur and include both acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN).Campylobacter jejuni, a leading cause of acute gastroenteritis in humans, has been identified as the single most important predisposing factor associated with the development of GBS and occurs in up to 66% of patients (20, 27, 29, 47). Characteristically, 76% of the AMAN and 42% of the AIDP GBS patients have serologic evidence consistent with recent C. jejuni infection (17, 30).

C. jejuni can be serotyped based on differences in the saccharide structure (O side chain and core oligosaccharide [OS]) of the lipopolysaccharide (LPS; O antigen) of the bacterium (32, 45, 46). Some reports suggest that only specific C. jejuni serotypes are associated with GBS. A predominance of C. jejuni O:19, an uncommon serotype in gastroenteritis patients, has been found in Japanese GBS patients (23, 24). Similarly, Fujimoto et al. (11) described four C. jejuni isolates that belonged to serotype O:19. This same serotype has been isolated from GBS patients in the United States, where 33% of GBS isolates were of serotype O:19 (28). Other C. jejuni serotypes that have been identified in association with GBS include O:2, O:2/44, O:4/59, O:5, O:10, O:15, O:18, O:21, O:24, O:30, O:37, and O:64 (24, 37, 39, 43, 49). C. jejuni O:2, O:10, and O:23 (19, 52, 66) have been found in association with Miller-Fisher syndrome, a variant of GBS comprising areflexia, ataxia, and ophthalmoplegia without limb weakness (50).

Serum antibodies against gangliosides have been observed in about 30% of GBS patients (27, 70). A predominance of the major human gangliosides are shown in Fig. 1. Antiganglioside antibodies to gangliosides, especially the GM₁ ganglioside, occur in GBS patient sera after C. jejuni infection during the acute phase of the illness (14, 19, 41, 42, 54, 64, 69, 70). Conversely, antiganglioside antibodies, including those in sera from GBS patients, cross-react with LPSs of C. jejuni serotypes with GBS (19, 54). Antiganglioside antibodies may be involved in the pathogenesis of GBS because some individuals have developed GBS-like symptoms after the administration of gangliosides (10, 18, 59) and, moreover, because plasma exchange and administration of intravenous immunoglobulin (Ig) elicit a beneficial response (59).

Chemical studies on LPS extracted from C. jejuni have shown that the structures of the terminal regions of the core OSs of specific serotypes mimic the structures of human gangliosides (2, 5, 6, 37), and research has focused on the view that molecular mimicry may be a factor in the pathogenesis of GBS (37). Furthermore, the core OSs of LPSs of C. jejuni O:19 isolates have been shown to mimic human gangliosides GM₁, GD₁ α, GT₁ β, and GD₃ (2, 3, 33, 64, 68). GM₁-like OS structures occur in LPSs from serostrains O:1, O:23, and O:36 (6), whereas the core OS of C. jejuni serostrain O:4 mimics the GD₃ ganglioside (6, 69). However, mimicry of C. jejuni O:2 is limited to that of a disaccharide which is present in a range of gangliosides including GD₁ α (4).

The present study describes the characterization of C. jejuni strains belonging to serotype O:41, three recovered from patients who developed GBS and one recovered from a patient who developed enteritis only. In particular, the presence of ganglioside-like epitopes in the LPSs of these strains was in-
Confluent monolayers (about 10^6 cells) were washed 30 min prior to infection with DMEM without supplements. A bacterial suspension in DMEM was added to each dish to give a multiplicity of infection of 10^3 bacteria per cell. Control experiments were performed with DMEM alone. Infected monolayers were incubated at 37°C for 2 h in an atmosphere of 95% air-5% CO_2, to allow bacteria to adhere to the cells. After incubation, monolayers were washed five times with DMEM and reincubated for another 3 h with DMEM containing 250 µg of gentamicin per ml. Following this second incubation, the monolayers were washed three times with phosphate-buffered saline (PBS; pH 7.4; Oxoid) and lysed in 0.5% Triton X-100. The suspensions were diluted, and the numbers of CFU were calculated. Colonies were confirmed as being C. jejuni by standard CFU procedures (33, 46, 56).

**Protease K digestion.** A procedure modified from that of Hitchcock and Brown (16) was used for the enzymatic digestion of whole-cell lysates. Bacteria were cultured on blood agar, harvested in PBS (pH 7.4), and diluted to an A_540 of 0.3. Quantities of 1.5 ml were transferred to microtubes and centrifuged at 13,000 × g for 5 min. The resultant pellets were solubilized in 200 µl of lysis buffer (20% glycerol, 5% 2-mercaptoethanol, 4.6% sodium dodecyl sulfate [SDS], 0.125 M Tris-hydrochloride-buffered saline [pH 6.8], 0.004% bromophenol blue). The lysate was heated to 100°C for 5 min and cooled to room temperature, and 40 µl of a 2 mg/ml protease K solution (Sigma Chemical Co., St. Louis, Mo.) was added. The incubation was performed at 37°C for 1 h and subsequently heated at 100°C for 5 min prior to electrophoresis.

**SDS-PAGE and immunoblotting.** The discontinuous buffer system of Laemmli (25) was used to fractionate the lysates prepared by protease K digestion of whole-cell lysates and also to examine purified LPS. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a stacking gel of 5% acrylamide and a separation gel of 15% acrylamide containing 3.2 M urea (BDH Laboratory Supplies, Poole, England). Samples were electrophoresed at a constant current of 35 mA until the tracking dye was within 0.5 cm of the bottom of the gel. After SDS-PAGE, the gels were fixed and the LPS was detected by silver staining, as described previously (55). Alternatively, LPS fractionated by SDS-PAGE was electrotransferred from gels to nitrocellulose membranes (pore size, 0.45 µm; Bio-Rad Laboratories, Hercules, Calif.) by using the buffer system of Towbin et al. (57). Visualization of nitrocellulose blots was performed with rabbit antiserum as the first antibody and goat anti-rabbit IgG-horseradish peroxidase conjugate (Bio-Rad) as the second antibody (46).

**TLC.** Gangliosides (Sigma) and LPS were analyzed by thin-layer chromatography (TLC) on precoated silica gel 60 glass plates (Merck, Darmstadt, Germany). Solvent systems consisting of chloroform-methanol-0.2% CaCl_2 : 2H_2O (50:45:10 [vol/vol/vol]) (51) and n-propanol-water–25% NH_4OH (60:30:10 [vol/vol/vol]) (54, 65) were used as developing solvents for gangliosides and LPS, respectively. Gangliosides and LPS were visualized by spraying plates with resorcinol-HCl reagent (55).

**Immunostaining.** TLC with immunostaining was performed by the procedure of Saito et al. (51), as modified by Schwerer et al. (54). Briefly, developed TLC plates were dried for 30 min in a vacuum desiccator, fixed in 1% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), and dried as before. Lanes were overlaid with either rabbit antiserum to ganglioside GM_1, GM_2, or asialoGM_1 (Matreya Inc., Pleasant Gap, Pa.) or rabbit antiserum to C. jejuni O:41 diluted 1:100 in a solution of PBS containing 0.3% gelatin. The enzyme-treated lysates were incubated at 37°C overnight in a humid chamber washed three times with cold PBS, overlaid with peroxidase-conjugated anti-rabbit IgG (Sigma) diluted 1:500 in gelatin-PBS and incubated in a humid chamber at room temperature for 1 h with gentle agitation. The plates were washed with cold PBS and immersed in a horseradish peroxidase color development solution (Bio-Rad) consisting of 60 ml of 4-chloro-1-naphthol dissolved in 20 ml of methanol and added to 100 ml of Tris-hydrochloride-buffered saline containing 60 µl of cold 30% aqueous H_2O_2 until the immunoreactants became visible. The substrate reaction was stopped by washing the plates with cold PBS.

Binding experiments with cholera toxin (CT)-peroxidase conjugate (Sigma) and peanut agglutinin (PNA)-peroxidase conjugate (Kem-En-Tec, Copenhagen, Denmark) were performed under the same conditions as those described for immunostaining. However, only one overlay step with CT-peroxidase conjugate at a dilution of 1:1,000 or PNA-peroxidase conjugate at a dilution of 1:50 in gelatin-PBS was used. Inhibition experiments were performed by a modified TLC technique whereby the B subunit of CT (Sigma) was diluted 1:250 in gelatin-PBS from a stock solution (1 mg/ml) and overlaid onto TLC plates. The plates were incubated for 1 h at room temperature with gentle rocking. Antiserum was subsequently overlaid overnight at 4°C onto the plates, and detection of immunoreactants was performed with horseradish peroxidase color development solution.

**Immunoadsorption.** Antiserum to C. jejuni O:41 was incubated with 200 µg of ganglioside GM_1 or 200 µg of LPS for 2 h at 37°C with rotation of the test tubes (Cell Major Mixer, model CM200). Immunoprecipitates were removed by centrifugation (10,000 × g, 10 min), and supernatants were tested. The adsorbed antiserum was diluted 1:100 in gelatin-PBS before tests to measure residual IgG binding activity to GM_1 and LPS. Similar experiments were performed with anti-GM_1, antiserum by immunoadsorption before being tested with 200 µg of LPS or 200 µg of GM_1.

**Materials and Methods.**

**Patients.** The clinical details of patients at Groote Schuur Hospital (GSH) and Red Cross Hospital (RXH) in Cape Town, South Africa, from whom C. jejuni was isolated have been described previously (26). Briefly, a 26-year-old male patient (A) from whom C. jejuni 16971.94RXH was isolated developed GBS 10 days after an episode of diarrhea. A cerebrospinal fluid (CSF) study performed in the first 48 h of illness was normal, and electromyogram studies showed evidence of a severe predominately motor polyneuropathy with axonal loss. There was no sensory or bulbary involvement. A 22-month-old female patient (B) from which C. jejuni 260.94RXH was isolated from a formed stool developed a more severe type of GBS. After ventilation and prolonged hospitalization, the patient progressed well with no relapse. A 29-year-old female patient (C), from whom C. jejuni 28134.94RXH was isolated, had suffered an episode of GBS at the age of 10 years. She had a history of upper respiratory tract infections. Her CSF characteristics were normal, and neurological tests showed evidence of a diffuse motor neuropathy with both demyelinating and axonal elements. C. jejuni 176.83 was isolated from a 9-year-old female patient (D) who developed enteritis but did not subsequently develop GBS.

**Bacterial strains and growth conditions.** Isolation of Campylobacter strains involved filtration and incubation in an H_2-enriched microaerobic atmosphere (GasPak BR38 [Oxoid Ltd., London, England] without a catalyst) according to an established protocol (26). Gram-negative, motile, spiral or curved rods were cultured and maintained on blood agar (tryptose agar base [Oxoid] with 10% unlysed horse blood) under microaerobic conditions as described previously (38). C. jejuni serotyprsa O2 (ATCC 43340), O3 (ATCC 43431), and O19 (ATCC 43446) were obtained from the American Type Culture Collection (Manassas, Va.). Strains were routinely grown on blood agar in a manner identical to that described above. Bacterial biomass was harvested, and bulk extraction of LPS was performed by the phenol-water extraction procedure described previously (38, 61).

**Biotyping and serotyping.** Bacterial identification was accomplished by established procedures (33, 46, 56). Isolates were biotype by the scheme of Skirrow and Benjamin (56). Serotyping on the basis of theophile somatic O antigens was performed with the 66 antisera of the Penner scheme (46) and an additional 30 antisera to new serotypes not included in the Penner scheme. Hemolysis assay. Hemolytic activity was screened by inoculating bacteria onto blood agar (tryptose agar base [Oxoid] with 10% unlysed horse blood) under microaerobic conditions as described previously (38). C. jejuni serotypes O2 (ATCC 43340), O3 (ATCC 43431), and O19 (ATCC 43446) were obtained from the American Type Culture Collection (Manassas, Va.). Strains were routinely grown on blood agar in a manner identical to that described above. Bacterial biomass was harvested, and bulk extraction of LPS was performed by the phenol-water extraction procedure described previously (38, 61).

**Invasion assay.** The invasiveness of C. jejuni isolates was determined according to the method of Wassenaar et al. (60). Briefly, intestinal cells (INT-407; Flow Laboratories, Herts, England) were maintained in Dulbecco’s modified Eagle's medium (DMEM; Gibco BRL, Grand Island, N.Y.), supplemented with 10% (vol/vol) fetal calf serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). The invasion assay was performed in 35-mm-diameter tissue culture dishes.
Electrophoretic patterns of C. jejuni serotype O:41 LPS. Silver-stained SDS-PAGE gels of water-phase LPS from C. jejuni O:41 exhibited a pattern of bands migrating near the bottom of the gel, corresponding to low-molecular-weight (low-\(M_r\)) rough-form LPS composed of core OS and lipid A, but bands characteristic of high-\(M_r\) LPS with O side chains were absent (Fig. 2). As C. jejuni high-\(M_r\) LPS is not visualized by the staining procedure of Tsai and Frasch (58), immunoblotting was performed with C. jejuni O:41 serotyping antiserum to visualize this high-\(M_r\) material (46). As with silver staining, no high-\(M_r\) LPS was visualized in any of the C. jejuni O:41 strains, and only low-\(M_r\) LPS was apparent (data not shown). Similar banding patterns were observed with low-\(M_r\) LPSs of proteinase K-treated whole-cell lysates and purified LPS, thus indicating that the phenol-water extraction did not alter the structure of the low-\(M_r\) LPS. The LPSs from all of the C. jejuni O:41 bacterial isolates, including the enteritis isolate, had similar mobilities, each migrating as one distinct band at the bottom of the gel. The serotype O:41 LPSs migrated to the same region of the gel as serostrain O:19 LPS, indicating similar molecular weights of core OSs in C. jejuni O:41 and O:19 LPSs.

**TABLE 1. Reactions of anti-ganglioside, anti-C. jejuni antiserum and ligands with gangliosides**

<table>
<thead>
<tr>
<th>Antibody or ligand</th>
<th>Strength of reaction* with ganglioside:</th>
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<tbody>
<tr>
<td></td>
<td>GM(_1)</td>
</tr>
<tr>
<td>Rabbit antibodies</td>
<td>Anti-GM(_1)</td>
</tr>
<tr>
<td></td>
<td>Anti-asialo-GM(_1)</td>
</tr>
<tr>
<td></td>
<td>Anti-GM(_2)</td>
</tr>
<tr>
<td></td>
<td>Anti-C. jejuni</td>
</tr>
<tr>
<td></td>
<td>16971.94GSH</td>
</tr>
<tr>
<td></td>
<td>260.94RXH</td>
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<tr>
<td>Ligands</td>
<td>CT</td>
</tr>
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<td></td>
<td>PNA</td>
</tr>
</tbody>
</table>

* **++++**, very strong reaction; **+++**, strong reaction; **++**, moderate reaction; **+**, weak reaction; (•), barely visible reaction; ---, no reaction. ND, not done.
TABLE 2. Binding of antiganglioside, anti-*C. jejuni* antisera and ligands with *C. jejuni* LPS

<table>
<thead>
<tr>
<th>Antibody or ligand</th>
<th>Strength of reaction* with LPS of <em>C. jejuni</em> strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O:41 16971.94GSH O:41 260.94RXH O:41 28134.94GSH O:41 176.83 O:2 O:3 O:19</td>
</tr>
<tr>
<td>Rabbit antibodies</td>
<td></td>
</tr>
<tr>
<td>Anti-GM1</td>
<td>++ ++ ++ ++ ++ + — ++</td>
</tr>
<tr>
<td>Anti-asialoGM1</td>
<td>++ (+) (+) (+) ++ + — ++</td>
</tr>
<tr>
<td>Anti-GM2</td>
<td>— — — — — — — —</td>
</tr>
<tr>
<td>Anti-C. <em>jejuni</em> O:41 16971.94GSH</td>
<td>+++ + ++ ++ ++ (+) — +</td>
</tr>
<tr>
<td>Anti-C. <em>jejuni</em> O:41 260.94RXH</td>
<td>+++ + ++ ++ ++ (+) — +</td>
</tr>
<tr>
<td>Ligands</td>
<td></td>
</tr>
<tr>
<td>PNA</td>
<td>++ ++ ++ ++ ++ ++ ++ — +</td>
</tr>
<tr>
<td>CT</td>
<td>++++ +++++ +++++ +++++ — ++</td>
</tr>
</tbody>
</table>

* ++++, very strong reaction; ++++, strong reaction; ++, moderate reaction; +, weak reaction; (+), barely visible reaction; —, no reaction.

are shown in Table 2. Anti-GM1 antiserum bound to purified serotype O:41 LPSs from the three GBS-associated isolates but also bound to serotype O:41 LPS purified from the enteritis isolate, suggesting the presence of a GM1-like epitope in these LPSs. The anti-GM1 antibodies cross-reacted with serotype O:19 LPS and cross-reacted weakly with serostrain O:2 LPS. Anti-GM2 antisera did not react with the LPS of *C. jejuni* O:3, a strain whose LPS is not sialylated and which is not associated with the development of GBS (1, 38).

Anti-asialoGM1 antisera showed a strong reaction with *C. jejuni* 260.94RXH LPS and weaker binding with each of the other serotype O:41 LPSs, including that from the enteritis isolate (Table 2). A strong reaction of this antisera was observed with serostrain O:2 LPS, and a weaker reaction occurred with serostrain O:19 LPS.

Anti-GM2 antisera bound the GM2 ganglioside (Table 1) but did not bind to any serotype O:41 LPS, suggesting that this LPS does not have a GM2-like epitope (Table 2). This antisera did not bind to serostrain O:2 or O:19 LPSs, and anti-ganglioside antisera did not bind to serostrain O:3 LPS (Table 2). Control rabbit antisera did not bind to LPS or gangliosides (data not shown).

**Binding of rabbit anti-*C. jejuni* O:41 antisera to gangliosides and LPS.** Rabbit antisera against *C. jejuni* 16971.94GSH showed a strong reaction with GM1 and a much weaker reaction with asialo-GM1 (Table 1), suggesting the presence of cross-reactive epitopes in serotype O:41 LPSs and the GM1 ganglioside. As shown in Fig. 3, only one band of immunoreactive material was observed when anti-*C. jejuni* 16971.94GSH antisera reacted with serotype O:41 LPSs from GBS patients. The antisera also reacted with serostrain O:19 LPS and reacted weakly with serostrain O:2 LPS, but again no reaction was observed with serostrain O:3 LPS (Table 2). The antisera against *C. jejuni* 260.94RXH yielded the same results. Results are summarized in the tables.

**Binding of CT and PNA to gangliosides and LPS.** CT, a ligand for the GM1 ganglioside, and PNA, a ligand for the disaccharide moiety Galβ1–3GalNAc, were tested for their abilities to react with a variety of gangliosides (Table 1) and *C. jejuni* LPS (Table 2). In addition to a reaction with GM1, CT showed a weaker binding to asialoGM1, GM2, and GD1α. On the other hand, PNA bound strongly to asialoGM1 as well as showing a weaker reaction with GM1. With respect to purified LPS, CT reacted strongly with serotype O:41 LPSs (those of strains 16971.94GSH, 260.94RXH, and 176.83) and strongly with serostrain O:19 LPS, and a weaker reaction was observed with LPS of *C. jejuni* 28134.94GSH. A weak reaction was observed with serostrain O:2 LPS, but no reaction was observed with serostrain O:3 LPS.

*PNA showed strong binding to serostrain O:2 and O:19 LPSs and all the *C. jejuni* O:41 LPSs (Table 2), suggesting the presence of the Galβ1–3GalNAc disaccharide in the serotype O:41 LPS. However, PNA did not react with any of the other LPSs tested.*

**Immunoadsorption.** Antisera to *C. jejuni* 16971.94GSH and to *C. jejuni* 260.94RXH which had been preadsorbed with the respective LPSs were subsequently tested by TLC with immunostaining. Adsorption with serotype O:41 LPS removed antibody binding to the GM1 ganglioside and reduced the binding to serotype O:41 LPS. The lack of reaction of anti-GM1 antisera subsequent to immunoadsorption with serotype O:41
LPS confirms our earlier findings that GM₃ and O:41 LPSs have similar epitopes.

Composition and structure of LPS from *C. jejuni* 16971. 94GSH. An analysis of de-O-acylated LPS and material derived from hydrolyzed LPS and subsequent gel chromatography revealed saccharide chains consistent with the core OS of LPS molecules. Structural analyses established the structure of the core OS as shown in Fig. 4. This core OS has a terminal tetrasaccharide mimicking that of ganglioside GM₁ (Fig. 1); this mimicry resembles that observed in certain *C. jejuni* O:19 GBS isolates (3, 33, 68). The complete details of the chemical characterization of *C. jejuni* 16971.94GSH LPS will be published elsewhere (35).

**DISCUSSION**

*C. jejuni* is a major cause of human gastroenteritis that is usually self-limiting, but in a small minority of cases GBS develops within 1 to 3 weeks after infection (20, 27). The association of GBS with the preceding infection has led to a search for candidate bacterial antigens which may precipitate autoimmune responses in the host (15, 47, 62, 63). Gangliosides have been extensively studied as possible host antigens for autoimmune disease since serum antibodies against gangliosides, especially GM₁, are found during the acute phase of GBS when preceded by *C. jejuni* infection (14, 41, 48, 67, 70). Molecular mimicry between core OSs of certain *C. jejuni* serotypes associated with GBS (O:2, O:4, and O:19) and gangliosides has been established elsewhere (35).

By SDS-PAGE, *C. jejuni* serotype O:41 LPS from the three GBS isolates and the LPS from the enteritis isolate showed virtually identical rates of migration, reflecting core molecules with the same molecular weight. The serotype O:41 LPSs exhibited a profile characteristic of rough-form LPS, i.e., composed only of a lipid A region and core OS. LPSs from the O:19 serostrain migrated to the same point in the gel as O:41 LPS, indicating that serostrain O:19 LPS and serotype O:41 LPSs have core OSs with similar molecular weights. The high-*Mₙ* LPSs of *C. jejuni* strains are not visualized by silver staining (46), but bands corresponding to *C. jejuni* high-*Mₙ* LPSs with O side chains are visualized by immunoblotting with homologous antisera (44, 46). However, even by immunoblotting, no high-*Mₙ* bands were observed in immunoblots of any of the *C. jejuni* O:41 strains with O:41 typing antisera, consistent with previous observations (46). Preliminary chemical studies indicate the presence of high-*Mₙ* polysaccharides containing arabinose, fucose, altrose, and 6-deoxy-altro-heptose in the *C. jejuni* O:41 strains. However, to date, it remains unclear whether these polysaccharides are attached as O side chains to the core OS of *C. jejuni* O:41 LPS or are independent of LPS.

Sialic acid, i.e., Neu5Ac, is not commonly found in LPS but has been identified in *C. jejuni* LPS (6, 31, 38). When present in LPS, Neu5Ac is more commonly encountered as a constituent of the core OS (6, 21, 22, 34, 38) than the O side chain (12, 13). Neu5Ac was detected in *C. jejuni* O:41 LPS from the GBS patients in amounts ranging from 101 to 200 mmol/mg. The LPS from *C. jejuni* 28134.94GSH contained only half the amount of Neu5Ac than was present in *C. jejuni* 16971.94GSH LPS. Studies of the migration patterns of mutants of *Neisseria* species LPS in SDS-PAGE gels have shown that LPSs differing in the presence or absence of no more than one sialic acid residue can exhibit different mobilities in SDS-PAGE gels (53). However, no difference in migration pattern between the serotype O:41 LPSs was observed in the SDS-PAGE gel.

Antibodies against GM₁ ganglioside reacted strongly with all serotype O:41 LPSs, including LPS from the enteritis isolate, suggesting the presence of a GM₁-like epitope in serotype O:41 LPSs. Cross-reactivity was observed with serostrain O:19 and O:2 LPSs, and this indicates that the core OSs of the LPSs of serostrains O:19 and O:2 and that of serotype O:41 LPSs have similar structures. This concurs with the previously reported epitope mimicry between the LPS of the O:19 serostrain and the GM₁ ganglioside (3, 24, 64, 65). Structural analyses have shown that *C. jejuni* O:19 LPS from the serostrain contains a 1:1 mixture of core OS mimicking GM₁ and GD₁₉ (3). However, the core OSs of LPSs of two GBS serotype O:19 isolates were examined and found to be not only different from each other but different also from that of the O:19 serostrain LPS (3). The GBS isolates, *C. jejuni* OH4384 and *C. jejuni* OH4382, had core OSs with terminal regions mimicking GT₁₀ and GD₁₀, respectively (3). This finding demonstrates the heterogeneity that can exist between LPSs within the same serotype. In addition to *C. jejuni* O:19 LPS, other serotypes mimic gangliosides. *C. jejuni* O:2 LPS shares a terminal disaccharide, Neu5Ac(α2-3)Gal, with many of the major gangliosides (4). Reports have shown that O:4 LPS shares a terminal pentaaccharide with the GD₁₉ ganglioside (6). Yuki et al. (69) reported that O:4 LPS bears GM₁-like and GD₁₉-like epitopes. The report observed that the fraction of O:4 LPS that bore the GM₁-like epitope and that with the GD₁₉-like epitope had different mobilities in TLC, thus indicating the heterogeneity of structures in their LPS preparations.

Anti-asialoGM₁ antibodies recognized serostrain O:2 LPS strongly and serostrain O:19 LPS weakly. These antibodies showed a strong reaction with *C. jejuni* 260.94RXH LPS and displayed moderate binding to other serotype O:41 LPSs, including LPS from the enteritis isolate, suggesting the presence of an asialoGM₁-like epitope. In addition to *C. jejuni* O:41 LPSs, other serotypes mimic gangliosides. *C. jejuni* O:2 LPS shares a terminal disaccharide Neu5Ac(α2-3)Gal, with many of the major gangliosides (4). Reports have shown that O:4 LPS shares a terminal pentaaccharide with the GD₁₉ ganglioside (6). Yuki et al. (69) reported that O:4 LPS bears GM₁-like and GD₁₉-like epitopes. The report observed that the fraction of O:4 LPS that bore the GM₁-like epitope and that with the GD₁₉-like epitope had different mobilities in TLC, thus indicating the heterogeneity of structures in their LPS preparations.

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Junji O:3 LPS. This antiserum bound strongly to the GM₁ ganglioside, supporting earlier results that O:41 LPS shares an epitope with the GM₁ ganglioside. Similar results were obtained with anti-\textit{C. jejuni} 260.94RXH antiserum.

\textbf{CT}, a ligand for GM₁, was found to react with a variety of gangliosides. In addition to binding to GM₁, it also bound asialoGM₁, GD₁a, and GM₂, as these gangliosides share the GalNAcβ₁-4Galβ₁-3Glc trisaccharide. CT did not recognize GT₁a, GD₁a, and GQ₁b, and thus the presence of a terminally sialylated galactose residue may prevent its binding. CT reacted with serostrain O:19 and O:2 LPSs and binds avidly to Yuki et al. (65) observed the binding of CT to serotype O:19 GBS-associated LPS. This phenomenon has previously been limited to strains associated with GBS, since LPS from the \textit{C. jejuni} ganglioside in the core OS of \textit{C. jejuni} exhibited by other \textit{C. jejuni} glioside in the core OS of \textit{C. jejuni} associated with GBS (33). Although the mimicry of the GM₁ ganglioside by the core OS or in the presentation of epitopes in the different LPSs. Whether these factors play a role in disease development requires further investigation.


