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Lipopolysaccharides from *Campylobacter jejuni* Associated with Guillain-Barré Syndrome Patients Mimic Human Gangliosides in Structure

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Lipopolysaccharides extracted from *Campylobacter jejuni* serostrains (serotype reference strains) for serotypes O:4 and O:19 were found to have core oligosaccharides with terminal structures resembling human gangliosides G_{M1} and G_{D1a}. High-molecular-weight molecules that reflected the presence of O chains were shown in immunoblots to be immunologically specific for each serostrain. The O:19 antiserum also reacted strongly with core oligosaccharides of two isolates from patients with Guillain-Barré syndrome (GBS), but the banding patterns and molecular structures were different from those of the O:19 serostrain. A neuraminobiose disaccharide unit is attached to the terminal Gal residue in one isolate, and the other isolate lacked terminal *N*-acetyl glucosamine and galactose with attached sialic acid so that the sialic acid residues were present in a neuraminobiose unit linked to the only remaining galactose. Analysis of the high-M_r lipopolysaccharides of the O:19 serostrain and the two isolates from GBS patients revealed the presence of a hyaluronic acid-like polymer with disaccharide-repeating units consisting of β-D-glucuronic acid amidated with 2-amino-2-deoxyglycerol and *N*-acetyl glucosamine. The results confirm a potential role for the core oligosaccharides in the etiology of GBS but also suggest that the O-chain polysaccharide may be a contributing factor.

The development of acute polyradiculoneuritis, more commonly known as Guillain-Barré syndrome (GBS), subsequent to infection with *Campylobacter jejuni* has been the focus of much recent investigation (14). An association of GBS with a previous infection has been recognized for several decades, but a mechanism for eliciting the onset of GBS has escaped detection largely because of the fact that a wide variety of agents, including several bacterial species and at least seven viruses and strains of mycoplasma, have been implicated as the etiological agents of the infection preceding GBS (8, 14). In the last decade, following the general recognition of the genus *Campylobacter* as a leading cause of human enteritis, an association of *C. jejuni*-induced diarrhea with onset of GBS has been also reported by several investigators (9, 13). Two particularly significant findings centered on *C. jejuni*-associated GBS have stimulated renewed interest in the pathogenesis of the disease. Several reports have shown that the *C. jejuni* isolates obtained from diarrheic patients prior to the onset of GBS belonged to serotype O:19 (7, 9, 10). In examinations of the structures of lipopolysaccharides (LPS) extracted from *C. jejuni*, Aspinall et al. (5, 6) found that the terminal regions of core oligosaccharides (OSs) of several serotypes of *C. jejuni* reflected the structures of human gangliosides. Serotype O:4 is particularly interesting in that it yielded OS structures resembling the G_{M1} and G_{D1a} gangliosides (5). Recently, Yuki et al. (15) suggested that the anti-G_{M1} antibody has a role in the development of GBS, and structural studies by Yuki et al. (16) have shown that an isolate of serotype O:19 from a GBS patient has a terminal tetrasaccharide which is the same as that in the human ganglioside G_{M1}. Since anti-G_{M1} antibodies in human sera may be a significant factor in producing the symptoms of GBS, an important step in elucidating the etiology of GBS is determining the compositions and molecular structures of the LPS of the O:19 serostrain (serotype reference strain) and isolates of this serotype that have been recovered from patients who subsequently developed GBS.

Two serostrains, O:4 (ATCC 43432) and O:19 (ATCC 43446), from the O-serotyping system of Penner et al. (11) were obtained from stock cultures maintained in the laboratory in Toronto, Canada. Two isolates of *C. jejuni* (4382 and 4384, both serotype O:19) were recovered from siblings in Japan who developed GBS. Isolate 4382 was obtained from a 19-month-old patient who experienced a rapidly progressive weakness of the lower extremities 9 days after an acute episode of diarrhea. Neurological examination revealed areflexia and paraplegia without sensory signs or symptoms. Respiratory muscles and cranial nerves were unaffected. She recovered completely 5 months after the onset of symptoms. *C. jejuni* isolate 4384 was isolated from her older sister (3 years and 9 months of age), who experienced diarrheal illness 10 days after her sister. Three days after the diarrhea ceased, she developed GBS symptoms. A weakness beginning in the legs progressed to flaccid quadriparesis. Physical examination revealed areflexia in both lower and upper extremities, but sensory function was intact. After admission to the hospital, she required endotracheal intubation and mechanical ventilation because of progressive bulbar palsy and respiratory failure. Complete recovery occurred after 6 months.

Bacteria were cultured at 37°C for 48 h under microaerophilic conditions on Columbia blood agar (Oxoid Ltd., London, England) as described previously (11).

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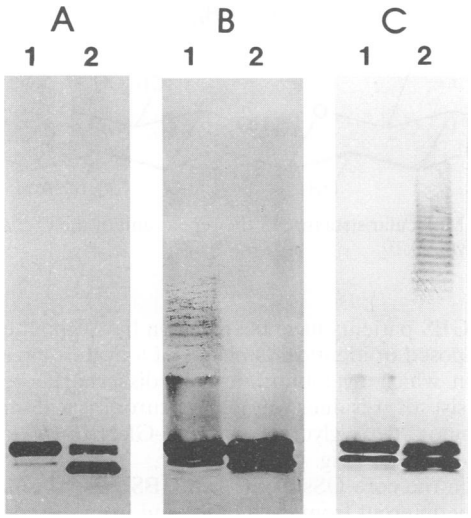

FIG. 1. Silver staining (A) and immunoblotting with O:4 (B) and O:19 (C) antisera of electrophoresed proteinase K-digested whole-cell lysates of *C. jejuni* serostrains O:4 (lanes 1) and O:19 (lanes 2).

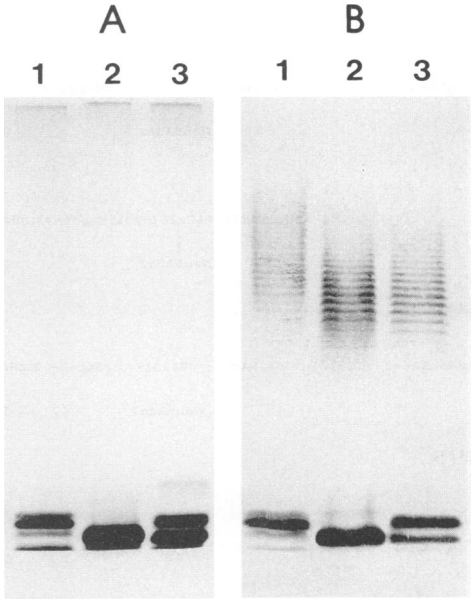

FIG. 2. Silver staining (A) and immunoblotting with O:19 antiserum (B) of electrophoresed proteinase K-digested whole-cell lysates of *C. jejuni* serotype O:19. Lanes: 1, isolate 4384; 2, isolate 4382; 3, O:19 serostrain.

Evidence from the study of Yuki et al. (15) indicated that isolates of serotype O:19 elicited the production of anti-G_{M1} antibodies, and earlier studies by Aspinall et al. (5, 6) showed that low-M_r LPS (core OS) of serostrain O:4 consisted of molecules resembling human gangliosides G_{D1a} and G_{M1}. To provide preliminary evidence that the O:19 also possessed G_{M1}- or G_{D1a}-like structures, the LPS from serostrains O:4 and O:19 were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining (Fig. 1A) and immunoblotting with O:4 and O:19 antisera (Fig. 1B and C, respectively) in a comparative study using methods previously described (12).

Two bands corresponding to low-M_r LPS with characteristic core (OS) regions were visualized in both preparations in silver-stained gels after SDS-PAGE. The uppermost bands of OS from each sample showed virtually identical rates of migration, reflecting core molecules with the same molecular weight; considerable differences in the intensities of the bands indicated that the O:4 LPS contained more of the higher-molecular-weight OS molecules and fewer of the lower-molecular-weight OS molecules than did the O:19 LPS. However, as expected from previous observations of *C. jejuni* LPS, bands corresponding to high-M_r LPS with O chains were not detectable by the silver-staining procedure (12).

In immunoblotting experiments with either anti-O:4 antiserum or anti-O:19 antiserum, low-M_r LPS of both serostrains were observed to have patterns of banding almost indistinguishable from those seen in the silver-stained gels (Fig. 1B and C). The virtually identical reactions of the two antisera with the low-M_r LPS indicated the presence of antibodies in both antisera directed against core OS epitopes of both serostrains. This supported the supposition that O:19 LPS, like O:4 LPS, possessed core structures mimicking those of G_{M1} or G_{D1a} gangliosides.

In high-M_r LPS, bands with ladder-like patterns were observed for each serostrain in immunoblots only with the homologous antiserum, signifying the presence in each serostrain of LPS with O chains of serotypic specificity. From these results, it is clear that serostrains O:4 and O:19 share the same or very similarly structured core OS components but have O chains with different specificities that serve as the antigenic markers for differentiating isolates of these serotypes.

Electrophoresed LPS from the O:19 serostrain and two O:19 isolates from GBS patients (4382 and 4384) were examined after silver staining and immunoblotting with O:19 antiserum (Fig. 2). It was noted that the low-M_r LPS of the isolates did not have banding patterns similar to those of the O:19 serostrain. The low-M_r LPS of isolate 4382 consisted of at least three components, whereas the material from isolate 4384 consisted of a single intensely stained component that migrated at a rate indistinguishable from that of the fast-migrating component of the O:19 low-M_r LPS. The O:19 antiserum reacted with low-M_r LPS from the serostrain and with low-M_r LPS from the isolates, producing the same banding patterns as those visualized in the silver-stained gels. From these results, it was evident that the core OSs differ in molecular weight and structure from each other, but each bears an array of epitopes, some or all of which react with antibodies in the O:19 antiserum. The O:19 antiserum reacted with high-M_r LPS from each of the preparations, yielding ladder-like banding patterns. Comparisons of the banding profiles showed that although the spacings between the bands were the same, the bands were not coincident in their electrophoretic mobilities. These results suggested that the LPS possessed the same O-chain structures linked to core OSs with different molecular weights.

Procedures for determining the compositions and molecular structures of the low-M_r LPS and high-M_r LPS are similar to those published previously for investigations of *C. jejuni* serotypes O:1, O:2, O:4, O:23, and O:36 (1, 4, 5). Details of the physical and chemical procedures for investigating LPS of *C. jejuni* serotype O:19 have been reported elsewhere (2, 3). Only the molecular structures determined in these studies are presented here and are those from the most extended studies (Fig. 3). In all materials isolated, there is evidence for the presence of some less-complete molecules, the relative propor-

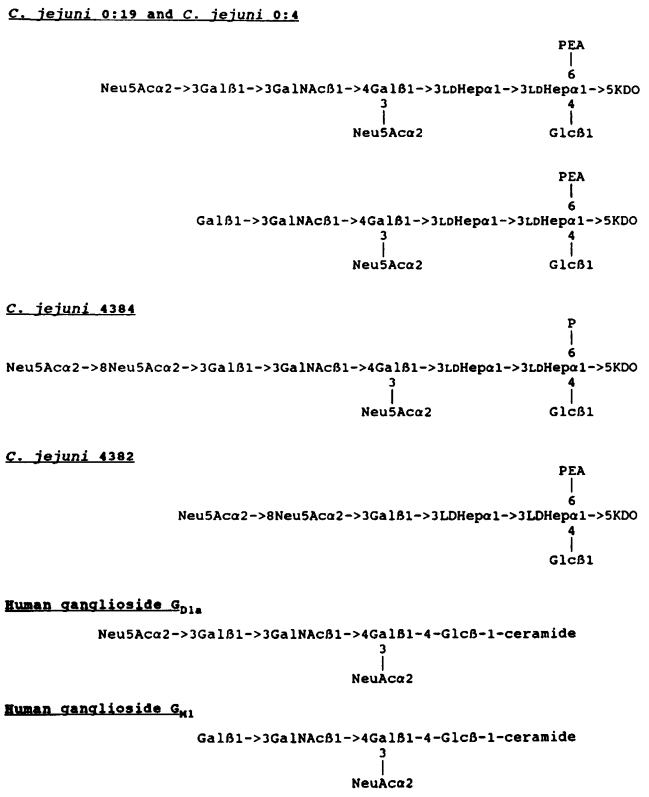

FIG. 3. Molecular structures of OSs of *C. jejuni* serotypes O:4 and O:19 and two serotype O:19 isolates, 4382 and 4384, from patients who developed GBS. The structures of human gangliosides G_{D1a} and G_{M1} are also shown. P, phosphate; PEA, *O*-phosphoethanolamine; KDO, 3-deoxy-D-*manno*-octulosonic acid; LDHep, L-*glycero*-D-*manno*-heptose.

tions of which may vary naturally from one sample to another of ostensibly the same material. In this connection, the terminal unit of the core OS in the LPS from serostrain O:4 has been reported to be identical to that of the human ganglioside G_{M1} (5). More recently, it was recognized that a second terminally linked residue of sialic acid (Neu5Ac) in the O:4 OS was readily lost during chemical manipulation and that this second Neu5Ac residue was present in >90% of the intact LPS molecules in a terminal unit with the structure of G_{D1a}, a ganglioside closely related to G_{M1} (6). By using similar analytical procedures for the analysis of low-M_r LPS from serostrain O:19, identical structures were revealed in the native LPS but with approximately equal proportions of G_{M1}- and G_{D1a}-like terminal units (3).

Analytical procedures were also carried out for low-M_r LPS extracted from the isolates of two patients who later developed GBS. The core OS of *C. jejuni* 4384 differed slightly from that of the serostrain in that a disaccharide, neuraminobiose (Neu5Ac → 8Neu5Ac), instead of a single residue of Neu5Ac, was terminally linked to galactose. The core OS of *C. jejuni* 4382 is a shorter molecule lacking the terminal Gal and GalNAc residue to which Neu5Ac residues may be linked. A terminal neuraminobiose is linked at that point, as opposed to that of the neuraminobiose unit in *C. jejuni* 4384, which is attached to the outer of the two Gal residues.

In contrast to the variability among the three core OSs, the high-M_r LPS of the O:19 serostrain and the two O:19 isolates

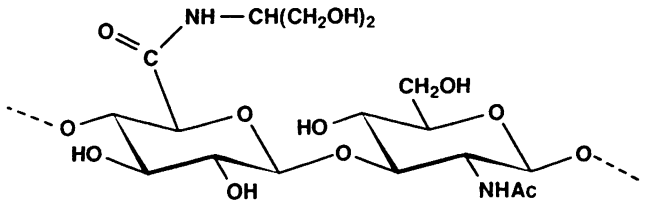

FIG. 4. Molecular structure of the repeat unit of the O chain for *C. jejuni* serotype O:19.

from the GBS patients have been shown by Aspinall et al. (2) to be composed of identical O chains of a hyaluronic acid-like polymer in which hyalobiuronic acid disaccharide-repeating units consist of residues of β-D-glucuronic acid amidated with 2-amino-2-deoxyglycerol and β-D-GlcNAc (*N*-acetylglucosamine) residues (Fig. 4).

Although the core OSs of the two GBS patient isolates are structurally different from each other and from the core OS of the O:19 serostrain, the observation that O:19 antibodies reacted with them in the immunoblot (Fig. 2) indicated the presence of epitopes common to each of the three strains (Fig. 2). Until their specificities have been rigorously defined, it would be premature to assume, therefore, that antibodies elicited by the O:19 serostrain recognize only G_{M1} and do not recognize related members of the ganglioside family, such as G_{D1a}, or closely related structures found in the two GBS patients' isolates. It appears, therefore, that the complete G_{M1}- or G_{D1a}-like terminal regions of the core OSs are not essential for the stimulation of cross-reactive antibodies by strains associated with the development of GBS. The lack of reports of GBS cases associated with the frequently isolated diarrhea-causing *C. jejuni* serotype O:4, which also has core OSs with terminal regions reflecting G_{M1} and G_{D1a} structures (Fig. 3), supports the premise that precise mimicking of a ganglioside structure is not the determining factor for the development of GBS. Moreover, GBS has been found to develop, although much less frequently, subsequent to infections with *C. jejuni* serotype O:2 (10), which, like isolate 4382, does not have a core OS structurally resembling the outer regions of either the G_{M1} or G_{D1a} ganglioside (4). Further understanding of the role of the core OSs of *C. jejuni* associated with GBS requires the identification of the cross-reactive epitope at the molecular level. The most consistent feature of the O:19 strains is the unique structure of the O-chain repeat unit (2). The fact that it reflects the structure of hyaluronic acid, a common human tissue component, indicates that it is imprudent to preclude the O chain as a contributing factor in the development of GBS until the outcome of further investigations is available.

REFERENCES

1. **Aspinall, G. O., A. G. McDonald, and H. Pang.** 1992. Structures of the O chains from lipopolysaccharides of *Campylobacter jejuni* serotypes O:23 and O:36. Carbohydr. Res. **231**:13–20.
2. **Aspinall, G. O., A. G. McDonald, and H. Pang.** 1994. Lipopolysaccharides of *Campylobacter jejuni* serotype O:19. Structures of O antigen chains from the serostrain and two bacterial isolates from patients with the Guillain-Barré syndrome. Biochemistry **33**:250–255.
3. **Aspinall, G. O., A. G. McDonald, H. Pang, L. A. Kurjanczyk, and J. L. Penner.** 1994. Lipopolysaccharides of *Campylobacter jejuni* serotype O:19: structures of the core oligosaccharide regions from the serostrain and two bacterial isolates from patients with the Guillain-Barré syndrome. Biochemistry **33**:241–249.
4. **Aspinall, G. O., A. G. McDonald, T. S. Raju, H. Pang, L. A. Kurjanczyk, J. L. Penner, and A. P. Moran.** 1993. Chemical

structure of the core region of *Campylobacter jejuni* serotype O:2 lipopolysaccharide. Eur. J. Biochem. **213:**1029–1037.

5. **Aspinall, G. O., A. G. McDonald, T. S. Raju, H. Pang, S. D. Mills, L. A. Kurjanczyk, and J. L. Penner.** 1992. Serological diversity and chemical structures of *Campylobacter jejuni* low-molecular-weight lipopolysaccharides. J. Bacteriol. **174:**1324–1332.
6. **Aspinall, G. O., A. G. McDonald, T. S. Raju, H. Pang, A. P. Moran, and J. L. Penner.** 1993. Chemical structure of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23 and O:36 lipopolysaccharides Eur. J. Biochem. **213:**1017–1027.
7. **Fujimoto, S., N. Yuki, T. Itoh, and K. Amako.** 1992. Specific serotype of *Campylobacter jejuni* associated with Guillain-Barré syndrome. J. Infect. Dis. **165:**183. (Letter.)
8. **Kaldor, J., and B. R. Speed.** 1984. Guillain-Barré syndrome and *Campylobacter jejuni*. Br. Med. J. **288:**1867–1870.
9. **Kuroki, S., T. Haruta, M. Yoshioka, Y. Kobayashi, M. Nukina, and H. Nakanishi.** 1991. Guillain-Barré syndrome associated with *Campylobacter* infection. Pediatr. Infect. Dis. J. **10:**149–151.
10. **Kuroki, S., T. Saida, M. Nukina, T. Haruta, M. Yoshioka, Y. Kobayashi, and H. Nakanishi.** 1993. *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome belong mostly to Penner serogroup 19 and contain β-N-acetylglucosamine residues. Ann. Neurol. **33:**243–247.
11. **Penner, J. L., J. N. Hennessy, and R. V. Congi.** 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. Eur. J. Clin. Microbiol. **2:**318–383.
12. **Preston, M. A., and J. L. Penner.** 1987. Structural and antigenic properties of lipopolysaccharides from serotype reference strains of *Campylobacter jejuni*. Infect. Immun. **55:**1806–1812.
13. **Rhodes, K. M., and A. E. Tattersfield.** 1982. Guillain-Barré syndrome associated with *Campylobacter* infection. Br. Med. J. **285:**173–174.
14. **Ropper, A. H.** 1992. The Guillain-Barré syndrome. N. Engl. J. Med. **326:**1130–1136.
15. **Yuki, N., S. Handa, T. Taki, T. Kasama, M. Takahashi, K. Saito, and T. Miyatake.** 1992. Cross-reactive antigen between nervous tissue and a bacterium elicits Guillain-Barré syndrome: molecular mimicry between ganglioside G_{M1} and lipopolysaccharide from Penner's serotype 19 of *Campylobacter jejuni*. Biomed. Res. **13:** 451–453.
16. **Yuki, N., T. Taki, F. Inagaki, T. Kasama, M. Takahashi, K. Saito, S. Handa, and T. Miyatake.** 1993. A bacterium lipopolysaccharide that elicits Guillain Barré syndrome has a G_{M1} ganglioside-like structure. J. Exp. Med. **178:**1771–1775.