A Bacterium Lipopolysaccharide That Elicits Guillain-Barré Syndrome Has a GM1 Ganglioside-like Structure

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Summary

There is a strong association between Guillain-Barré syndrome (GBS) and Penner's serotype 19 (PEN 19) of Campylobacter jejuni. Sera from patients with GBS after C. jejuni infection have autoantibodies to GM1 ganglioside in the acute phase of the illness. Our previous work has suggested that GBS results from an immune response to cross-reactive antigen between lipopolysaccharide (LPS) of the Gram-negative bacterium and membrane components of peripheral nerves. To clarify the pathogenesis of GBS, we have investigated whether GM1-oligosaccharide structure is present in the LPS of C. jejuni (PEN 19) that was isolated from a GBS patient. After extraction of the LPS, the LPS showing the binding activity of cholera toxin, that specifically recognizes the GM1-oligosaccharide was purified by a silica bead column chromatography. Gas-liquid chromatography-mass spectrometric analysis has shown that the purified LPS contained Gal, GalNAc, and NeuAc, which are sugar components of GM1 ganglioside. 1H NMR methods [Carr-Purcell-Meiboom-Gill (CPMG), total correlation spectroscopy (TOCSY), and nuclear Overhauser effect spectroscopy (NOESY)] have revealed that the oligosaccharide structure [Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ] protrude from the LPS core. This terminal structure [Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ] is identical to the terminal tetrasaccharide of the GM1 ganglioside. This is the first study to demonstrate the existence of molecular mimicry between nerve tissue and the infectious agent that elicits GBS.

Guillain-Barré syndrome (GBS) is the most common cause of acute neuromuscular paralysis in developed countries and it affects 1-2/100,000 people annually (1). Anti-neural antibodies may function in the development of GBS because plasma exchange elicits a beneficial response (1). Gangliosides, cell surface components of nerve tissue, are considered the target antigens of anti-neural antibodies because some patients develop GBS after being administered them (2–6). The dependence of GBS on "molecular mimicry" between infectious agents and surface components of peripheral nerves has been postulated (1). 15–40% of the GBS patients develop the syndrome after being infected by the Gram-negative bacterium Campylobacter jejuni, a leading cause of acute gastroenteritis in humans (7, 8). Sera from GBS patients who have had C. jejuni enteritis contain autoantibodies to GM1 ganglioside (5, 6, 9–11). The bacterium can be serotyped on the difference in the carbohydrate structure in the lipopolysaccharides (LPS) which are major constituents of the outer membrane of Gram-negative bacteria (12, 13, 14, 15). The specific serotype of Penner's 19 (PEN 19) of C. jejuni is very frequently isolated from GBS patients (8, 16); whereas, it is rarely isolated in the case of C. jejuni enteritis. We recently found antigenic similarity between the GM1 ganglioside and the LPS of C. jejuni (PEN 19) (17). Present study of immunochemical and NMR analyses on the LPS of C. jejuni has now confirmed molecular mimicry between the carbohydrate structure of this LPS and that of GM1 ganglioside.
Materials and Methods

Case Report. A 24-yr-old man developed watery diarrhea with fever, which improved over 4 d. 6 d after the resolution of this illness, he developed distal muscle weakness, and on day 4, proximal muscle weakness. He was admitted to the hospital 7 d after the onset of neurologic symptoms. Neurologic examination was significant for areflexia and distal-dominant muscle weakness without superficial or deep sensory disturbances. Muscles of respiration and cranial nerves were unaffected. The cerebrospinal fluid protein level was increased with normal cellularity. Serial electrophysiologic studies indicated that the predominant mechanism was axonal degeneration of the motor nerve. C. jejuni was isolated from his stool. The agent of antecedent infection was serologically determined using a complement fixation test; a high serum antibody titer against C. jejuni and a more than fourfold change in titer indicated a preceding C. jejuni infection. In the acute phase of this neurologic disease, the patient's serum antibody reacted strongly with GM1, and very faintly with GD1b as previously reported (17), but did not react with GD1a, GT1b, GM2, and GQ1b.

Bacterium. The bacterium used in this study was C. jejuni (PF-90-26) from this GBS patient. Serotyping of C. jejuni was performed by the method of Penner and Hennessey (12) and the strain belonged to PEN 19. Stock culture was maintained at ~80°C in glycerol and brain heart infusion broth (15:85, by volume, Difco Laboratories Inc., Detroit, MI). Cultures were grown on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) at 37°C for 48 h in an atmosphere of 5% O2, 10% CO2, and 85% N2. The bacterium was harvested by centrifugation at 10,000 g (4~ 30 min), and washed three times with physiological saline.

Extraction of Crude LPS. LPS was extracted from 20 g (wet weight) of C. jejuni (PEN 19) by the hot phenol-water technique (18). After keeping the fraction at 4°C overnight, the aqueous layer was dialyzed against distilled water. 2 vol of methanol and 1 vol of chloroform were added to the aqueous phase, after which 1 vol of chloroform, followed by 1 vol of water was added. The chloroform layer was evaporated, and the dry residue was dissolved in 25% NH4OH, and then incubated at 56°C for 48 h. After dialysis against distilled water, the crude LPS was lyophilized.

Purification of LPS with GM1-oligosaccharide Structure. 100 mg crude LPS was dissolved in a solvent mixture of n-propanol-water-25% NH4OH (75:15:10, by volume). This LPS solution was applied to a column (2 x 100 cm) packed with lactobeads (6RS-8060; Iatron, Co., Tokyo, Japan) and eluted with a gradient of n-propanol-water-25% NH4OH (75:15:10) to 64:1 (900 ml, by volume). Each fraction eluted from the column was subjected to thin-layer chromatography (TLC) and made visible by spraying precoated Silica Gel 60 plates (E. Merck, Darmstadt, Germany) with ninhydrin, orcinol reagents for hexose, and resorcinol reagents for phosphorous groups (data not shown). GC/MS analysis showed that the purified LPS from C. jejuni (PEN 19) contained the following sugar components galactose (Gal), glucose, N-acetylgalactosamine (GalNAc), N-acetyleneuraminic acid (NeuAc), and heptose (Hep), as well as 3-deoxy-2-octulosonic acid and the fatty acid components, 3-hydroxyamyristic acid and palmitic acid. These fatty acids are known to be the components of the lipid A from C. jejuni (23).

Results and Discussion

Fig. 1 shows chromatographic elution profile of the LPS from the lactobeads column. Fractions from 94 to 104 showed homogeneous bands when they were stained by the binding activity of cholera toxin and by ninhydrin reagents for amino groups, orcinol reagents for hexose, and resorcinol reagents for deoxy-keto sugar (Fig. 1), as well as Dittmer reagents for phosphorous groups (data not shown). GC/MS analysis showed that the purified LPS from C. jejuni (PEN 19) contained the following sugar components galactose (Gal), glucose, N-acetylgalactosamine (GalNAc), N-acetyleneuraminic acid (NeuAc), and heptose (Hep), as well as 3-deoxy-2-octulosonic acid and the fatty acid components, 3-hydroxyamyristic acid and palmitic acid. These fatty acids are known to be the components of the lipid A from C. jejuni (23).
Figure 1. Chromatographic profile of the LPS. Fractions eluted from the latex beads column were subjected to TLC and made visible by spraying the plate with (a) ninhydrin, (b) orcinol, and (c) resorcinol reagents, and by the binding of (d) cholera toxin.

Ethanolamine also was detected by the amino acid analysis (data not shown).

Fig. 2a shows the normal NMR spectrum of the purified LPS. The 3ε and 3α proton resonances of NeuAβ are present. Anomeric proton resonances a, b, c, and d suggest the presence of sugars Galβ, Galβ, GalNαβ, and Hepα, respectively. The spectrum in Fig. 2a has resonances with narrow and broad line widths, indicative of two components with different mobilities. This is supported by measurement of the CPMG spectrum (Fig. 2b), in which resonances with broad line widths were suppressed due to fast spin–spin relaxation times, whereas those with sharp line widths were enhanced. The resonances in Fig. 2b correspond to protons located in the mobile region of the LPS. From the intensity of the anomeric protons in the CPMG spectrum, the mobility of the sugar residues was estimated to the Galβ(a)>GalNαβ(c)>Galβ(b)-Hepα (d), which supports the contention that these oligosaccharides extend from the LPS core. Fig. 3a shows TOCSY spectrum of the LPS sample. Cross peaks produced by magnetization transfer from the anomeric protons are present. On the basis of these cross peak patterns, the anomeric protons a, b, c, and d were assigned to the sugar types Galβ, Galβ, GalNαβ, and Hepα shown in Fig. 3a, respectively (21, 22). It should be noted that the H3 of Galβ(b) shifted as much as 0.5 ppm to the lower field, which is characteristic of the chemical shift change caused by the linkage formation of NeuAα2-3Gal. We therefore concluded that the LPS sample had the NeuAα2-3Galβ(b) moiety. The other sugar linkages then were studied by NOESY. The cross peaks developed from the anomeric protons are connected by dotted lines in Fig. 3. Taking into account the chemical shifts of the individual sugar proton resonances obtained in the TOCSY experiment, we identified the interresidual (boxed with solid lines) as well as the intraresidual NOESY cross peaks. Galβ(b) linked to the 2-position of the manno-type sugar. No mannoose was detected by GC/MS, Hep being the only sugar with the manno-configuration in this sample. The linkage of Galβ(a)1-3GalNαβ(c)1-4(NeuAα2-3)Galβ(b)1-2Hepα(d) was confirmed from the interresidual cross peaks. On the basis of the mobility of the sugar residues (shown in Figs. 2 and 3), this oligosaccharide chain must protrude from the LPS core, because of which the GM1-oligosaccharide moiety [Galβ(a)1-3GalNαβ(c)1-4(NeuAα2-3)Galβ(b)] may be exposed (Fig. 4). This terminal structure [Galβ1-3GalNαβ1-4 (NeuAα2-3)Galβ] is identical to the terminal tetrasaccharide of GM1 ganglioside (Fig. 4), thereby enabling this LPS to interact with the cholera toxin.

GM1 is enriched in the membrane of the motor nerve ter-
Figure 3. Two-dimensional 1H NMR spectra of the LPS sample. (a) TOCSY spectrum. TOCSY cross peaks developed from the anomeric protons are connected by dotted lines. The assignments of the cross peaks also are shown. (b) NOESY spectrum. Interresidual NOESY cross peaks are boxed with solid lines. The sugar linkages are identified as Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer NeuAcα2

Figure 4. Molecular mimicry between GM1 ganglioside and the LPS of C. jejuni (PEN 19). The same terminal tetrasaccharide (underline) occupies the nonreducing end of GM1 ganglioside and the LPS (PEN 19).

minal which lacks the blood-nerve barrier, and to which anti-neural antibodies easily gain access (24). In a coculture system of rat motoneurons and human muscle cells in monolayer (25), monoclonal anti-GM1 antibodies and serum anti-GM1 antibodies from GBS patients, who previously had been in-
fected by C. jejuni, suppressed spontaneous firing and end-
plate potentials; whereas, monoclonal anti-GD1a, anti-GD1b, anti-GT1b, and anti-GQ1b antibodies did not (Kobayashi et al., unpublished data). This finding indicates that the anti-
GM1 antibody inhibits motoneuron excitability.

Administration of GM1 ganglioside sometimes causes the development of GBS associated with anti-GM1 antibody (2, 5, 6). Inoculation of rabbits with GM1 and certain adjuvants can cause peripheral neuropathy (26, 27). LPS is a potent poly-
clonal adjuvant of immune responses, its lipid A moiety being a known B cell mitogen (28). The LPS of C. jejuni (PEN 19), which bears the GM1-oligosaccharide structure, appears to have high immunogenic activity to produce anti-GM1 anti-
body. Sera from the GBS patient from whom C. jejuni (PEN 19) was isolated had antibodies to GM1, but did not have antibodies to GD1a, GT1b, GM2, or GQ1b. In contrast, rabbit anti-GM1 antibody did not react with LPSs from other sero-
types of C. jejuni, PEN 3, 5, or 11 (Yuki et al., unpublished data). Very recently, Aspinall et al. determined chemical struc-
tures of side chains of LPSs from C. jejuni PEN 1, 2, 4, 23, and 36. The side chains described by them have terminal sugar structures of GM2, GM3, GD1a, or GM1b; they do not have that of GM1. Therefore, we speculate that infection by C. jejuni (PEN 19) induces high production of anti-GM1 anti-
body in patients with an immunogenetic background, thereby leading to the abolishment of tolerance, and that the anti-
GM1 antibody binds to motor nerve terminals causing motoneuron inexcitability and the eventual development of muscular weakness. Ours is the first study to demonstrate the existence of molecular mimicry between nerve tissue and the infectious agent that elicits GBS (Fig. 4). The results reported here should be of use in establishing the mechanism of the pathogenesis of GBS after infection as well as the mech-
anisms of other autoimmune diseases.

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