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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 \( \textit{Campylobacter jejuni} \)

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ABSTRACT

We investigated the lipopolysaccharide (LPS) from Penner’s serotype 19 (PEN 19) of \( \textit{C. jejuni} \) that had been isolated from a patient with the Guillain-Barré syndrome (GBS). The LPS was extracted from the bacterium and separated by a silica beads column chromatography. One homogeneous fraction showing reactivity with the patient’s serum, rabbit anti-\( G_{M1} \) antibody, and also with cholera toxin, which binds oligosaccharide of \( G_{M1} \), was obtained. By gas-liquid chromatography-mass spectrometric analysis, the purified LPS was found to contain D-galactose, D-glucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetylneuraminic acid, 3-deoxy-2-octulosonic acid, heptose and fatty acids (3-hydroxy-myristic acid and palmitic acid). The result indicates that sugar components of the \( G_{M1} \)-oligosaccharide are present in the purified LPS. Chemical analysis and immunological binding experiment show that the LPS from \( \textit{C. jejuni} \) (PEN 19) has the oligosaccharide structure in common with \( G_{M1} \) ganglioside. These results suggest that some GBS patients associated with anti-\( G_{M1} \) antibody are caused by an autoimmune mechanism after infection by \( \textit{C. jejuni} \) (PEN 19) which has the \( G_{M1} \)-oligosaccharide structure.

The Guillain-Barré syndrome (GBS) is characterized as an acute, symmetrically progressive, inflammatory polyneuropathy (10). Approximately two thirds of the GBS patients develop the syndrome following various infections. Humoral autoimmunity is likely because of the therapeutic effect of plasmapheresis. GBS sometimes develops after infection by \( \textit{Campylobacter jejuni} \), a gram-negative bacterium that frequently causes acute enteritis. Ganglioside \( G_{M1} \) is a component of human nervous tissue. Since our first report that two GBS patients had autoantibody against \( G_{M1} \) after having been suffered from \( \textit{C. jejuni} \) enteritis (15), evidence for close association between anti-\( G_{M1} \) antibody and GBS subsequent to \( \textit{C. jejuni} \) infection has accumulated (5, 8, 11, 14). In contrast, no anti-\( G_{M1} \) antibody has been detected in patients who had \( \textit{C. jejuni} \) enteritis, but not followed by GBS (5, 15).

The pathogen \( \textit{C. jejuni} \) can be serotyped on the difference in carbohydrate structure in the lipopolysaccharide (LPS) (1, 9). Our group (3, 13) and another (6) have shown that the specific serotype of Penner’s 19 (PEN 19) of \( \textit{C. jejuni} \) is very frequently isolated from GBS patients; 11 of 12 isolates (92%) from GBS patients belonged to PEN 19, but less than 2% in patients who had \( \textit{C. jejuni} \) enteritis not followed by GBS. An immunoabsorption test suggested the presence of a cross-reactive antigen between \( \textit{C. jejuni} \) and \( G_{M1} \) (8). We have investigated whether the \( G_{M1} \)-like structure is present in the LPS from \( \textit{C. jejuni} \) (PEN 19) to clarify the mechanism that produces anti-\( G_{M1} \) antibodies as well as the pathogenetic significance of \( \textit{C. jejuni} \) (PEN 19) in eliciting GBS.

LPS was extracted from \( \textit{C. jejuni} \) (PEN 19; CF
Fig. 1 Binding of serum from a GBS patient, of rabbit anti-\( \text{G}_{\text{M}1} \) antibody, and of cholera toxin to LPS from \( C. \text{jejuni} \) (PEN 19). Panel A: a TLC stained with the resorcinol-HCl reagent. Panels B and C: immunostained chromatogram that first was overlaid with serum from a patient with GBS following \( C. \text{jejuni} \) (PEN 19) infection (1:100) (panel B) and the rabbit anti-\( \text{G}_{\text{M}1} \) antibody (1:250) (panel C), then with peroxidase-conjugated second antibodies (Tago, CA, U.S.A.) (1:1,000). Panel D: binding of the biotinylated cholera toxin B-subunit (1:250), and the subsequent avidin-biotin-peroxidase complex system (Vector, CA, U.S.A.). Lane 1: crude LPS from \( C. \text{jejuni} \) (PEN 19) isolated from the GBS patient. Lane 2: the LPS after mild alkaline treatment. Lane 3: the purified LPS. Lane 4: bovine brain ganglioside mixture. Lane 5: \( \text{G}_{\text{M}1} \). The TLC plate was developed with \( n \)-propanol-water-25% \( \text{NH}_4\text{OH} \) (6:3:1, by volume), then with chloroform-methanol-0.2% \( \text{CaCl}_2 \) (60:40:9, by volume). Serum from the GBS patient reacted strongly with the LPS from \( C. \text{jejuni} \) (PEN 19) and \( \text{G}_{\text{M}1} \), and faintly with \( \text{G}_{\text{D}1, \text{b}} \). The rabbit anti-\( \text{G}_{\text{M}1} \) antibody reacted with the LPS, \( \text{G}_{\text{M}1} \), and \( \text{G}_{\text{D}1, \text{b}} \). Cholera toxin reacted with both the LPS and \( \text{G}_{\text{M}1} \).

90–26) that had been isolated from a GBS patient, by using the hot phenol-water technique (12). The LPS-containing fraction was dialyzed against water and lyophilized. The crude LPS was treated with 0.1 N NaOH at 37°C for 1 h, dialyzed against water, and lyophilized. The LPS was dissolved in a solvent mixture of \( n \)-propanol-water-25% \( \text{NH}_4\text{OH} \) (75:15:10, by volume) and applied to a silica beads column, which had been preequilibrated with the same solvent mixture. The LPS was eluted with a gradient of \( n \)-propanol-water-25% \( \text{NH}_4\text{OH} \) (75:15:10–6:4:1, by volume). Each fraction was monitored by use of the binding activity of rabbit anti-\( \text{G}_{\text{M}1} \) antibody (Dia-iatron, Tokyo) and with the resorcinol-HCl reagent on thin-layer chromatography (TLC). Fractions that showed homogeneity on TLC were obtained (Fig. 1, lane 3). TLC with immunostaining (15) revealed that the patient’s serum and the rabbit anti-\( \text{G}_{\text{M}1} \) antibody, as well as cholera toxin, which recognizes the \( \text{G}_{\text{M}1} \)-oligosaccharide (4), reacted with \( \text{G}_{\text{M}1} \) and the LPS from \( C. \text{jejuni} \) (PEN 19) (Fig. 1). Normal control sera (human, \( n=10 \); rabbit, \( n=3 \)) did not react with the LPS and gangliosides (data not shown). Anti-\( \text{G}_{\text{M}1} \) antibody titer in the GBS patient decreased concurrently with the clinical improvement (data not shown).

The LPS was evaporated and dissolved in physiological saline. The purified LPS was applied to a \( \text{C}_{18} \) Sep-Pak cartridge (Millipore, MA, U.S.A.), washed with water, and eluted with methanol. The eluted LPS was subjected to methanolysis (2). Fatty acid methyl esters were extracted with \( n \)-hexane and analyzed by gas-liquid chromatography-mass spectrometry (GC-MS). The lower layer was used for the analysis of sugars, which were converted to trimethylsilyl derivatives after \( N \)-acylation (2). GC-MS analysis demonstrated that the purified LPS contained \( \text{n-galactose, d-glucose, N-acetyl-d-galactosamine, N-acetyl-d-glucosamine, N-acetylneuraminic acid, 3-deoxy-2-octulosonic acid, heptose and fatty acids (3-hydroxy-myristic acid and palmitic acid). Chemical analysis together with immunological observation suggest that the LPS from \( C. \text{jejuni} \) (PEN 19) has oligosaccharide structure Galβ1-3GalNAcβ1-4(NeuAca2-3)Galβ1-4Glcβ1-in common with \( \text{G}_{\text{M}1} \) ganglioside.

The patient with GBS following \( C. \text{jejuni} \) (PEN 19) enteritis transiently had high titer of autoantibody against \( \text{G}_{\text{M}1} \) ganglioside (14). Immunological and chemical studies have revealed that LPS from
C. _jejuni_ (PEN 19) contains the G_{M1}-oligosaccharide structure. The mechanism for production of anti-G_{M1} antibody has been unknown. However, this study suggests that anti-G_{M1} antibody in patients with GBS following _C. jejuni_ infection is produced by the bacterial antigen cross-reacting with G_{M1} ganglioside. Patients who have developed GBS associated with anti-G_{M1} antibody after the administration of gangliosides, including G_{M1}, have been reported (7, 8). We speculate that patients who have specific immunogenetic backgrounds (13, 14) develop GBS associated with anti-G_{M1} antibody after infection by _C. jejuni_ (PEN 19) which has the G_{M1}-oligosaccharide structure.

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