Molecular mimicry in *Campylobacter jejuni*: role of the lipo-oligosaccharide core oligosaccharide in inducing anti-ganglioside antibodies

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Abstract

*Campylobacter jejuni* is recognized as the most common identifiable pathogen associated with the development of Guillain–Barré syndrome (GBS), an acute autoimmune-mediated disease affecting the peripheral nervous system. The immune response to ganglioside-like structures in lipo-oligosaccharides (LOs) of certain *C. jejuni* strains is thought to cross-react with human nerve gangliosides and induce GBS. To study the involvement of LOs in the pathogenesis of *Campylobacter*-induced GBS, we created truncated LOS molecules by inactivating the *waaF* gene in a GBS-associated isolate of *C. jejuni*. Gas Chromatography–MS analysis of the *waaF* mutant LOSs revealed a marked reduction in sugar content, including sialic acid and galactose. GM1 and GD1a-like mimicry was not detected in the *waaF* mutant by Western blot analysis with cholera toxin B and anti-GD1a antibodies. Mice immunized with the *waaF* mutant failed to develop anti-GM1 or anti-GD1a antibodies. The *waaF* mutant also showed reduced adherence to and invasion of INT-407 cells. The results indicate that the LOS of *C. jejuni* HB93-13 is essential for adherence and invasion as well as for anti-ganglioside antibody induction.

Introduction

*Campylobacter jejuni* has been recognized as the leading cause of acute gastroenteritis in the developed and developing world (Taylor, 1992; Friedman et al., 2000). Infection with *C. jejuni* usually results in uncomplicated gastroenteritis, but some patients may also have extra-intestinal manifestations including bloodstream infections (Skirrow & Blaser, 2000). In addition, *C. jejuni* infection is now recognized as the most common identifiable illness preceding Guillain–Barré syndrome (GBS), a group of immune-mediated, neuropathic disorders, including Fisher syndrome, affecting the peripheral nervous system (Ho et al., 1998). The pathogenesis of *Campylobacter*-induced GBS is complex and involves bacterial virulence factors as well as host susceptibility factors (Moran & O’Malley, 1995; Yuki et al., 1995; Bersudsky et al., 2000; Magira et al., 2003). Certain *C. jejuni* serotypes possess sialylated LOS structures, confined to the outer core of the LOS molecules, which mimic a wide range of peripheral nerve gangliosides including GM1, GA1 (asialo-GM1), GM1b, GalNAc-GM1b, GM2, GD1a, GalNAc-GD1a, GT1a, GQ1b and GD3 (Aspinall et al., 1993a, b, 1994a, b, c; Yuki et al., 1993, 1996; Moran & O’Malley, 1995; Kusunoki et al., 1996; Bersudsky et al., 2000; Nachamkin et al., 2002). This molecular mimicry in *C. jejuni* is hypothesized to elicit an immune response in susceptible hosts that results in demyelination or axonal damage of peripheral nerves, partly by the anti-ganglioside antibody response (Yuki, 2001).

A variety of Penner heat-stable (HS) serotypes of *C. jejuni* have been isolated from patients with GBS, but two types, HS:19 and HS:41, have been found to be particularly associated with GBS (Kuroki et al., 1991; Yuki et al., 1992; Aspinall et al., 1994a; Goddard et al., 1997; Wirguin et al., 1997; Nachamkin et al., 1998; Prendergast et al., 1998; Endtz et al., 2003; Wassenaar et al., 2000). However, HS:19 and HS:41 are not commonly found in diarrhoeic patients (Lastovica et al., 1997; Nishimura et al., 1997) and are reported to be clonal populations, whereas non-HS:19/HS:41 serotypes have been found to be genetically
heterogenous (Nishimura et al., 1997; Endtz et al., 2000; Wassenaar et al., 2000; Nachamkin et al., 2001).

The role of Campylobacter LOSs in generating anti-ganglioside antibodies is poorly understood. Furthermore, it is unknown if any other cell-surface component(s), such as flagella or capsular polysaccharide (CPS), in addition to LOSs, play a role in anti-ganglioside antibody induction. To investigate this, truncated LOS molecules in C. jejuni HB93-13 (ATCC 700297), serotype HS:19, isolated from a Chinese patient with the axonal form of GBS (Sheikh et al., 1998) were created, by means of insertional mutagenesis of the waaF gene. The waaF gene corresponds to the ORF Cj1148 in the C. jejuni NCTC11168 genome sequence. Inactivation of the waaF gene is known to create truncated LOS molecules in C. jejuni, and the gene was previously characterized in C. jejuni NCTC11168 by Oldfield et al. (2002). Mice challenged with the HB93-13 waaF mutant failed to elicit either anti-GM1 or anti-GD1a antibodies. Hence, additional experimental evidence is presented to show that C. jejuni LOS molecules are primarily involved in triggering anti-ganglioside antibody generation in an experimental mouse model without the apparent involvement of other cell-surface components.

Materials and methods

Bacterial strains

The bacterial strains used in this study are summarized in Table 1.

<table>
<thead>
<tr>
<th>Strain, plasmid or primer</th>
<th>Description</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni strains</td>
<td>HB93-13 Penner 19 serotype, ATCC 700297</td>
<td>Sheikh et al. (1998)</td>
</tr>
<tr>
<td>HB93-13 waaF waaF mutant</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>HB93-13 R2 Flagella mutant</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>81116 Penner 6 serotype</td>
<td>Palmer et al. (1983)</td>
<td></td>
</tr>
<tr>
<td>81116 R2 Flagella mutant</td>
<td>Wassenaar et al. (1991)</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli strains</td>
<td>DH5α F′endA1 hsdR17(ωrKm−) subE44 thi-1 recA1 gyrA (NaR) relA1 D (lacZYA-argF)U169 (80lacZΔM15)</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>Plasmids</td>
<td>pBl Cloning vector pBluescript</td>
<td>Short et al. (1988)</td>
</tr>
<tr>
<td>pMW2 Vector with ampicillin and kanamycin resistance cassettes</td>
<td>Wösten, unpublished</td>
<td></td>
</tr>
<tr>
<td>pVNF100 pBl containing a 1.68-kb fragment from C. jejuni HB93-13 in the direction of the LacZ promoter</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>pVFM pBl containing a 1.45-kb fragment from C. jejuni HB93-13</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>pVFMKmR pVFM containing a kanamycin resistance cassette inserted into the 1.4-kb fragment cloned</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Primers</td>
<td>11a-F TTAAGTCGACATCTTTTTTAAAGCACTTCG</td>
<td>This study</td>
</tr>
<tr>
<td>waaF-Rev GCCTTTAGATCCTCTTCGATGATTGGT</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>ghmA-Rev GAGCGGATCCAGCCCTAATGTTGCAGC</td>
<td>This study</td>
<td></td>
</tr>
</tbody>
</table>

PCR isolation, cloning and sequencing of the waaF gene

The C. jejuni HB93-13 waaF gene was isolated using primers 11a-F and ghmA-R for complementation and 11a-F and waaF-Rev for insertional mutagenesis (Table 1). The primers were designed from the sequenced C. jejuni HB93-13 LOS biosynthesis gene cluster (GenBank accession no AY297047) and from the genome sequence of C. jejuni NCTC 11168 (Parkhill et al., 2000). The waaF gene was PCR-amplified and cloned into vector pBluescript (Stratagene) using unique restriction sites introduced by means of the primers. The gene was cloned in the direction of the Lac2 promoter of pBluescript (pVNF100). The waaF gene was sequenced with the Big Dye terminator cycle sequencing kit (Applied Biosystems) on an ABI 373 DNA sequencer. Sequence analysis was performed using NCBI BLAST. All enzymes used were obtained from Promega unless specified, and were used according to the manufacturer’s instructions.

Knock-out mutagenesis of the C. jejuni HB93-13 waaF gene

The PCR product amplified from primers 11a-F and waaF-Rev was cloned into pBluescript to form pVFM, which contained a HindIII site at nucleotide 558 of the 1038-nucleotide coding region of the waaF gene. A kanamycin resistance cassette was inserted into the HindIII site to form pVFMKmR. The HB93-13 parent strain was transformed with pVFMKmR via natural transformation as described previously (Wassenaar et al., 1993). The kanamycin-
resistant transformants were screened by PCR and Southern blot analysis to confirm that the incoming plasmid DNA had integrated by a double crossover event with the genomic DNA.

**LOS and CPS extraction and SDS-PAGE**

LOS from *C. jejuni* was isolated as described previously (Lugtenberg *et al.*, 1975). For gas chromatography–MS (GC–MS) analysis, LOS was prepared by subjecting the isolated LOS samples to DNase (200 µg mL⁻¹), RNase (50 µg mL⁻¹) and proteinase K (50 µg mL⁻¹) digestions. The samples were then subjected to hot phenol–water extractions to remove all the proteins, followed by dialysis to remove traces of phenol. Crude preparations of CPS from *C. jejuni* were prepared as described previously (Karlyshev & Wren, 2001).

LOS and CPS samples were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with the discontinuous buffer system described by Laemmli (1970). LOS was visualized by silver staining (Tsai & Frasch, 1982), and CPS by alcian blue staining as described previously (Karlyshev & Wren, 2001). Samples for Western blot analysis were transferred to Immobilon-PVDF membranes (Millipore Corporation), blocked with 3% bovine serum albumin (BSA), and, for the detection of the GM1-like structures, incubated with horseradish peroxidase (HRP)-labelled cholera toxin subunit B (Sigma). For detecting the GD1a-like epitope, two monoclonal antibodies, GD1a-1 and GD1a-2a, that react with GD1a were used (Schnaar *et al.*, 2002).

**Knock-out mutagenesis of flaA and flaB genes in C. jejuni HB93-13**

Ten micrograms of genomic DNA isolated from *C. jejuni* 81116 R2 mutant was used to transform *C. jejuni* HB93-13 parent strain via natural transformation (Wassenaar *et al.*, 1993). The resulting mutants were grown in the presence of kanamycin.

**GC–MS analysis**

GC–MS analysis of the isolated and purified LOS samples was performed as described previously (Linton *et al.*, 2000).

**Animal studies**

Ten-week-old C3H/HEN mice (Charles River) were used for immunization studies (Bowes *et al.*, 2002). *Campylobacter jejuni* HB93-13 was grown on Campy CVA medium (Becton Dickinson) at 42 °C under microaerobic conditions for 48 h. Bacteria were harvested in distilled water, washed once, and the pellet was vacuum-dried. The dried whole bacterial cells were subjected to grinding and resuspended in 0.8% NaCl. The protein content was measured and adjusted to 1.3 mg mL⁻¹, aliquoted into 2-mL samples, and stored at –70 °C until use. Whole-cell antigen was prepared in the RIBI adjuvant system (Sigma). Two millilitres of frozen bacterial antigen was mixed with adjuvant according to the manufacturer’s instructions. Two groups of mice were used, comprising eight mice per group. One group received wild-type HB93-13 whole-cell antigen and the other group received the waaF mutant-prepared whole-cell antigen. Each animal received 100 µL of whole-cell antigen into two different sites subcutaneously. Booster injections were given after 7 days, and weekly thereafter for the following 3 weeks. Blood samples were collected via the tail at weekly time points. After 28 – 30 days, animals were euthanized and the final blood sample collected.

**Serological studies**

Mouse serum antibody responses to *C. jejuni* surface antigen were measured, (PEN123), purified LOS from HB93-13, and gangliosides GM1 and GD1a by enzyme-linked immunosorbent assay (ELISA), performed as previously described (Ho *et al.*, 1999) with the following modifications. For surface antigen and LOS, the plate-coating concentrations were 0.5 µg well⁻¹ for surface antigen and 2 µg well⁻¹ for LOS diluted in carbonate buffer. Plates were blocked with PBS containing 1 mg gelatin mL⁻¹, 0.005% Tween-20 at 4 °C overnight. Gangliosides GM1 (Sigma) and GD1a (Sigma) were diluted in 100% ethanol and coating performed by air-drying at room temperature for 2 h. Plates were blocked with PBS containing 0.1% BSA for 2 h at 4 °C. Preliminary experiments were performed to optimize the serum dilution used for the assay; sera were diluted 1:50 for immunoglobulin A (IgA), 1:200 for IgG, and 1:100 for IgM. We used 3-ethylbenzothiazoline-6-sulfonic acid as the substrate for ELISA development, and wells were read in a microplate spectrophotometer at 405 nm.

**Adherence/invasion assay**

The invasiveness of *C. jejuni* HB93-13 and its mutants waaF and R2 was determined by means of the gentamicin protection assay using INT-407 cells as described previously (Wassenaar *et al.*, 1991).

**Results**

**PCR and cloning of the waaF gene from C. jejuni HB93-13**

The primers 11a-F and gmhA-R amplified a 1.68-kb fragment containing the *C. jejuni* HB93-12 waaF gene. The fragment was cloned into pBluescript to form pVNF100 and pVNF. The *C. jejuni* HB93-13 waaF gene was 1038 bp long. Sequence analysis of the gene showed 88% amino acid
sequence similarity (nucleotide similarity 87%) to that from C. jejuni NCTC11168, and 93% similarity (nucleotide similarity 94%) to that from C. jejuni NCTC 11828 (Oldfield et al., 2002; Fig. 1).

Knock-out mutagenesis of the C. jejuni HB93-13 waaF gene

Mutation of the waaF gene has been shown to create truncated LOS molecules in several bacterial species including C. jejuni NCTC 11168 (Oldfield et al., 2002). Hence, the waaF gene was inactivated to create truncated LOS in C. jejuni HB93-13, as this was essential for investigating the ability of such mutants to induce anti-ganglioside antibodies in immunized mice.

A 1.45-kb fragment was PCR-amplified from C. jejuni HB93-13 using primers 11a-F and waaF-Rev. This fragment was cloned into pBluescript and was inactivated by means of the insertion of a kanamycin resistance cassette. The mutants obtained after natural transformation were analysed for phenotypic expression of the LOS. Examination of silver-stained LOS after SDS-PAGE revealed that LOS from the waaF mutant was of faster electrophoretic mobility than the parent-strain LOS and hence was truncated (Fig. 2). To determine whether ganglioside-like mimicry was lost in the waaF mutant, we used cholera toxin, which binds to GM1, and antibodies to GD1a to probe for these LOS structures.

Both cholera toxin and anti-GD1a antibodies bound to HB93-13 LOS but not with that of its waaF mutant (Fig. 3). This indicates that the waaF mutation caused a structural alteration in the LOS that resulted in disruption of the GM1 and GD1a ganglioside mimics, and is consistent with the similar loss of ganglioside reactivity in the C. jejuni NCTC 11168 waaF mutant (Oldfield et al., 2002).

Evidence that the waaF mutation has not impaired CPS or motility in C. jejuni HB93-13

Prior to testing the waaF mutant for the ability to induce anti-ganglioside antibodies, the waaF mutant was...
analysed for alterations in CPS production and motility. CPS fractionated with SDS-PAGE followed by alcian blue staining revealed intact bands corresponding to those described by Karlyshev & Wren (2001) (Fig. 4). Thus, the C. jejuni HB93-13 waaF mutant possessed CPS molecules that were similar in size to those of the parent strain.

The swarming experiments showed that the parent strain and mutant HB-93-13 were both motile and swarmed in a similar way, indicating that the flagella in the waaF mutant were still intact and active (data not shown). In contrast, the C. jejuni HB93-13 R2 mutant (control mutant lacking flagella) failed to swarm in thioglycollate plates (data not shown). Thus, the results of the above experiments showed that the C. jejuni HB93-13 waaF mutant has intact flagella as well as CPS production.

GC–MS analysis

GC–MS analysis was performed in order to compare the sugar composition of the parent-strain LOS with that of the waaF mutant LOS. The sugars present in the C. jejuni HB93-13 parent-strain LOS and the waaF mutant LOS are shown in Table 2. The LOS sugar composition analysis showed that sugars such as glucose, galactose, GlcNAc and NANA were significantly lower in concentration in the C. jejuni waaF mutant than in the parent-strain LOS.

Adherence/invasion assay

LOS has been shown to be involved in several virulence mechanisms, including adherence to and invasion of host cells (Fry et al., 2000; Kanipes et al., 2004). Because the LOS of the waaF mutant seemed to lack the outer core and a part of the inner core of the LOS, it was an ideal model with which to examine the contribution of LOS towards adherence to and invasion of host cells. The waaF mutant as well as the C. jejuni HB93-13 R2 mutant showed significantly reduced adherence and invasion, and confirmed results recently reported by Kanipes et al. (2004) using a waaF mutant of C. jejuni 81–176 (Table 3).

Immunization of animals with parent and the waaF mutant

Mice immunized with the C. jejuni parent strain developed antibodies to both C. jejuni surface antigen and homologous LOS (Fig. 5a). Although animals immunized with the waaF mutant reacted with C. jejuni surface antigens, they did not develop serum antibodies to purified LOS, which suggests that the outer core is an immunodominant region of the LOS (Fig. 5a). An antibody response to both GM1 and GD1a gangliosides was apparent in animals immunized with the HB93-13 parent strain, with antibodies of the IgG, IgA and IgM isotypes detected (Fig. 5b). However, mice immunized with the waaF mutant exhibited a reduced immune response to both GM1 and GD1a gangliosides (Fig. 5b). These results are consistent with other data showing the lack of ganglioside-like reactivity in the outer LOS core in the waaF mutant.
Molecular mimicry between *C. jejuni* LOS molecules and human nerve-tissue gangliosides has been hypothesized to stimulate the production of antibodies that are auto-reactive against peripheral nerves, causing immune-mediated damage that subsequently results in acute paralysis (Yuki, 2001). Certain *C. jejuni* Penner serotypes, such as HS : 19 and HS : 41, have been shown to be over-represented among patients that develop GBS, suggesting that these serotypes possess unique virulence factors. Furthermore, *C. jejuni* strains from patients with GBS are more likely to express ganglioside-like epitopes, specifically GD1a, than strains from patients with uncomplicated enteritis (Nachamkin et al., 2002). However, the contribution of other bacterial

**Table 3.** Percentage adherence to and invasion of INT-407 cells by *Campylobacter jejuni* HB93-13 and the waaF mutant

<table>
<thead>
<tr>
<th></th>
<th>Adherence* (%)</th>
<th>Invasion* (%)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Campylobacter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB93-13</td>
<td>100</td>
<td>100</td>
<td>17.5451</td>
</tr>
<tr>
<td>waaF mutant</td>
<td>17.2</td>
<td>7.4</td>
<td>0.026</td>
</tr>
<tr>
<td>R2 mutant</td>
<td>7.2</td>
<td>0.26</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*Percentage adherence to or invasion of INT-407 cells by the *C. jejuni* waaF LOS and R2 flagella mutants compared with the parent strain HB93-13. The number of HB93-13 cells adhered to or invaded was taken as 100%. The assay was repeated three times and mean values were calculated. Percentage invasion was calculated relative to the number of cells adhered to.

![Fig. 5. Mouse immune responses to the gangliosides GM1 and GD1a following immunization with *Campylobacter jejuni* HB93-13 parent and the waaF mutant whole bacterial cells. (a) IgG antibody response to *C. jejuni* surface protein antigen (left) and homologous lipopolysaccharide (right). (b) Serum IgG, IgA and IgM responses to the GM1 and GD1a gangliosides.](image)

**Discussion**

Molecular mimicry between *C. jejuni* LOS molecules and human nerve-tissue gangliosides has been hypothesized to stimulate the production of antibodies that are auto-reactive against peripheral nerves, causing immune-mediated damage that subsequently results in acute paralysis (Yuki, 2001). Certain *C. jejuni* Penner serotypes, such as HS : 19 and HS : 41, have been shown to be over-represented among patients that develop GBS, suggesting that these serotypes possess unique virulence factors. Furthermore, *C. jejuni* strains from patients with GBS are more likely to express ganglioside-like epitopes, specifically GD1a, than strains from patients with uncomplicated enteritis (Nachamkin et al., 2002). However, the contribution of other bacterial
C. jejuni cell-surface molecules in eliciting anti-ganglioside like antibodies is not clear.

Campylobacter jejuni cell-surface molecules such as flagella have been shown to be antigenic and to carry sialic acid residues, a component of human nerve gangliosides (Linton et al., 2000). Campylobacter jejuni produces LOS molecules as well as an extracellular polysaccharide termed CPS (Karlyshev & Wren, 2001) that has been shown to be involved in its virulence (Bacon et al., 2001). Consequently, this study was conducted to determine experimentally if C. jejuni LOS molecules are directly involved in inducing anti-ganglioside antibodies without the apparent involvement of the aforementioned cell-surface molecules. The results suggest that CPS and flagella in HB93-13 are not major surface structures responsible for eliciting anti-ganglioside antibodies; however, one cannot rule out the possibility on the basis of our studies alone. Future experiments using purified components such as flagella and CPS should be performed.

The complete genome sequence of C. jejuni NCTC 11168 revealed the waaI LOS biosynthesis gene cluster spanning from the waaC to the waaF gene. The waaC gene encodes a heptosyltransferase I enzyme, and this has been characterized in Campylobacter jejuni and E. coli (Klena et al., 1998). The WaaC protein adds the first l-glycero-d-manno-heptose to the inner core of the LOS. Mutation of this gene has been unsuccessful in C. jejuni, indicating that the waaC gene is essential for the survival of C. jejuni (unpublished data, B.N. Fry). The waaF gene has been shown in E. coli and Salmonella to encode an enzyme heptosyltransferase II that is involved in adding a second l-glycero-d-manno-heptose moiety to the inner core of the LOS/lipopolysaccharide molecule (Sirisena et al., 1994; Gronow et al., 2000). We were thus able to create a C. jejuni waaF mutant that resulted in the smallest possible LOS moiety.

It has been shown that ganglioside-mimicking LOS epitopes are confined to the outer core of LOS molecules in C. jejuni (Linton et al., 2000; Oldfield et al., 2002). We predicted that the waaF mutant from C. jejuni HB93-13, a strain exhibiting ganglioside-like mimicry, isolated from a patient with GBS, would not be able to induce anti-ganglioside antibodies, assuming that there would be no contribution from other cell-surface components. The waaF mutant created in this study did not elicit antibodies to purified LOS, GM1 or GD1a gangliosides, despite its having intact CPS and flagella.

GC–MS analysis showed that glucose, galactose, GlcNAc, and NANA, also known as sialic acid, were significantly low in abundance in the waaF mutant LOS, indicating the truncation of the molecule. The presence of NANA, however, is probably the result of other sialylated bacterial structures, such as flagella (Linton et al., 2000). The presence of NANA in the LOS is a prerequisite for ganglioside mimicry, because it is a constituent of human nerve gangliosides. The markedly reduced amount of NANA and other core-containing sugars is consistent with the lack of anti-ganglioside antibody response in animals immunized with the waaF mutant.

Adherence and invasion assays were performed to determine further the contribution of C. jejuni HB93-13 LOS and flagella to C. jejuni pathogenesis. The invasiveness would also indicate the ability of the organisms to trigger a humoral response that could result in the induction of cross-reactive antibodies. The HB93-13 waaF mutant showed reduced adherence to and invasion of INT-407 cells. Furthermore, the C. jejuni HB93-13 R2 mutant, which is devoid of flagella, showed reduced adherence to INT-407 cells, and their invasive ability was diminished. The reduced invasiveness of HB93-13 waaF is consistent with the recent findings by Kanipes et al., who found that a waaF mutant created from the highly invasive strain C. jejuni 81-176 showed decreased invasion of INT407 cells in vitro (Kanipes et al., 2004). This strongly suggests that the presence of intact LOS, in addition to flagella, is vital for C. jejuni adherence to and invasion of host cells.

Mutation of the waaF gene resulted in the loss of GM1- and GD1a-like ganglioside mimicry in the LOS, based on the lack of binding with cholera toxin and anti-GD1a antibodies. Previous studies have shown that purified LOS from C. jejuni could elicit ganglioside antibodies in a variety of animals including mice (Goodyear et al., 1999; Bowes et al., 2002) and rabbits (Ang et al., 2001, 2002). Godschalk et al. (2004) showed that mice immunized with nonsialylated LOS mutants of C. jejuni GB11 failed to induce anti-GD1a antibodies in comparison to the wild-type strain. However, they did detect high titres of asialo-GM1 antibodies following immunization of mice with nonsialylated LOS mutants. This was probably the result of the mutated LOS structures becoming asialo-GM1 mimics. In this study, animals immunized with the wild-type HB93-13 induced anti-GM1 and anti-GD1a antibodies, whereas the waaF mutant failed to produce antibodies directed at intact LOS or at GM1 and GD1a gangliosides, findings that confirm that the outer LOS core contains these ganglioside-like moieties. We did not determine if the waaF mutant could induce asialo-GM1 antibodies in mice. The above results suggest that the core LOS region is responsible for the induction of anti-ganglioside antibodies in the host; however, further studies are needed to rule out the requirement of other surface structures that could exhibit ganglioside-like mimicry.

The incidence of GBS in the general population has been estimated at 1–4/100,000 (Hughes & Rees, 1997). While precise data are unavailable, it is estimated that GBS occurs in one per 1000 patients with Campylobacter infection, and in one per 180 patients infected with serotype HS:19.
Isolates of *C. jejuni* expressing ganglioside-like mimicry from patients without GBS occur frequently (Nachamkin, 2002), and thus the presence of ganglioside-like mimicry alone is not responsible for the development of GBS. Host susceptibility to GBS is likely to be a critical component, and recent studies by Magira et al. (2003) suggest that a specific DQB epitope is associated with GBS pathogenesis. Nevertheless, this mimicry does not induce cross-reactive antibodies. The lack of an appropriate animal model that mimics human GBS has greatly hampered the possibility of determining their ability to induce anti-ganglioside antibodies. Therefore, further experiments should be performed with flagella and capsule mutants with intact LOS in order to determine their ability to induce anti-ganglioside antibodies. The lack of an appropriate animal model that mimics human GBS has greatly hampered the possibility of unravelling the mechanisms behind anti-ganglioside antibody induction.

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**References**


Campylobacter jejuni and the Guillain–Barré syndrome


Sheikh KA, Nachamkin I, Ho TW et al. (1998) Campylobacter jejuni lipopolysaccharides in Guillain–Barré syndrome:


