Human Monoclonal Immunoglobulin M Antibodies to Ganglioside GM₁ Show Diverse Cross-Reactivities with Lipopolysaccharides of Campylobacter jejuni Strains Associated with Guillain-Barré Syndrome

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We examined the reactivity of a panel of anti-GM₁ immunoglobulin M monoclonal antibodies (MAbs) cloned from multifold motor neuropathy patients with lipopolysaccharides (LPSs) of Campylobacter jejuni strains, including serotype O:41 strains associated with Guillain-Barré syndrome. The MAbs reacted with ganglioside GM₁ to different degrees, and these differences in fine specificities for GM₁ were reflected in the different degrees of reactivity with each of the C. jejuni LPSs tested. Antibodies could also be discriminated by the varying patterns of inhibition by cholera toxin (a GM₁ ligand) in LPS binding studies. These results indicate that there is a substantial heterogeneity among C. jejuni O:41 strains in their expression of GM₁-like epitopes and among the fine specificities of different neuropathy-associated anti-GM₁ antibodies.

Guillain-Barré syndrome (GBS), an acute polynuropathy, is now the most common cause of generalized paralysis and in two-thirds of cases is preceded by a respiratory or gastrointestinal infection (8, 12). Campylobacter jejuni, a leading cause of acute gastroenteritis, has been identified as the most frequent infectious agent associated with the development of GBS (up to 66% of patients) (8, 17). C. jejuni can be serotyped based on differences in the polysaccharide structure (O side chain and core oligosaccharide [OS]) of the lipopolysaccharide (LPS) (O antigen) of the bacterium (16). Reports have shown that the C. jejuni isolates obtained from diarrheic patients prior to the onset of GBS belonged to serotype O:19, an uncommon serotype in gastroenteritis patients (6, 9, 23). Other C. jejuni serotypes commonly identified in association with GBS include O:2, O:2/44, O:4/59, O:15, O:18, O:21, O:24, O:30, O:37, and O:53 (6, 10, 12, 17).

Autoreactive antibodies to gangliosides, especially GM₁, are found in 20% of GBS patient sera, particularly after C. jejuni infection (8, 12, 19, 25), and are also found in the sera of 50% of patients with the chronic neuropathy termed multifocal motor neuropathy (14). Neuropathy-associated GM₁ antisera have been shown to cross-react with C. jejuni LPSs (19, 22, 23). It is thus currently hypothesized that antiganglioside antibodies may be induced as a result of molecular mimicry between peripheral nerve gangliosides and structurally similar C. jejuni LPSs (19, 23). Although there are indications that anti-GM₁ antibodies may lead to the activation of inflammatory pathways and act by disrupting membrane ion channel function at nodes of Ranvier (20), experimental proof of involvement in disrupting nerve function has been difficult to conclusively demonstrate. However, since anti-GM₁ antibodies in human sera are likely to be a contributory factor in the induction of GBS, an important step in elucidating the pathogenesis of the disease is determining the structure of the immunogenic epitopes in ganglioside-mimicking C. jejuni LPSs.

Chemical studies of the LPS extracted from C. jejuni O:19 have shown that the terminal regions of the LPS core mimic human gangliosides GM₁, GD₂, GT₁, and GD₃ (2, 9, 24). GM₁-like OS structures occur in LPS from O:1, O:23, and O:36 (4), whereas the core OSs of C. jejuni O:4 and O:41 mimic gangliosides GD₂ and GM₁, respectively (4, 15). Mimicry of C. jejuni O:2 LPS is limited to a disaccharide present in a range of gangliosides (3).

The authors of several studies have previously investigated the reactivities of human and animal anti-GM₁ antisera with C. jejuni LPSs and demonstrated the principle of cross-reactivity. However, no information is available on the extent to which antibodies with different fine specificities of epitope recognition for GM₁ are capable of binding GM₁-like LPSs. In this study, we aimed to use a set of human monoclonal antibodies (MAbs) that are reactive with GM₁ and have been characterized as structurally distinct (13), in conjunction with a panel of well-defined LPSs, to determine the degree to which ganglioside GM₁ and C. jejuni LPSs share immunoreactive epitopes. C. jejuni serostrains O:2 (ATCC 43430), O:3 (ATCC 43431), O:4 (ATCC 43432), O:19 (ATCC 43446), and O:41 (ATCC 43460) were obtained from the American Type Culture Collection (Manassas, Va.). The details concerning three GBS patients and one enteritis patient from whom C. jejuni O:41 strains (16971.94GSH, 28134.94GSH, 260.94RXH, and 176.83, respectively) were isolated have been described previously (7, 15). Isolates and serostrains were routinely cultured on blood agar under microaerobic conditions at 37°C for 48 h. The biomass was harvested, and the bulk extraction of LPS was performed by phenol-water extraction procedure (11). In addition, LPSs from two GBS isolates, C. jejuni OH4382 and OH4384, which exhibit mimicry of gangliosides GD₃ and GT₁, respectively (2), were a generous gift from G. O. Aspinall (York University, Toronto, Ontario, Canada). The immunoglobulin M (IgM) anti-GM₁ MAbs termed BO₁,1, BO₃,1, SM₁,8, and WO₁,4 were cloned from peripheral blood lymphocytes of three multifocal motor neuropathy patients, all of whom had abnor-

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TABLE 1. Binding and inhibition studies with anti-GM₁ antibodies and C. jejuni LPS or gangliosides

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CT binding</th>
<th>PNA binding</th>
<th>BO₁₁</th>
<th>BO₃₁</th>
<th>SM₁₈</th>
<th>WO₁₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganglioside GM₁</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++++</td>
<td>-</td>
</tr>
<tr>
<td>C. jejuni LPS</td>
<td>16971.94GSH</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>28134.94GSH</td>
<td>++++</td>
<td>+++</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>260.94RXH</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>176.83</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Serostain O:41</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Serostain O:2</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Serostain O:3</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Serostain O:4</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Serostain O:19</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*+++*, very strong reaction; **++*, strong reaction; +++, moderate reaction; +, weak reaction; −, no reaction. ND, not determined.

nally elevated anti-GM₁ antibody titers, and have been described previously (13, 21). The MAbs were purified by the ultrafiltration of culture supernatants and checked for monoclontality by isoelectric focusing (21).

Gangliosides (Sigma Chemical Co., St. Louis, Mo.) and LPSs were analyzed by thin-layer chromatography (TLC) on precoated silica gel 60 glass plates (Merck, Darmstadt, Germany) by using solvent systems of chloroform–methanol–80% (vol/vol/vol) (19) as the primary antibody and peroxidase-conjugated anti-human IgM (Dako, Cambridge, United Kingdom) diluted 1:1000 as the secondary antibody. Binding experiments with cholera toxin-peroxidase conjugate (CT-HRP; Sigma) and peanut agglutinin (PNA)-HRP conjugate (Kem-En-Tec, Copenhagen, Denmark) were carried out with only one TLC overlay step by using CT-HRP or PNA-HRP at dilutions of 1:1000 and 1:50, respectively. Inhibition experiments were performed by using the B subunit of CT (Sigma) at 1 μg/ml to overlay separated gangliosides on TLC plates and incubating the plates at room temperature for 3 h, immunoprecipitates were removed by centrifugation (10,000 × g for 10 min), and the supernatants were reacted with intact C. jejuni 176.83 LPS.

The binding patterns of the four MAbs to ganglioside GM₁ and C. jejuni LPSs are summarized in Table 1. The specificities of the MAbs were confirmed by their binding to GM₁ and are consistent with previous results (13, 21). The MAbs reacted to different degrees with each of the serotype O:41 LPSs tested. BO₁₁ and BO₃₁ both recognized the LPS of the enteritis strain (176.83), whereas BO₁₁ and BO₃₁ separately bound LPSs of two different GBS-associated strains (28134.94GSH and 260.94RXH, respectively). In addition, both MAbs bound serostain O:2 LPS but did not react with serostain O:41 LPS or with any of the other C. jejuni LPSs. SM₁₈, which reacted with ganglioside GM₁ only, recognized all C. jejuni O:41 LPSs with different intensities, including those from the three GBS-associated strains (Fig. 1), but did not bind to any of the other C. jejuni LPSs. WO₁₄ recognized three of the four serotype O:41 LPSs (excluding that from the enteritis strain) and the serostain O:41 LPS but did not react with the other C. jejuni LPSs. Previously, we undertook investigations of the structures of LPSs from the C. jejuni O:41 strains and, in particular, established chemically that the core OS structure of C. jejuni 16971.94GSH shared a tetrasaccharide with ganglioside GM₁ (15). The putative regions of binding of MAbs SM₁₈ and WO₁₄ in C. jejuni 16971.94GSH LPS are shown in Fig. 2. MAbs BO₁₁ and BO₃₁ are not indicated in Fig. 2, since no reaction was observed with this particular C. jejuni O:41 LPS. However, the binding patterns of BO₁₁ and BO₃₁ are more comparable to that of MAb WO₁₄ than that of MAb SM₁₈.
Therefore, ganglioside GM1 and C. jejuni O:41 LPSs share an immunoreactive epitope, but because the patterns of MAb reactivity varied with individual C. jejuni O:41 LPSs (including those MAbs which were specific for GM1 only), there must be a degree of structural variability present within this epitope(s). Alternatively, there may be differences in the surface topography or density of the GM1-like molecule(s) in the C. jejuni O:41 LPSs. Furthermore, the influence of an O side chain in C. jejuni O:41 LPSs (Table 1) on MAb binding can be excluded, since electrophoretic and immunoblotting analyses have shown that C. jejuni O:41 strains produce low-Mₕ LPS without an O chain (15) and chemical analyses indicate the presence of only high-Mₕ extracellular polysaccharides independent of LPS (5, 15).

Interestingly, none of the anti-GM1 MAbs reacted with serostrain O:4 or O:19 LPSs. The core OS of serostrain O:4 LPSs contains a pentasaccharide in common with ganglioside GD1ₐ (4). None of the MAbs react with ganglioside GD1ₐ (13, 21), and so the lack of a reaction with serostrain O:4 LPS is expected. Although GM1 mimicry, along with mimicry of ganglioside GD1ₐ, is present in the core OS of serostrain O:4 LPS (2), the lack of MAb reactivity may be explained by the presence of an O side chain in this LPS (2, 10), which affects epitope accessibility. As expected, no reaction occurred when the MAbs were reacted with LPSs of the C. jejuni O:19 GBS-associated strains (OH4382 and OH4384) which have been shown to mimic GD₃ and GT₁₆, respectively (2). Furthermore, none of the MAbs reacted with free lipid A, and MAb preparations immunoadsorbed with the isolated core OS of C. jejuni 176.83 did not bind with the intact LPS of this strain. This provides conclusive evidence that it is the core OS region to which the antibodies bind and not lipid A.

CT, a specific ligand for ganglioside GM₁ₓ and PNA, a ligand for the disaccharide structure Gaβ1-3Gal, asialo-GM₁, and GD₁₆, respectively (2), did not react with serostrain O:19 LPSs. This demonstrates that CT and MAb WO1-4 do not recognize the same epitope in this LPS, a further indication that differences occur in the C. jejuni O:41 LPSs. Likewise, CT did not inhibit the binding of BO₃₋₁ to serostrain O:2 LPS.

This study demonstrates the cross-reactivity of human monoclonal IgM anti-GM1 antibodies with C. jejuni LPSs associated with GBS. The MAbs are known to have different specificities for ganglioside GM₁, some of which may also occur in asialo-GM₁ and GD₁₆ (21). Our results are in accordance with those of others who demonstrated that IgM anti-GM1 MAbs from patients with chronic motor neuropathy reacted with LPSs of C. jejuni O:4, O:19, and O:50 serostrains (22). The MAbs also differed in their relative reactivities with the C. jejuni LPSs tested, in particular with regard to serotype O:41 LPSs. The results not only show the existence of a GM1-like epitope(s) in C. jejuni O:41 LPSs but also reveal the existence of differences within the serotype O:41 LPSs. The pattern of binding of the IgM anti-GM1 MAbs to C. jejuni O:41 LPSs indicates that either slight differences in sugar substitution occur in the core OS of the various C. jejuni O:41 LPSs or variation occurs in how the LPSs present these structures to the antibodies, possibly as a function of antigen density, and hence affect antibody recognition.

Furthermore, in another study, CT recognized serostrain O:2, O:4, and O:19 LPSs (19, 22, 23) and CT bound avidly to all C. jejuni O:41 LPSs, including that of the serostrain and the enteritis isolate (C. jejuni 176.83). Yuki et al. observed the binding of CT to serostrain O:19 LPS and deduced that it had a GM1-like structure (23), which has been confirmed by structural analyses (2). However, LPSs of two C. jejuni O:19 strains from GBS patients (OH4382 and OH4384) which mimic GD₃ and GT₁₆, respectively (2), did not react with CT or any other ligands or antibodies used in this study. The binding of IgM MAbs to ganglioside GM₁ and C. jejuni LPSs was inhibited by the CT B subunit; thus, CT and the MAbs recognize the same or a structurally overlapping epitope in ganglioside GM₁ and GBS-associated C. jejuni LPSs. This concurs with the results in a previous report where the binding of GBS sera to O:19 LPS was blocked with CT (22). However, in this study, CT did not inhibit the binding of some of the MAbs to one serotype O:41 LPS, a further indication that slight differences in structure occur within the core OS of C. jejuni O:41 LPSs. Since our inhibition experiments using PNA were considered technically unreliable, we cannot confirm the evidence of others (22) that
PNA does not inhibit the binding of anti-GM1 antibodies to either ganglioside GM1 or C. jejuni LPs.

In conclusion, the evident mimicry between C. jejuni LPs and gangliosides may act as a trigger to stimulate the production of anti-ganglioside antibodies which may play a role in the pathogenesis of GBS. The mimicry of gangliosides is not limited to those strains associated with GBS, as LPs from the C. jejuni O:41 enteritis isolate reacted in a way similar to that seen with LPs from the GBS-associated strains. This phenomenon has previously been observed by us with C. jejuni O:19 LPs, whereby an enteritis isolate mimics both gangliosides GM1 and GD1b, suggesting that, in addition to mimicry, other host or bacterial factors are involved in disease pathogenesis and require further investigation.

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REFERENCES