ORIGINAL ARTICLE INFECTIOUS DISEASES

Siglec-7 specifically recognizes *Campylobacter jejuni* strains associated with oculomotor weakness in Guillain–Barré syndrome and Miller Fisher syndrome

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Abstract

Due to molecular mimicry, *Campylobacter jejuni* lipo-oligosaccharides can induce a cross-reactive antibody response to nerve gangliosides, which leads to Guillain–Barré syndrome (GBS). Cross-reactive antibodies to ganglioside GQ1b are strongly associated with oculomotor weakness in GBS and its variant, Miller Fisher syndrome (MFS). Antigen recognition is a crucial first step in the induction of a cross-reactive antibody response, and it has been shown that GQ1b-like epitopes expressed on the surface of *C. jejuni* are recognized by sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7). We aimed to determine the epitope specificity of *C. jejuni* binding to Siglec-7, and correlate the outcome to disease symptoms in GBS and MFS patients. Using a well-defined GBS/MFS-associated *C. jejuni* strain collection, which included three sialic acid knockout strains, we found that Siglec-7 exclusively binds to *C. jejuni* strains that express terminal disialylated ganglioside mimics. When serological and diagnostic patient records were correlated with the Siglec-7-binding properties, we observed an association between Siglec-7 binding and the presence of anti-GQ1b antibodies in patient serum. In addition, Siglec-7 binding was associated with oculomotor weakness in GBS and MFS patients. Lipo-oligosaccharide-specific binding of *C. jejuni* to Siglec-7 may be an initiating event in immune recognition and presentation, and lead to anti-GQ1b antibody production and the development of ocular weakness in GBS or MFS.

Keywords: *Campylobacter jejuni*, disialylation, lipo-oligosaccharide, Miller Fisher Syndrome, Siglec-7
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Introduction

Guillain–Barré syndrome (GBS) is an antibody-mediated autoimmune disease of the peripheral nerves, which mainly arises after gastrointestinal infection [1–3]. GBS is characterized by rapidly progressing acute ascending paralysis, which can result in complete systemic paralysis and the need for artificial respiration [4]. Miller Fisher syndrome (MFS) is a restricted variant of GBS, characterized by paralysis of the eye muscles (oculomotor weakness), lack of coordination and loss of tendon reflexes, without limb weakness [5]. GBS–MFS overlap syndrome may also occur in patients who display a combination of limb and oculomotor weakness [6,7].

Campylobacter jejuni is the predominant infection preceding the onset of weakness and paralysis in GBS and MFS [8,9]. *C. jejuni* strains isolated from GBS patients frequently express lipo-oligosaccharide (LOS) structures that contain glycan moieties which mimic gangliosides from the human peripheral nervous system [10]. In these patients and in animal models, the antibodies raised during the immune response to *C. jejuni* LOS can cross-react with various gangliosides and lead to complement-dependent nerve destruction and paralysis [11,12].

Guillain–Barré is a syndromic disease entity with a heterogeneous presentation of symptoms [2]. Ganglioside mimicry in *C. jejuni* is strongly associated with the specificity of the cross-reactive antibody response and the clinical neurological phenotype. *C. jejuni* can express monosialylated and disialylated

LOS with α(2,3)- or α(2,3/2,8)-linked sialic acid residues, respectively. Monosialylated *C. jejuni* strains are predominantly isolated from the stools of patients with GBS. In agreement with this observation, antibodies against monosialylated structures, including GM1a, GM1b, GD1a and GalNAc-GD1a, are frequently detected in the serum of GBS patients [13,14]. In contrast, *C. jejuni* strains with disialylated LOS that mimic GQ1b-like epitopes including GD1c and GD3 are closely associated with MFS patients, who often have cross-reactive antibodies directed against GQ1b [15,16]. Interestingly, the human oculomotor nerves, which innervate the eye muscles and are affected in MFS, have a relatively high content of GQ1b, which could explain their vulnerability to damage mediated by anti-GQ1b antibodies [15].

Antigen recognition is a determining initial step in the development of immune responses leading to GBS or MFS. Sialylation of *C. jejuni* LOS is an important determinant for the development of GBS and MFS [17]. Therefore, sialic acid-binding immunoglobulin-like lectins (Siglecs) expressed on immune-related cells may play a decisive role in immune recognition. Siglecs comprise a family of surface exposed receptors that are involved in sialic-acid-dependent cell-to-cell interactions and ligand binding [18]. Additionally, Siglecs function as endocytic receptors in immune recognition of both bacteria and viruses [19–21].

We recently demonstrated that GBS-related *C. jejuni* strains specifically bind to sialoadhesin (Siglec-1) [22]. Furthermore, other researchers have shown that *C. jejuni* strains expressing disialylated LOS structures can bind to Siglec-7 [23]; however, a limited number ($n = 4$) of strains were examined and no correlation was made with the clinical phenotype.

In this study, we determined the epitope specificity of *C. jejuni* for Siglec-7 binding. We examined a large and unique collection of *C. jejuni* strains ($n = 29$) derived from GBS and MFS patients, for which detailed information was available on the ganglioside mimicking structures expressed. In particular, we investigated the relationship between Siglec-7 binding and the presence of anti-ganglioside antibodies in patient serum and the specific clinical phenotypes. This study demonstrates that Siglec-7 specifically recognizes the *C. jejuni* strains associated with oculomotor weakness in GBS or MFS.

Materials and Methods

Bacterial strains and culture conditions

A group of 29 successive and well-characterized *C. jejuni* strains isolated from the stools of either GBS or MFS patients (see Supplementary material, Table S1), three previously described sialic acid transferase (*cst-II*) knockout mutants of GBS-associated strains (GB2Δ*cst-II*, GB11Δ*cst-II* and GB19Δ*cst-II*) [24,25] and the reference strain NCTC 11168 were used in this study [22,24,26]. Strains GB13, GB14, GB26 and GB27 were cultured from the diarrhoeal stools of the family members of two GBS patients after a family outbreak of *C. jejuni* enteritis [27] (Table S1). The GBS-related and MFS-related strains predominantly originate from Dutch patients. Two strains from the Netherlands Antilles and one Belgian strain were included. *C. jejuni* strains were cultured from stocks held at − 80°C and maintained on Colombia blood agar plates (Becton Dickinson BV, Alphen aan den Rijn, the Netherlands) supplemented with 10 mg/L vancomycin in a microaerobic atmosphere at 37°C. Chloramphenicol (20 mg/L) was added to the Δ*cst-II* mutant strain culture plates. For each experiment, all strains were freshly cultured for 2 days on blood agar plates containing only vancomycin. The LOS outer core structures of most GBS/MFS-associated strains used in this study have been described previously [10]. The LOS structures of *C. jejuni* GB29, GB30 and GB33 were determined using mass spectrometry analysis, as previously described [10]. Genotyping by PCR was performed to verify the LOS classes, as previously described [24].

Serology and diagnosis

Serum samples obtained within 2 weeks of the onset of weakness and before treatment, were tested for the presence of IgM and IgG antibodies to the ganglioside GQ1b using a validated ELISA with predefined cut-off values, as previously described [28]. The diagnosis of GBS or MFS was made by specialized neurologists, based on previously described criteria [29,30].

Preparation of Siglec-7-Fc-conjugate

Chinese hamster ovary (CHO) cells expressing the full extracellular region of human Siglec-7 fused to recombinant Fc protein (CHO-Siglec-7-Fc) were generated [31] and Siglec-7-Fc was produced as previously described [22]. Briefly, CHO-Siglec-7-Fc cells were cultured in glutamine-free Glasgow Minimal Essential Medium (Sigma-Aldrich, Zwijndrecht, the Netherlands) containing 100 μM L-methionine sulphoximine (Sigma-Aldrich), GS supplement (Sigma-Aldrich), penicillin/streptomycin and 10% dialysed fetal calf serum (Invitrogen, Leek, the Netherlands). Once the cells reached 80% confluency, the fetal calf serum concentration was adjusted to 2% and, eventually, the cells were cultured in X-VIVO-10 serum-free media (Lonza, Verviers, Belgium) and the medium was harvested weekly. The concentration of Siglec-7-Fc was determined using an Fc-specific ELISA, as previously described [22].

Siglec-7-Fc ELISA

Two-day *C. jejuni* cultures grown on blood agar plates were harvested, washed and the optical density at 600 nm (OD_{600}) was adjusted to 0.2 in phosphate-buffered saline (PBS) containing 2 mM $MgCl_2$ (PBS-Mg). After heat inactivation at 56°C for 45 min, 100 μL of each sample was plated in triplicate in 96-well Maxisorp ELISA plates (NUNC Inc., Uden, the Netherlands). The plates were kept open overnight at 37°C to allow the fluid to evaporate. After washing, the wells were blocked for 1 h using 1% bovine serum albumin in PBS at 37°C. Simultaneously, 1 mg/L Siglec-7-Fc conjugate was precomplexed with peroxidase-conjugated anti-human IgG (IgG-PO; Sigma-Aldrich) diluted 1/3000 in PBS containing 0.05% normal goat serum for 1 h at room temperature with shaking. After washing, 100 μL precomplexed Siglec-7-Fc was added per well, the plates were incubated for 2 h at room temperature, washed four times with PBS containing 0.05% Tween 20 and developed using 100 μL 3′,3′,5′,5′-tetramethylbenzidine substrate (Sigma-Aldrich) per well. After an appropriate incubation time (5–10 min), the reaction was stopped by adding 100 μL of 2 M H_2SO_4 per well and signal intensity was measured spectrophotometrically at 450 nm using a 96-well microplate reader (Bio-Rad, Veenendaal, the Netherlands). With respect to the Siglec-7 inhibition experiment, equal amounts (300 ng/well) purified bovine brain GQ1b (Sigma-Aldrich) were coated on an ELISA plate and blocked to avoid non-specific binding. In parallel, precomplexed Siglec-7-Fc was incubated for 1 h with twice the number of bacteria we normally use in our Siglec-7 ELISA to coat the wells. Siglec-7-bound- or free bacteria were removed by centrifugation. The supernatant (i.e. non-adsorbed Siglec-7) was transferred to the GQ1b-coated plate and binding of Siglec-7 was determined as described above.

Statistical analysis

Two-tailed *t* tests and Mann–Whitney *U* tests were performed using PRISM software (GraphPad, La Jolla, CA, USA) as indicated; $p \leq 0.05$ was considered statistically significant.

Results

Recognition of *C. jejuni* by Siglec-7 is sialic acid-specific

Although it has been shown that disialylated ganglioside-like structures expressed on the surface of *C. jejuni* can bind to Siglec-7 in a sialic acid-dependent manner, the possibility of low-affinity Siglec-7 binding to monosialylated structures or complexes could not be excluded [23]. Therefore, we aimed to determine the precise requirements of ganglioside-like structures for Siglec-7 binding.

Sialic acid-specific Siglec-7 binding was determined using three *C. jejuni* strains GB2, GB11 (both $GM1a^+$ $GD1a^+$) and GB19 ($GD1c^+$), and their sialic acid mutants GB2Δ*cst-II*,

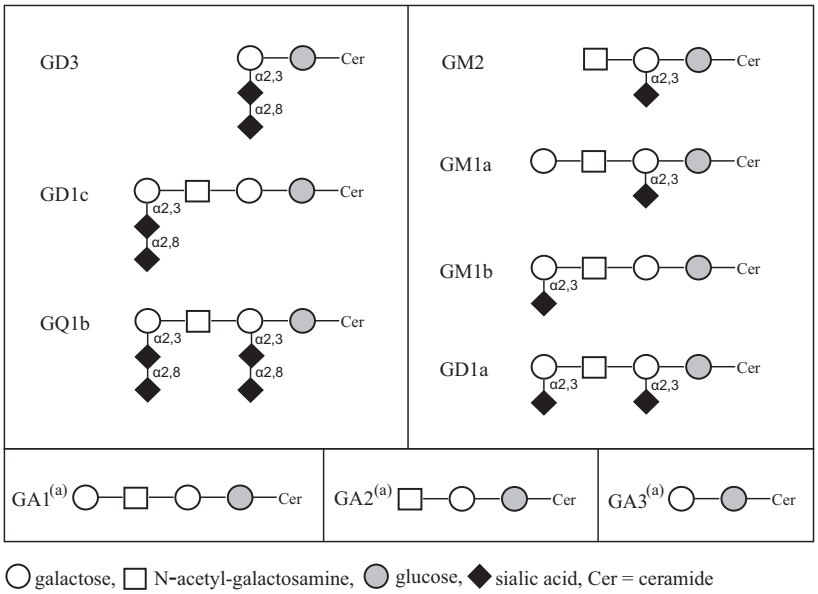
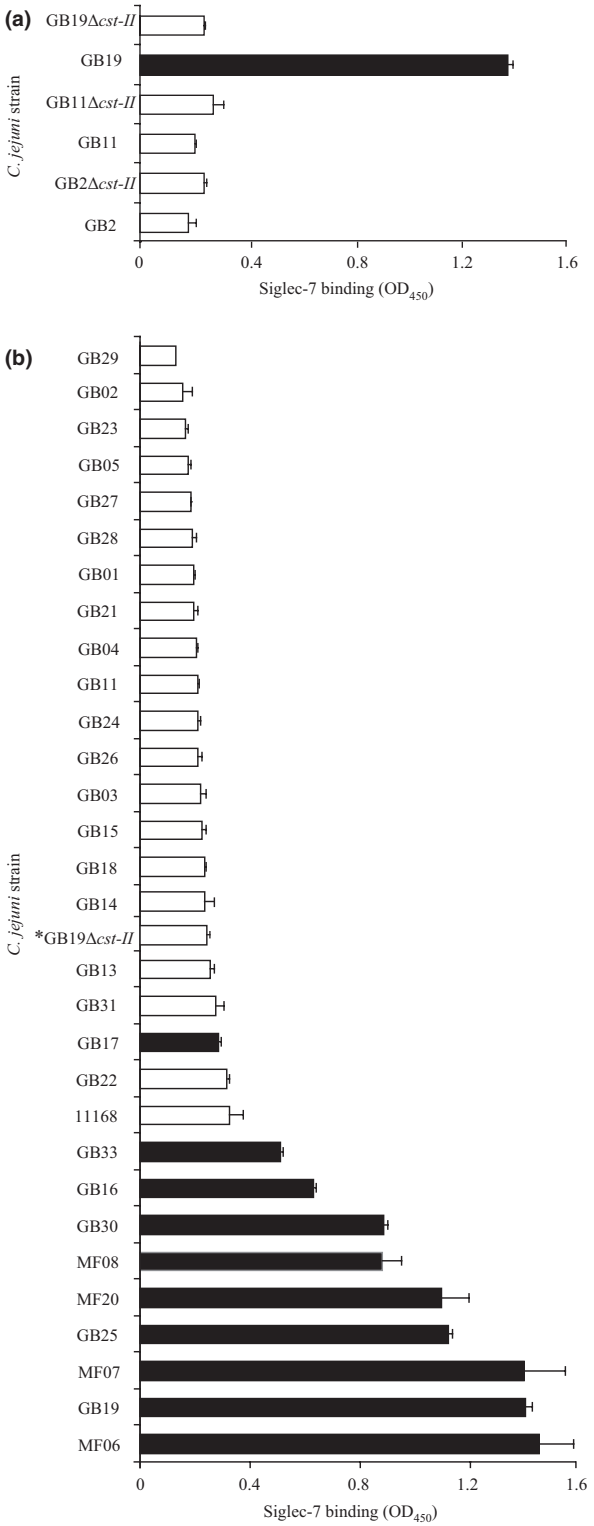


FIG. 1. Schematic illustration of the ganglioside structures discussed in this study. These structures can be mimicked by the *Campylobacter jejuni* outer core lipo-oligosaccharides (LOS). However, instead of the ceramide-bound glucose, the *C. jejuni* LOS has a heptose, followed by an inner sugar core, and *C. jejuni* LOS has a lipid A transmembrane tail instead of a ceramide tail. Disialylated structures with α(2,3/2,8)-linked sialic acid residues are represented in the left panel; monosialylated structures with α(2,3)-linked sialic acid residues are represented in the right panel. (a) GA1, GA2 and GA3 (or asialo GM1, -GM2 and -GM3) contain no sialic acids and are considered not to be gangliosides.

GB11Δ*cst-II* (both GA1$^+$ GA2$^+$ GA3$^+$) and GB19Δ*cst-II* (GA1$^+$; Fig. 1). Strain GB19 showed high Siglec-7 binding affinity in a whole cell Siglec-7-Fc ELISA (Fig. 2a). In agreement with reports that disialylated carbohydrate structures specifically bind to Siglec-7 [23,32], GB19 (GD1c$^+$) is disialylated at the terminal galactose of the oligosaccharide chain. GB19Δ*cst-II* demonstrated reduced Siglec-7 binding, indicating that the binding was sialic acid-specific. Comparable background levels of Siglec-7 binding were observed for the monosialylated strains GB2 and GB11, and their respective non-sialylated Δ*cst-II* mutants (Fig. 2a). To address whether Siglec-7 binds a similar epitope on ganglioside GQ1b, an inhibition ELISA was performed. Compared with GB19Δ*cst-II*, Siglec-7 binding to GQ1b was significantly (p 0.0005; *t* test) reduced when strain GB19 was used for adsorption, demonstrating that strain GB19 inhibits Siglec-7 binding to GQ1b (see Supplementary material, Fig. S1). GB19Δ*cst-II* showed a reduction in the signal when compared with the non-blocking situation. This reduction was similar to that observed with GB11 and GB11Δ*cst-II*, indicating that this effect was not dependent on sialic acid.

We concluded that Siglec-7 can bind to the disialylated GD1c-like structure present on *C. jejuni* LOS in a sialic acid-dependent manner; however, Siglec-7 cannot bind monosialylated GM1a-like and GD1a-like structures.



In a large collection of GBS/MFS-associated *C. jejuni* strains, only disialylated *C. jejuni* strains bind Siglec-7

To further study ganglioside mimic-specific Siglec-7 binding, 25 GBS-related and four MFS-related *C. jejuni* strains with known LOS structures, and the reference strain NCTC11168 were tested in the Siglec-7-Fc ELISA. A clear diversity in Siglec-7 binding was observed, with various strains showing high or low Siglec-7 binding capacity (Fig. 2b). In particular, strains MF06, GB19, MF07, GB25, MF20, MF08, GB30, GB16 and GB33 strongly bound Siglec-7. Strikingly, all of these strains have terminally disialylated LOS structures (Fig. 2b). The GD1c-like structure is disialylated in strains MF06, GB19, GB25, GB16 and GB33; whereas disialylation is present in the GD3-like structure of strains MF07 and MF08 (Fig. 1; Table S1).

FIG. 2. Evaluation of the binding of Siglec-7-Fc to *Campylobacter jejuni* strains using an ELISA. The strains were heat-inactivated, coated on ELISA plates, incubated with precomplexed Siglec-7 conjugate and visualized using 3′,3′,5′,5′-tetramethylbenzidine substrate. The bars represent a single experiment that was repeated at least three times, with means and standard deviations of triplicate measurements. Strains GB2Δ*cst-II*, GB11Δ*cst-II* and GB19Δ*cst-II* are the non-sialylated *Campylobacter* sialic acid transferase (*cst-II*) knockout mutants of the parental wild-type strains GB2, GB11 and GB19, respectively. White bars, non-/monosialylated lipo-oligosaccharides (LOS); black bars, disialylated LOS. (a) Siglec-7 binding to parental wild-type and sialic acid transferase knockout *C. jejuni* strains. (b) Siglec-7 binding to GBS- and MFS-associated *C. jejuni* strains. *Strain GB19Δ*cst-II* was included as a reference and it is considered to be a negative control for Siglec-7 binding.

The exact structures of the ganglioside mimics present on strain GB30 could not be determined because mass spectrometry analysis yielded a complex profile. However, mass spectrometry analysis confirmed the presence of monosialic and disialic acids in the LOS outer core of strain GB30. Based on these results, it is probable that the GB30 LOS outer core contains GD3-like structures (M. Gilbert, personal communication).

When the non-sialylated and monosialylated strains (n = 20) were compared with the disialylated strains (n = 10), we observed significantly higher Siglec-7 binding for the disialylated strains (p <0.0001; Mann–Whitney *U* test; Fig. 3a). Binding of the non-sialylated and monosialylated strains was low and comparable to the binding of strain GB19Δ*cst-II*, which lacks sialic acid. Strain GB17 did not strongly bind Siglec-7, despite the presence of disialylated ganglioside-like structures. It is possible that this strain contains additional structures that hinder Siglec-7 binding. Therefore, we concluded that only *C. jejuni* equipped with terminally disialylated ganglioside-like structures can bind to Siglec-7.

Siglec-7 binding correlates with the presence of anti-GQ1b antibodies in the serum of patients with GBS

We determined whether Siglec-7 binding correlated with the presence of anti-GQ1b antibodies in the serum of GBS patients. The strains isolated from patients with a high anti-GQ1b antibody titre demonstrated significantly higher Siglec-7 binding than the strains isolated from patients who did not have anti-GQ1b antibodies (p 0.0002; Mann–Whitney *U* test; Fig. 3b; Table S1). Seven of the nine strains that showed strong binding to Siglec-7 (78%) were isolated from GBS/MFS patients expressing anti-GQ1b antibodies; no patient serum was available for testing from the other two strains. Three strains (GB4, GB17 and GB22) that did not bind Siglec-7 were isolated from patients with anti-GQ1b antibodies. Strain GB4 expresses a class E LOS and therefore does not carry the genes necessary for sialylation, which is an essential determinant for ganglioside mimicry. We hypothesize that this strain (GB4) was not involved in triggering the patient's immune system and the subsequent development of GBS. Strain GB17 ($GM1b^+$ $GD1c^+$ $GA1^+$) contains disialylated LOS but did not bind to Siglec-7; however, the patient had (low) anti-GQ1b antibodies. Strain GB22 ($GD1a^+$ $GM1a^+$) does not express disialylated LOS; therefore, it probably does not bind to Siglec-7. In addition to anti-GQ1b antibodies, the patient from whom strain GB22 was isolated also had antibodies against GM1a (data not shown), suggesting that strain GB22 may contribute to the induction of anti-GM1a antibodies but perhaps not anti-GQ1b antibodies.

Campylobacter jejuni Siglec-7 binding is associated with oculomotor weakness and MFS

As disialylated *C. jejuni* strains and anti-GQ1b antibodies are associated with oculomotor weakness [15,33], we determined whether Siglec-7 binding also correlated with oculomotor weakness. Strikingly, all of the patients with oculomotor weakness (7/7; 100%) were infected with *C. jejuni* strains that showed a high binding affinity for Siglec-7 (p 0.0002; Mann–Whitney *U* test; Fig. 3c; Table S1). Three of these strains were isolated from GBS patients and four were isolated from MFS patients. All of the patients with MFS had been infected with strains that had a high Siglec-7 binding affinity (4/4; 100%). Two other strains that bound Siglec-7, GB30 and GB33, were isolated from GBS patients for whom no information on oculomotor weakness was available.

Discussion

In the present study, we report that sialylated structures on the surface of *C. jejuni* can bind to Siglec-7, a receptor of the siglec family that is expressed on immune cells including dendritic cells. We demonstrated that the binding of *C. jejuni* to Siglec-7 is sialic acid-dependent, as a sialic acid transferase knockout strain

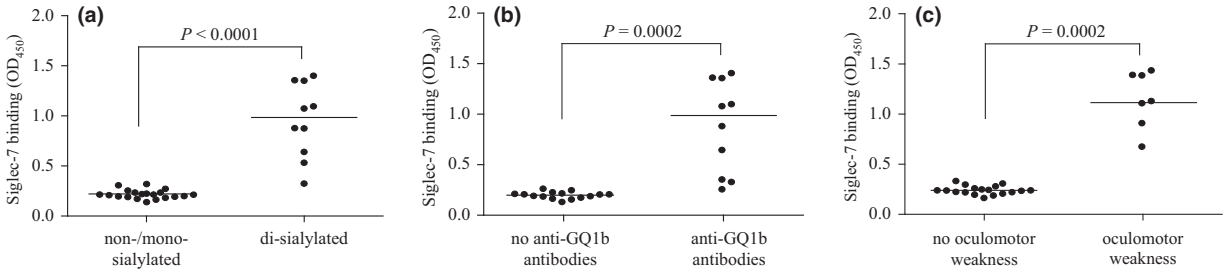


FIG. 3. Relationship between Siglec-7 binding by *Campylobacter jejuni* and (a) lipo-oligosaccharide (LOS) sialylation; (b) the presence of anti-GQ1b antibodies in Guillain–Barré syndrome (GBS) and Miller Fisher syndrome (MFS) patient serum and (c) oculomotor weakness in GBS and MFS patients. Siglec-7 binding was measured using an ELISA. Four individual bacterial strains were cultured from multiple individuals within two separate families (see Supplementary material, Table S1); data from only one strain isolated from each family are included in (b) and (c). The median values are indicated by the horizontal line; p values <0.05 were considered statistically significant (Mann–Whitney *U* test).

could not bind Siglec-7 whereas the parental wild-type strain could. Siglec-7 has a preference for binding disialylated sialic acid conjugates, such as those present in the ganglioside GQ1b [32]. Indeed, only strains expressing disialylated ganglioside-like structures could bind Siglec-7; specifically, the GD1c-like or GD3-like disialylated ganglioside-mimics present in the *C. jejuni* strain collection used in this study. Similar to GQ1b, both GD1c and GD3 are disialylated at the terminal galactose of the carbohydrate chain. Infection with GD1c-positive or GD3-positive *C. jejuni* strains has been previously associated with the presence of cross-reactive anti-GQ1b antibodies in the serum of GBS or MFS patients [16,33,34]. Upon screening a large panel of GBS-related and MFS-related *C. jejuni* strains, we observed an association between Siglec-7 binding and the presence of anti-GQ1b antibodies in patient serum. Furthermore, we found that Siglec-7 selectively recognized the *C. jejuni* strains that were isolated from GBS or MFS patients diagnosed with oculomotor weakness, strongly suggesting that the specific binding of *C. jejuni* to Siglec-7 is a marker for GBS and MFS with oculomotor weakness.

Our findings are in concordance with previous studies that reported that Siglec-7 can interact with terminally disialylated ganglioside structures, including GD3, GT1b and GQ1b [32,35]. An interaction of Siglec-7 with *C. jejuni* strains expressing disialylated LOS structures was also previously demonstrated using ELISA and CHO-cell adhesion assays [23]; however, a limited number of strains were tested and the correlation with clinical phenotypes was not examined.

It has been suggested that the presence of other ganglioside mimics influences the interaction with Siglec-7 [23]. Polyvinylidene difluoride glycoarray-based experiments revealed that a 1:1 complex of either GM1, GM2, GD1a, GD1b or GT1a with GD3 attenuated Siglec-7 binding [35]. Therefore, the binding of Siglec-7 to the GD3-like structure of the strains MF07 ($GM2^+$ $GD2^+$ $GD3^+$) and MF08 ($GM2^+$ $GD3^+$) used in this study could potentially be affected by the presence of GM2. As Siglec-7 binding was clearly observed for these strains, complex attenuation is apparently not a major issue. However, complex attenuation may explain why strain GB17 ($GM1b^+$ $GD1c^+$ $GA1^+$) did not bind to Siglec-7. It should be noted that in serum of patient GB17, complex reactivity against GM1/GD1a, GD1a/GD1b and GD1a/GQ1b was observed [26]. This suggests that the ganglioside-like epitopes on GB17 LOS form complexes. The formation of these complexes might prevent Siglec-7 binding. It is also possible that the GD1c-like structure was expressed in low levels on the surface of GB17 under the current culture conditions, resulting in low Siglec-7 binding.

The consequence of pathogen interactions with Siglec-7 is largely unknown. Siglec-7 is a member of the CD33-related Siglecs, which contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tail. Pathogen interactions with Siglec-7 could therefore exert an inhibitory effect on immune activation, as ITIM signalling has been shown to restrain Siglec internalization via ITIM phosphorylation [36]. However, Siglec-related pathogen uptake has also been reported [19]. It is possible that *cis* interaction of Siglec-7 with self-ligands results in an inhibitory response; whereas pathogen interactions with Siglec-7 overrule this signal, possibly through activation of co-receptors and cytokine secretion, or a higher receptor affinity [37]. Evidence for a Siglec-7-activated immune response was recently demonstrated, as Siglec-7 interactions resulted in the skewing of dendritic cells towards T helper type 1 polarization, due to LOS-mediated OX40 ligand induction [38]. However, the mechanisms by which this process could eventually lead to an anti-ganglioside antibody response and result in GBS or MFS with oculomotor weakness remain to be elucidated.

In conclusion, we demonstrate that oculomotor weakness in GBS and MFS is associated with *C. jejuni* strains that bind Siglec-7. Binding of *C. jejuni* to Siglec-7 may be an event that mediates anti-GQ1b antibody activation, leading to oculomotor weakness in patients with GBS or MFS. Identification of *C. jejuni* on the basis of Siglec-7 binding could be of diagnostic value for the detection of strains with the potential to induce neurological symptoms. In cases of *C. jejuni* infection where a Siglec-7 binding strain is cultured from faecal samples, antibiotic treatment could be prescribed to prevent postinfections neurological complications. Additional studies are necessary to identify the occurrence of Siglec-7 binding strains in uncomplicated enteritis.

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Transparency Declaration

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. *Campylobacter jejuni*-mediated inhibition of Siglec-7 binding to ganglioside GQ1b.

Table S1. Guillain–Barré syndrome- and Miller Fisher syndrome-associated *Campylobacter jejuni* strains used in this study.

References

1. Hahn AF. Guillain–Barré syndrome. *Lancet* 1998; 352: 635–641.
2. Hughes RA, Cornblath DR. Guillain–Barré syndrome. *Lancet* 2005; 366: 1653–1666.
3. Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A, Rodrigues LC. Guillain–Barré syndrome and preceding infection with *Campylobacter*, influenza and Epstein–Barr virus in the general practice research database. *PLoS ONE* 2007; 2: e344.
4. van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain–Barré syndrome. *Lancet Neurol* 2008; 7: 939–950.
5. Fisher M. An unusual variant of acute idiopathic polyneuritis: syndrome of ophthalmoplegia, ataxia and areflexia. *N Engl J Med* 1956; 255: 57–65.
6. Funakoshi K, Kuwabara S, Odaka M, Hirata K, Yuki N. Clinical predictors of mechanical ventilation in Fisher/Guillain–Barré overlap syndrome. *J Neurol Neurosurg Psychiatry* 2009; 80: 60–64.
7. Rajabally YA, Hassan-Smith G, Notturno F *et al.* Motor and sensory conduction failure in overlap of Guillain–Barré and Miller Fisher syndrome: two simultaneous cases. *J Neurol Sci* 2011; 303: 35–38.
8. Jacobs BC, Rothbarth PH, van der Meché FGA *et al.* The spectrum of antecedent infections in Guillain–Barré syndrome: a case–control study. *Neurology* 1998; 51: 1110–1115.
9. Koga M, Gilbert M, Li J *et al.* Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. *Neurology* 2005; 64: 1605–1611.
10. Godschalk PC, Kuijf ML, Li J *et al.* Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain–Barré and Miller Fisher syndromes. *Infect Immun* 2007; 75: 1245–1254.
11. Hafer-Macko CE, Sheikh KA, Li CY *et al.* Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann Neurol* 1996; 39: 625–635.
12. Yuki N, Susuki K, Koga M *et al.* Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain–Barré syndrome. *Proc Natl Acad Sci USA* 2004; 101: 11404–11409.
13. Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. *Brain* 2002; 125: 2591–2625.
14. Hiraga A, Kuwabara S, Ogawara K *et al.* Patterns and serial changes in electrodiagnostic abnormalities of axonal Guillain–Barré syndrome. *Neurology* 2005; 64: 856–860.
15. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain–Barré syndrome: clinical and immunohistochemical studies. *Neurology* 1993; 43: 1911–1917.
16. Ang CW, Laman JD, Willison HJ *et al.* Structure of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity and clinical features of Guillain–Barré and Miller Fisher patients. *Infect Immun* 2002; 70: 1202–1208.
17. van Belkum A, van den Braak N, Godschalk P *et al.* A *Campylobacter jejuni* gene associated with immune-mediated neuropathy. *Nat Med* 2001; 7: 752–753.
18. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol* 2007; 7: 255–266.
19. Jones C, Virji M, Crocker PR. Recognition of sialylated meningococcal lipopolysaccharide by Siglecs expressed on myeloid cells leads to enhanced bacterial uptake. *Mol Microbiol* 2003; 49: 1213–1225.
20. Rempel H, Calosing C, Sun B, Pulliam L. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS ONE* 2008; 3: e1967.
21. Vanderheijden N, Delputte PL, Favoreel HW *et al.* Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages. *J Virol* 2003; 77: 8207–8215.
22. Heikema AP, Bergman MP, Richards H *et al.* Characterization of the specific interaction between sialoadhesin and sialylated *Campylobacter jejuni* lipooligosaccharides. *Infect Immun* 2010; 78: 3237–3246.
23. Avril T, Wagner ER, Willison HJ, Crocker PR. Sialic acid-binding immunoglobulin-like lectin 7 mediates selective recognition of sialylated glycans expressed on *Campylobacter jejuni* lipooligosaccharides. *Infect Immun* 2006; 74: 4133–4141.
24. Godschalk PC, Heikema AP, Gilbert M *et al.* The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain–Barré syndrome. *J Clin Invest* 2004; 114: 1659–1665.
25. Louwen R, Heikema A, van Belkum A *et al.* The sialylated lipooligosaccharide outer core in *Campylobacter jejuni* is an important determinant for epithelial cell invasion. *Infect Immun* 2008; 76: 4431–4438.
26. Kuijf ML, Godschalk PC, Gilbert M *et al.* Origin of ganglioside complex antibodies in Guillain–Barré syndrome. *J Neuroimmunol* 2007; 188: 69–73.
27. Ang CW, van Doorn PA, Endtz HP *et al.* A case of Guillain–Barré syndrome following a family outbreak of *Campylobacter jejuni* enteritis. *J Neuroimmunol* 2000; 111: 229–233.
28. Kuijf ML, van Doorn PA, Tio-Gillen AP *et al.* Diagnostic value of anti-GM1 ganglioside serology and validation of the INCAT-ELISA. *J Neurol Sci* 2005; 239: 37–44.
29. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain–Barré syndrome. *Ann Neurol* 1990; 27: S21–S24.
30. Miller Fisher C. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). *N Engl J Med* 1956; 225: 57–65.
31. Vinson M, van der Merwe PA, Kelm S, May A, Jones EY, Crocker PR. Characterization of the sialic acid-binding site in sialoadhesin by site-directed mutagenesis. *J Biol Chem* 1996; 271: 9267–9272.
32. Rapoport E, Mikhalyov I, Zhang J, Crocker P, Bovin N. Ganglioside binding pattern of CD33-related Siglecs. *Bioorg Med Chem Lett* 2003; 13: 675–678.
33. Yuki N. *Campylobacter* sialyltransferase gene polymorphism directs clinical features of Guillain–Barré syndrome. *J Neurochem* 2007; 103 (suppl 1): 150–158.
34. Yuki N. Infectious origins of, and molecular mimicry in, Guillain–Barré and Fisher syndromes. *Lancet Infect Dis* 2001; 1: 29–37.
35. Rinaldi S, Brennan KM, Goodyear CS *et al.* Analysis of lectin binding to glycolipid complexes using combinatorial glycoarrays. *Glycobiology* 2009; 19: 789–796.
36. Walter RB, Raden BW, Zeng R, Hausermann P, Bernstein ID, Cooper JA. ITIM-dependent endocytosis of CD33-related Siglecs: role of intracellular domain, tyrosine phosphorylation, and the tyrosine phosphatases, SHP1 and SHP2. *J Leukoc Biol* 2008; 83: 200–211.
37. Paulson JC, Macauley MS, Kawasaki N. Siglecs as sensors of self in innate and adaptive immune responses. *Ann N Y Acad Sci* 2012; 1253: 37–48.
38. Bax M, Kuijf ML, Heikema AP *et al. Campylobacter jejuni* lipooligosaccharides modulate dendritic cell-mediated T cell polarization in a sialic acid linkage-dependent manner. *Infect Immun* 2011; 79: 2681–2689.