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Short communication

Campylobacter coli enteritis and Guillain–Barré syndrome: No evidence of molecular mimicry and serological relationship

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Abstract

Campylobacter coli was isolated from two Guillain–Barré syndrome (GBS) patients who had anti-GM1 and anti-GD1 IgG antibodies. Although both this bacteria and *Campylobacter jejuni* are common causes of diarrheal illness, previous studies have focused only on *C. jejuni* as the causal agent of GBS. To determine whether *C. coli* also is a causative agent, we examined the hypothesis that production of anti-ganglioside antibodies is induced by ganglioside-mimics on that bacterial lipo-oligosaccharide (LOS), as in *C. jejuni*-associated GBS. LOSs of both *C. coli* isolates had very weak reactivities with anti-GM1 and anti-GD1a IgG monoclonal antibodies, whereas those of some GBS-related *C. jejuni* isolates had strong reactivities. Anti-GM1 and anti-GD1a IgG antibodies from the two patients were not absorbed as much by the LOSs of their isolates as were those of GBS-related *C. jejuni* strains. These findings do not support the hypothesis of ganglioside mimicry on *C. coli* isolates' LOSs. We next made a serological assay of recent *C. coli* infection in 74 patients with GBS, 26 with Fisher syndrome (FS), 49 with other neurological diseases (OND), and 37 normal controls (NC) using the bacterial outer membrane protein as antigen. Eight (11%) GBS and two (8%) FS patients had two or three classes of IgG, IgM, and IgA anti-*C. coli* antibodies. Anti-*C. jejuni* IgG and IgA antibody titers were significantly higher than those of anti-*C. coli* (respectively, $p = 0.03$ and 0.01). This suggests that anti-*C. coli* antibodies cross-react with *C. jejuni* protein. We concluded that a *C. coli* infection was not the cause of GBS in our patients. Both isolation of a microorganism from, and the positive infectious serology of, GBS patients do not always indicate the causal agent.

Keywords: Guillain–Barré syndrome; *Campylobacter coli*; Molecular mimicry; Ganglioside

1. Introduction

Guillain–Barré syndrome (GBS) is an immune-mediated peripheral neuropathy, characterized by acute onset of limb weakness and loss of tendon reflexes, that develops 1–2 weeks after various infections [1]. *Campylobacter jejuni*, a leading cause of acute diarrhea, is the most common antecedent infectious pathogen [2], and the pathogenesis of *C. jejuni*-related GBS has been extensively investigated. Most patients who develop GBS after *C. jejuni* infection have IgG autoantibodies against gangliosides, in particular GM1 and GD1a, during the acute phase of illness [3]. A *C. jejuni* isolate (CF90-26) from a GBS patient who had anti-GM1 antibody carried a GM1-like structure on its lipo-oligosaccharide (LOS) [4]. Moreover, a disease model of GBS associated with anti-GM1 antibody developed in rabbits inoculated with the GM1-like *C. jejuni* LOS [5]. These findings provide conclusive evidence that anti-GM1 IgG antibody production is mediated by the GM1-mimicry of *C. jejuni* LOS.

Other *Campylobacter* species, *Campylobacter upsaliensis* and *Campylobacter curvus*, have been isolated from GBS patients [6–8]. Our serological analysis, however, found no evidence that these campylobacters are causal agents of GBS or of Fisher syndrome (FS) [7], characterized

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by acute onset of ophthalmoplegia, ataxia, and areflexia. In routine testing of stool specimens of GBS patients for the presence of *Campylobacter* species, we isolated two *Campylobacter coli* strains [9]. *C. coli* is closely related to *C. jejuni* in its bacterial and clinical features, and *C. coli* enterocolitis which clinically is indistinguishable from that of *C. jejuni*, accounts for 15–20% of campylobacter-associated diarrhea cases in Israel [10] and for 7.9% in the U.K. [11]. Unlike *C. jejuni*, little is known about post-infection diseases associated with *C. coli*.

Bersudsky et al. [10] reported a GBS patient, from whom *C. coli* had been isolated, who had anti-asialo-GM1 (GA1) IgG antibody. They showed that immunization with the LOS of the *C. coli* isolate obtained from the patient induced anti-GA1 antibody production in the rat, but failed to show the presence of a ganglioside mimic on the LOS. Our two patients with *C. coli*-related GBS, who had high anti-ganglioside antibody titers, led us to investigate there is ganglioside mimicry on the *C. coli* LOS. We then examined serologically recent *C. coli* infection in GBS and FS patient populations.

2. Case report

2.1. Patient 1

An 8-year-old girl had diarrhea with fever for 2 days. Ten days later she experienced increasing muscle weakness in all the limbs (day 1). Neurological examination on admittance showed that consciousness was alert and cranial nerves were intact. Upper and lower limb muscle strength was 3–4 on the Medical Research Council (MRC) scale. Deep tendon reflexes were decreased in all the limbs. Plantar responses were indifferent. There was no ataxia, and sensory disturbance. Cerebrospinal fluid showed 45 mg/dl protein with normal cellularity on day 7. Nerve conduction study results on day 7 were indicative of primary axonal neuropathy. *C. coli* (GC210) was isolated from a stool specimen on day 7. From day 8, she was given 0.4 g/kg human immunoglobulin intravenously (Venilon™, Teijin, Tokyo, Japan) daily for 5 days. Her muscle strength improved gradually from day 14, and she could walk without support on day 23.

2.2. Patient 2

A 25-year-old woman developed hand weakness (day 1). Nine days before, she had diarrhea for 3 days. Leg weakness appeared on day 2, and the next day she could walk only 20 m unaided. On examination (day 3), no cranial nerve abnormality was found except for the accessory nerves; muscle strength of bilateral sternocleidomastoid was 4 on the MRC scale. Muscle strength was mildly decreased (MRC 4), and deep tendon reflexes were decreased, in all the limbs. Plantar responses were indifferent. There was no ataxia and sensory disturbance. The result of motor nerve conduction studies on day 3 was indicative of primary axonal neuropathy. *C. coli* (GC146) was isolated from a stool specimen on day 3. From day 13, she was treated with the same regimen as Patient 1. Her strength gradually improved from day 17, and she could walk without support on day 22.

3. Materials and methods

3.1. Enzyme-linked immunosorbent assay for anti-glycolipid antibodies

Serum samples were taken from Patients 1 (day 7) and 2 (day 3). IgG and IgM antibodies to GA1, GM1, GM1b, GM2, GD1a, GalNAc-GD1a, GD1b, GD2, GD3, GT1a, GT1b, and GQ1b were measured in an enzyme-linked immunosorbent assay (ELISA), as described elsewhere [12]. Absorbance values at 492 nm were calculated by subtracting the optical densities (ODs) obtained for wells without antigen. Antibody titer was defined as the highest serum dilution at which the OD at 492 nm was 0.1 or more. Serum was considered positive if the titer was 500 or more.

3.2. Preparation of the bacterial LOS

Crude LOS fractions were prepared according to Hitchcock and Brown [13] with minor modifications [14]. *C. coli* strains (GC146 and GC210) isolated from Patients 1 and 2, strains (GC105, GC106, GC107, GC108, and GC109) from patients with uncomplicated enteritis, and *C. jejuni* (CF90-26, GC023, and GC207) from GBS patients with anti-GM1 and anti-GD1a IgG antibodies were used. An *Escherichia coli* isolate (DB1) from an enteritis patient also was used as the control of different species.

3.3. Detection of ganglioside-like LOS

We first examined whether bacterial LOSs are bound by monoclonal anti-ganglioside antibodies (mAb; GB2 [anti-GM1] and GB1 [anti-GD1a]) [5,14]. 1 μl portions of a bacterial LOS lysate in 100 μl of methanol were placed in individual wells of microtiter plates and evaporated. Monoclonal anti-ganglioside antibodies diluted 1 : 50 were added, and the whole incubated at 4 °C overnight. After the plates were washed, peroxidase-conjugated goat anti-mouse γ-chain-specific serum (Dako, Glostrup, Denmark; diluted 1 : 1000) was added. All the plates were incubated at room temperature for 1 h, washed, and developed.

Next we made an absorption study to determine whether the anti-ganglioside antibodies of Patients 1 and 2 cross-react with the LOS on their isolates. 100 μl portions of diluted serum, which gave an OD between 1.0 and 2.0 for the anti-GD1a IgG of Patient 1 and the anti-GM1 IgG of Patient 2, were incubated in LOS-precoated microtiter plate wells at 4 °C for 16 h. The supernatants were the primary