

Ganglioside reactive antibodies in the neuropathy associated with celiac disease

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Abstract

We tested patients with celiac disease (CD) for the presence of serum anti-ganglioside antibodies. Six of twenty-seven patient sera were reactive against brain gangliosides by an agglutination immunoassay. Neurological examination in all six revealed the presence of distal sensory loss, consistent with the diagnosis of peripheral neuropathy. When tested by ELISA for antibodies to isolated GM1, GM2, GD1a, GD1b, GT1b, and GQ1b gangliosides, all six were positive for IgG antibodies to at least one. The neuropathy of celiac disease may be autoimmune and associated with anti-ganglioside antibodies. The presence of IgG reactivity furthermore implicates a T cell-mediated response to ganglioside antigens.

1. Introduction

Celiac disease (CD) is a chronic inflammatory enteropathy, mediated by T-cell reactivity to ingested gluten proteins, resulting in villous atrophy of the small intestine (Farrell and Kelly, 2002). The disease is familial, and strongly linked to the expression of HLA-DQ2 and/or DQ8 molecules that are implicated in the presentation of gliadin to sensitized T cells infiltrating the lamina propria (Howell et al., 1986; Sollid et al., 1989).

Celiac disease is associated with several autoimmune phenomena, including autoantibodies to tissue transglutaminase (tTG) (Dieterich et al., 1998), and increased incidence of type I

diabetes, Sjogren syndrome, and autoimmune thyroid disease (Ventura et al., 1999; Kumar et al., 2001; Larizza et al., 2001). The mechanism of autoimmunity is unclear, but in the case of tTG, the antibodies are thought to be induced by gliadin-tTG complexes, with activation of tTG-specific B cells by T-cells that recognize gliadin in the complex (Sollid et al., 1997).

Approximately 10% of celiac patients exhibit evidence for neurological disease (Willis and Hovell, 1996), in particular peripheral neuropathy and cerebellar ataxia, but also ophthalmoplegia, epilepsy, and dementia (Cooke and Smith, 1966; Kaplan et al., 1988; Finelli et al., 1980; Chapman et al., 1978; Collin et al., 1991). These were assumed to be due to malabsorption, although nutritional deficiencies were rarely demonstrated, and no improvement of the neurological diseases was observed following vitamin therapy (Muller et al., 1996). Overall, very little research has been done into the mechanisms of the neurological complications of celiac disease.

As celiac disease is known to be linked to autoimmunity, we tested the patients for the presence of anti-ganglioside antibodies, which are associated with autoimmune neuropathies (O'Leary and Willison, 2000; Press et al., 2001). Sera were screened using a newly developed ganglioside agglutination immunoassay that detects antibodies to brain gangliosides, and the reactive sera were then tested by ELISA for binding to purified gangliosides. Patients with demonstrable serum ganglioside reactivity were evaluated neurologically for the presence of neuropathy or other neurological disease.

2. Materials and methods

2.1. Sera and diagnostic criteria

Serum samples were obtained from 27 unselected patients with celiac disease followed by the GI service. Diagnosis of celiac disease was based on the criteria of The European Society of Pediatric Gastroenterology and Nutrition (ESPGAN): characteristic histological features of villous atrophy, crypt hyperplasia, and inflammation in small intestinal biopsies, together with clinical and/or histological improvement on a gluten free diet. Sera from 6 patients with ALS, 20 patients with MS, and 40 normal subjects were tested as negative controls. Sera from Guillain Barre' syndrome patients with anti-ganglioside antibodies were used as positive controls (Alaedini et al., 2002). Patients whose

sera were found to have increased anti-ganglioside antibodies underwent neurological evaluation.

2.2. Ganglioside antibodies

2.2.1. Ganglioside agglutination assay

Polystyrene beads were coated with a bovine ganglioside extract as described previously (Alaedini et al., 2001), with minor modification. A 3 mg/ml solution of gangliosides was prepared by combining 750 µg of a total ganglioside extract (calcium salt) (Sigma, St. Louis, MO) dissolved in 105 µl of H₂O with 20 µl of methanol and 125 µl of 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.1) in a 1.7-ml conical tube (Corning Life Sciences, Corning, NY). Adsorption of gangliosides to latex beads was initiated by addition of 250 µl of a 1% suspension of 0.3 µm blue polystyrene particles (Seradyn Particle Technology, Indianapolis, IN) to the ganglioside solution, followed by gentle stirring for 4.5 h at room temperature. The suspension was then incubated for 72 h at 4 °C. The particles were washed twice with a solution of 1% bovine serum albumin (BSA) in 25 mM MES buffer (pH 6.1) by centrifugation at 9800 × *g* and 4 °C, and resuspended in the same solution. The microparticles were incubated for 48 h at 4°C before use.

The agglutination test was carried out on a 3-ring glass slide (Cell-Line, Newfield, NJ). On each ring, 5-µl aliquots of coated microparticles were added to 5µl of serum and mixed with a plastic applicator. The slide was rocked gently for 15 s. Positive agglutination, characterized by blue clumps of beads, indicated the presence of anti-ganglioside antibodies. Results were confirmed using a light microscope (×40 magnification) and scored from 1 to 3 according to the degree of agglutination, where 1 denoted weak agglutination and 3 strong agglutination. In the absence of agglutination, the reaction was considered to be negative.

2.2.2. Enzyme-linked immunosorbent assay (ELISA)

Sera were also tested by ELISA for presence of IgG, IgA, and IgM antibodies to GM1, GM2, GD1a, GD1b, GT1b, and GQ1b gangliosides. Wells in 96-well round-bottom polystyrene microtiter plates (Corning Life Sciences) were coated with 0.5 µg of the individual gangliosides (Sigma) in 100 µl of methanol. No ganglioside was added to control wells. After evaporation of the methanol, all wells were blocked by incubation with 300 µl of 1% BSA in 10 mM phosphate-buffered saline (154 mM NaCl, pH 7.4) (PBS) for 4 h at 4 °C. Wells were filled with 100 µL of BSA/PBS-diluted sera in duplicate, incubated overnight at 4 °C, and then washed with the BSA/PBS solution. To each well 100 µl of peroxidase-conjugated goat anti-human IgG, IgA, or IgM secondary antibody (ICN Biomedicals, Costa Mesa, CA) was added after the proper dilution, and the plates were incubated for 2 h at 4 °C. The wells were then washed as before, followed by the addition of 100 µl of developing solution comprised of 27 mM citric acid, 50 mM Na₂ HPO₄, 5.5 mM *o*-phenylenediamine, and 0.01% H₂O₂ (pH 5–5.5). The plates were incubated at room temperature for 30 min, before measuring the absorbance at 450 nm. The titer for each specimen was assigned as the highest dilution

in which the absorbance reading was 0.1 unit greater than in the corresponding control well. Sera with IgG and IgA titers of 100 and above were considered to be positive for presence of antibodies. Similarly, sera with IgM titers of 1600 and higher were considered positive.

2.3. Gliadin and tTG antibodies

Measurement of gliadin and tTG antibody levels in patient sera was performed by ELISA as previously described (Baldas et al., 2000).

3. Results

Of the 27 celiac patients, 6 (22%) were positive for anti-ganglioside antibodies by the agglutination assay. When tested by ELISA for IgG, IgM, and IgA antibodies to GM1, GM2, GD1a, GD1b, GT1b, or GQ1b gangliosides, the same six positive sera exhibited elevated IgG antibody levels to one or more of the gangliosides (Table 1).

Three of the six celiac patients with ganglioside antibodies had been on gluten-free diet for more than 6 months at the time of ganglioside antibody testing, and were found to be negative for gliadin and tTG antibodies. Of the rest, two patients were positive for IgA antibodies to gliadin and tTG, while one, with IgA deficiency, was only positive for IgG antibodies to gliadin (Table 1).

On neurological evaluation, all six patients with anti-ganglioside antibodies had numbness or paresthesias, with distal sensory loss in hands and feet. Three of the six suffered from neuropathic pain. EMG and nerve conduction studies showed mild abnormalities in two. In one of the patients (#18), sural nerve biopsy revealed changes of chronic axonopathy. None of the patients was found to have a nutritional deficiency or other known causes of neuropathy.

4. Discussion

Approximately 20% of the sera from patients with celiac disease had increased anti-ganglioside antibodies, all of whom were found to have a distal sensory neuropathy. It is not known if any of the other 22 celiac patients also had neuropathy or other neurological disease, as they were not examined. The incidence of neuropathy in celiac disease is unknown, and the diagnosis may be missed if the patients are not neurologically evaluated. Routine nerve conduction studies may be normal or only mildly abnormal in less severely affected cases (Oh et al., 2001), even with abnormal nerve biopsy studies, as was the case in one of our patients.

The presence of increased anti-ganglioside antibodies suggests that immune mechanisms may have a role in the development of neuropathy in patients with celiac disease.

However, although none of the patients was found to have

Table 1.

Clinical and laboratory characteristics of patients with celiac disease and antibodies to gangliosides.

Patient no.	Age	Sex	Gliadin antibodies (IgA/IgG)	tTG antibodies (IgA)	Time on gluten-free diet	Neurological evaluation		Ganglioside antibodies	
						Distal sensory loss	EMG	Ganglioside agglutination	Elisa (IgG titer) ^b
11	42	F	-/-	-	2 years	mild	normal	1	GM1 (400), GM2 (400), GD1b (100)
13	70	F	-/-	-	2 years	moderate	mild sensory motor axonal neuropathy	1	GM1 (100), GD1a (200)
16	53	F	+/+	+	7 months	moderate	normal	1-2	GM1 (400)
18 ^c	68	F	-/-	-	11 years	moderate	normal	1-2	GD1a (200)
19 ^d	44	M	-/+	-	0 month	moderate	mild right tibial mononeuropathy	1-2	GM2 (200)
21	78	F	+/-	+	0 month	severe	normal	1	GM1 (200), GM2 (100)

^a Results were scored from 1 to 3 according to the degree of agglutiation.^b Titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 unit greater than in the corresponding control wells.^c Nerve biopsy indicated a moderately severe axonopathy, with moderate loss of myelinated fibers.^d Patient was found to have an IgA deficiency.

nutritional deficiencies at the time of evaluation, it is impossible to determine whether such deficiencies could have been present in the past.

The cause for increased levels of ganglioside antibodies in celiac disease is not known, but the presence of IgG anti-ganglioside antibodies is suggestive of a T-cell mediated response (Ogino et al., 1995). Such antibodies might arise by: (1) possible immune reactivity to gliadin species that may have cross-reactive oligosaccharides, or that are com-plexed to gangliosides; (2) predisposition to co-infection with bacteria such as *Campylobacter jejuni* or *Haemophilus influenzae* that bear cross-reactive lipopolysaccharides as has been demonstrated in Guillain-Barre' syndrome (Yuki et al., 1993; Prendergast et al., 1998; Mori et al., 1999); or (3) predisposition to development of an anti-ganglioside immune response that is independent of the response to gliadin.

It is not known whether elimination of dietary gluten would result in reduction of the anti-ganglioside antibodies or amelioration of the neuropathy. Although no direct correlation was found between gluten ingestion and anti-ganglioside antibodies in this study, the possibility could not be ruled out, as strict adherence to a gluten-free diet as well as the monitoring of such adherence are difficult to achieve.

Further studies are needed to elucidate the incidence of neuropathy in celiac disease, and the relationship between gluten ingestion, anti-ganglioside antibodies, and neuropathy.

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