Amyotrophic Lateral Sclerosis with IgM Antibody against Gangliosides GM2 and GD2

Kotaro Mizutani, Nobuyuki Oka*, Susumu Kusunoki**, Ryuji Kaji***, Masutaro Kanda****, Ichiro Akiguchi and Hiroshi Shibasaki

Abstract

We report a case of amyotrophic lateral sclerosis (ALS) with IgM antibody against gangliosides GM2 and GD2. A 57-year-old woman presented with slowly progressive muscular weakness of the upper extremities and dysarthria. She fulfilled the clinical and electrophysiological criteria of ALS, and died from sudden suffocation about 3 years after the onset of illness. The patient’s serum IgM antibody was shown to recognize the structure shared by GM2 and GD2. Since anti-GM2 antibodies have been implicated in motor neuropathy or motor neuron syndrome, this rare case might contribute to the understanding of the immunological aspects of ALS.


Key words: anti-GM2 antibodies, antiganglioside antibodies, motor neuron disease

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by loss of motor neurons in the central nervous system. The etiology of sporadic ALS remains unclear, although abnormalities of the Cu/Zn superoxide dismutase gene have been detected in some familial cases (1, 2). Several reports have shown that immunoinflammatory processes are involved in the pathology of ALS (3–5). In addition, a variety of immunological abnormalities have been reported in some patients with ALS (6–9). Gangliosides (sialic acid-containing glycosphingolipids) are abundant in the nervous system, and serum antiganglioside antibodies are occasionally detected in some immunemediated neurological disorders (10–13). We encountered a sporadic ALS patient with serum IgM antibody against gangliosides GM2 and GD2.

Case Report

Patient

The patient was a 57-year-old woman who was hospitalized with a 5-month history of slowly progressive muscular weakness of the upper extremities and dysarthria. On examination, weakness was prominent in the right forearm and small hand muscles (2–3/5 on the Medical Research Council scale), but lower extremity strength was preserved. Muscular atrophy was noted in the right and left thenars. Deep tendon reflexes were exaggerated in the extremities with bilateral Babinski sign. Fasciculations were observed in the tongue. There were no sensory deficits. She had no skin lesions suggesting malignant melanoma, which is occasionally associated with chronic inflammatory demyelinating polyneuropathy and IgM anti-GM2 antibodies (14).

The level of fasting blood sugar was within the normal range. Thyroid function was normal. M-protein was not detected by serum immunoelectrophoresis. Hepatitis B surface antigen was positive with mild liver dysfunction. IgM anticytomegalovirus antibody was negative. The antibody test for human T-lymphotropic virus type I (HTLV-I) was negative, which ruled out the possibility of a pseudo-ALS form of HTLV-I-associated myelopathy (15). Examination of cerebrospinal fluid revealed a cell count of 1/μl and protein level of 36 mg/dl. Motor nerve conduction studies showed mark-
Figure 1. TLC-immunostaining with IgM in the patient’s serum, diluted 1:80. The binding activity of the IgM antibody of the patient’s serum to GM2 and GD2 is demonstrated (arrowheads). GN-GD1a: GalNAc-GD1a.

Methods

Serum antiganglioside antibodies (IgM and IgG) were investigated by enzyme-linked immunosorbent assay (ELISA) as described previously (12). Tested ganglioside antigens were GM1, GM2, GM3, GD1a, GD1b, GD3, GT1b, and GQ1b obtained from Funakoshi (Tokyo, Japan) and N-acetylgalactosaminyl GD1a (GalNAc-GD1a) obtained as described previously (12). Antibody titer was expressed as the maximal dilution factor which gave a corrected optical density of more than 0.1 (normal, less than 1:40). The results of ELISA were confirmed using thin-layer chromatogram (TLC)-immunostaining performed as described previously (12). ELISA using peroxidase-conjugated rabbit anti-human kappa or lambda light chain antibodies (DAKO, Glostrup, Denmark) as second antibodies was also performed to assess polyclonality or monoclonality of the patient’s serum antibody.

The absorption test was performed as follows. Each well of a 96-well microtiter plate was coated with 0.4 μg of each ganglioside. The wells were filled with 1% bovine serum albumin in phosphate-buffered saline for 30 minutes and emptied. The plate was incubated with the patient’s serum diluted 1:200 for 2 hours at room temperature then left overnight at 4°C. After absorption, the antiganglioside antibody titer of each sample was measured by ELISA.

Results

The patient’s serum IgM was reactive with GM2 (antibody titer of 1:640), but not with GM1, GM3, GD1a, GD1b, GD3, GalNAc-GD1a, GT1b, or GQ1b (less than 1:40). The binding activity of the antibody to GM2 was detected with both anti-human kappa and lambda light chain antibodies as second antibodies, suggesting that the antibody was polyclonal (data not shown). There were no IgG antibodies against any of the antigens tested. The anti-GM2 antibody titer remained high throughout the course of the illness. Since the IgM anti-GM2 antibody of the patient was not cross-reactive with GM1 or GalNAc-GD1a, cross-reactivity with GD2 was additionally examined. The patient’s serum IgM was also reactive with GD2 (1:320). Reactivities with GM2 and GD2 were confirmed by TLC-immunostaining (Fig. 1). Absorption with GM2 or GD2, but not with GM1 or GalNAc-GD1a, reduced the antibody activities (Table 1). Thirty other patients with ALS were also tested for serum anti-GM2 and anti-GD2 antibodies (IgM and IgG), and the

<table>
<thead>
<tr>
<th>Absorption Test</th>
<th>IgM anti-GM2 antibody</th>
<th>IgM anti-GD2 antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not absorbed</td>
<td>0.464</td>
<td>0.21</td>
</tr>
<tr>
<td>Absorbed with GM2</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Absorbed with GM1</td>
<td>0.385</td>
<td>0.259</td>
</tr>
<tr>
<td>Absorbed with GalNAc-GD1a</td>
<td>0.366</td>
<td>0.218</td>
</tr>
<tr>
<td>Absorbed with GD2</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
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Absorption of the patient’s serum was performed with 0.4 μg of each ganglioside. Data are optical density values.

Edly reduced compound muscle action potential amplitudes in the left median and the right median and ulnar nerves without slowed conduction velocity or conduction block. Needle electrode examination revealed evidence of active denervation in all extremities (first dorsal interosseous, extensor digitorum communis, and gastrocnemius muscles).

The patient did not respond to high-dose intravenous immunoglobulin treatment, which was administered considering the possibility of a treatable neuroimmunological disorder (13, 16, 17). She was clinically and electrophysiologically diagnosed as having ALS. After discharge, she developed dysphagia and muscular weakness of the lower extremities, and then became wheelchair bound. She died from sudden suffocation about 3 years after the onset of illness.
We recently reported that GM2 markedly enhances the properties in seven patients including five with GBS. One of these neurological disorders, and found high titers of the antibody (19, 20). Yuki et al (21) measured IgM and IgG anti-ganglioside antibodies in the serum of patients with GBS subsequent to cytomegalovirus infection (1:40 to 1:640).

**Discussion**

Cavanna et al (18) measured IgM anti-GM2 antibodies in the sera of 224 patients with different neuropathies and motor neuron disease (MND), and found high titers of the antibodies in eight patients with dysimmune neuropathies. All of those patients had a concomitant IgM reactivity with either GM1 or GalNAc-GD1a, the latter sharing the terminal trisaccharide with GM2 (Fig. 2). However, the present patient’s serum IgM was not reactive with GM1 or GalNAc-GD1a, but with GD2. The absorption test revealed that the IgM antibody mainly recognizes the moiety shared by GM2 and GD2 (Fig. 2). Similar reactivities with GM2 and GD2 have been observed in the serum antiganglioside antibodies of patients with GBS subsequent to cytomegalovirus infection (19, 20). Yuki et al (21) measured IgM and IgG anti-GD2 antibodies in the sera of 257 patients with various neurological disorders, and found high titers of the antibodies in seven patients including five with GBS. One of these GBS patients had IgM antibody against GD2, GM2, and GalNAc-GD1a.

GM2 may be an immunologically unique ganglioside. Tai et al (22) reported that GM2 appears to be the most immunogenic among gangliosides found on human melanoma cells. We recently reported that GM2 markedly enhances the production of tumor necrosis factor-α in peripheral blood mononuclear cell culture (23). In addition, GM2 is thought to be a possible major component of motor neuron gangliosides (24, 25).

Antibodies against several kinds of gangliosides other than GM2 have been detected in MND (26–28). O’Hanlon et al (29) recently reported three MND patients with IgM anti-GM2 antibodies whose cross-reactivity with GD2 was not described. Interestingly, high-titer IgM antibody against GM2 was detected in a patient who developed an ALS-like disorder after intramuscular treatment with bovine brain gangliosides for diabetic neuropathy (30). The IgM anti-GM2 antibody in that patient was cross-reactive with GalNAc-GD1a (31), but not with GD2 (30). In addition, Nakao et al (32) found two novel GM2-epitope containing gangliosides in bovine brain with that patient’s serum IgM. Similarly, there might be unknown gangliosides in the central nervous system that react with our patient’s serum IgM.

As one of the autoimmune hypotheses in ALS, autoreactive antibodies might be taken up at the nerve ending and transported within the axons (33). It was reported that immunoglobulins from ALS patients enhance spontaneous transmitter release from motor nerve terminals (7). IgM monoclonal antibody against terminal moiety of GM2 and GalNAc-GD1a has recently been shown to have effects on neurotransmitter release (34). These pathophysiological mechanisms involving motor nerve terminals may account for selective damage to motor nerves or motor neurons. Although the pathogenic significance of IgM anti-GM2 antibodies in MND is still undetermined (29), the present rare case might contribute to the understanding of the immunological aspects of ALS.

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