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Lower motor neuron syndrome associated with IgG anti-GM1 antibodies revisited ☆,☆☆

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ABSTRACT

A patient, who developed an amyotrophic lateral sclerosis-like disorder subsequent to ganglioside treatment, had IgM antibodies to GM2 as well as to minor gangliosides X1 and X2 containing GM2 epitope. These gangliosides as well as GM1 were tested in 655 sera obtained from patients who were suspected of having amyotrophic lateral sclerosis or motor neuron disease to find a treatable condition. Three patients had high titers of IgG anti-GM1 antibodies, but no IgM anti-GM1 antibodies. One of the patients also had IgG anti-X2 antibodies. The patients, being diagnosed with having lower motor neuron syndrome, had neither upper motor neuron signs nor multifocal conduction block. Both IgM and IgG anti-GM1 antibodies should be tested in patients who have lower motor neuron syndrome.

1. Introduction

Patients diagnosed with amyotrophic lateral sclerosis (ALS) or motor neuron disease (MND) may have autoantibodies against gangliosides, which makes both conditions treatable. IgM autoantibodies to GM1 gangliosides were first found in a patient diagnosed with lower MND (Freddo et al., 1986). IgM anti-GM1 antibodies were later found in 2 patients with multifocal motor neuropathy (MMN). The 2 patients had been initially diagnosed as having lower motor neuron (LMN) forms of ALS (Pestronk et al., 1988). Currently, intravenous immunoglobulin (IVIG) is the first line of treatment of MMN, and IgM anti-GM1 antibodies are used as a biomarker for the condition (Vlam et al., 2012).

In another instance, a patient developed progressive limb weakness gradually over a period of 6 months and showed signs of upper motor neuron weakness after receiving an intramuscular administration of a bovine ganglioside mixture (Yuki et al., 1991). His initial diagnosis was ALS. The patient's serum contained anti-GM2 antibodies capable of killing GM2-containing neuroblastoma cells in the presence of complement (Yuki et al., 1992). The patient improved after plasma exchange. Using the patient's serum, we found lacto-ganglio-series gangliosides X1 and X2 that bore the GM2 epitope in the bovine brain ganglioside mixture (Fig. 1A) (Nakao et al., 1993).

In order to discover immune-mediated and treatable patients, we synthesized the minor gangliosides X1 and X2 (Nakashima et al., 2011, 2012), and we tested antibodies to X1 and X2 in a large number of sera from patients who had been misdiagnosed with having ALS or MND. Unexpectedly, however, we found 3 patients with high titers of IgG anti-GM1 antibodies who presented with lower motor neuron syndrome because we tested the anti-GM1 antibodies to GM1 and GM2 as the controls.

2. Methods

2.1. Serum samples

The plasma sample for our study was obtained from the ALS-like disorder subsequent to ganglioside administration at his plasma exchange (Yuki et al., 1991), which was used to show whether synthesized X1 and X2 were suitable antigens on ELISA plates. His plasma was also used as a positive control for IgM anti-ganglioside antibodies on each microtiter plate. In addition, serum samples from patients who were suspected

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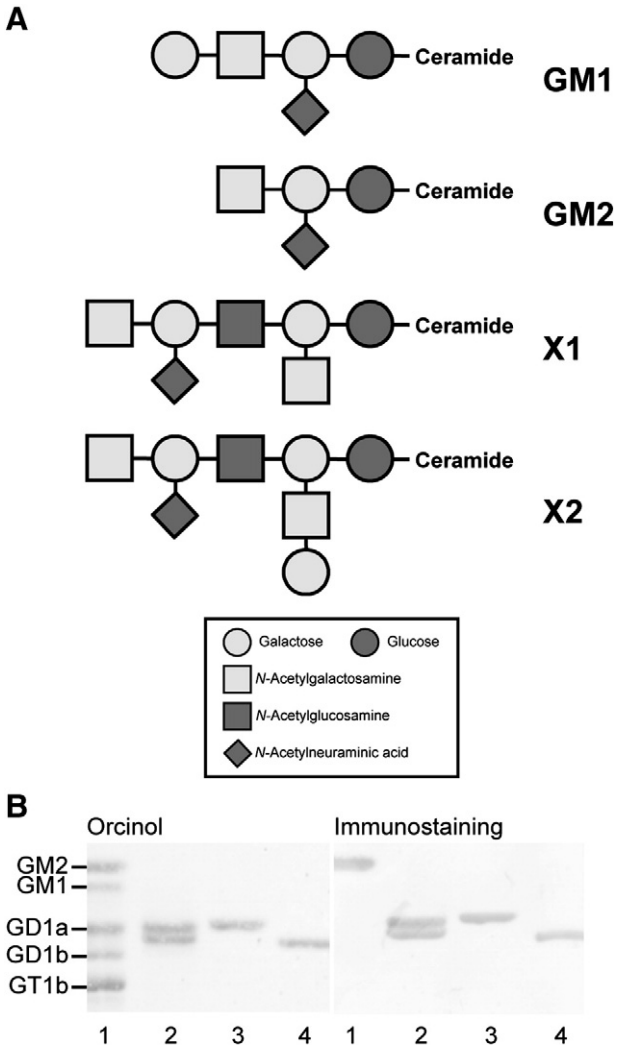

Fig. 1. (A) Schemed structure of gangliosides GM1, GM2 and GM2-epitope containing gangliosides X1 and X2. (B) An immunostained chromatogram that was overlaid first with serum from a patient with amyotrophic lateral sclerosis-like disorder, then with peroxidase-conjugated anti-human μ-chain specific antibodies. Lane 1, authentic gangliosides GM2, GM1, GD1a, GD1b and GT1b. Lane 2, GM2-epitope containing gangliosides, X1 and X2, from bovine brain. Lane 3, synthesized X1. Lane 4, synthesized X2. Serum IgM antibodies from the patient strongly bound to GM2, X1 and X2 from bovine brain, and to the synthesized X1 and X2.

of having ALS (n = 408), MND (n = 247) or MMN (n = 504) were sent from hospitals throughout Japan to the neuroimmunology laboratory at Dokkyo Medical University to be tested for IgM anti-GM1 antibody from 1994 to 2011. Written informed consent was obtained from each patient, and the ethics committee at Dokkyo Medical University approved the study. A serum sample from a patient with acute motor axonal neuropathy (AMAN) – an axonal subtype of Guillain–Barré syndrome associated with IgG anti-GM1 and -GD1b antibodies (Yuki et al., 1990) – was used as a positive control for IgG anti-ganglioside antibodies on each plate diluted in an enzyme-linked immunosorbent assay (ELISA) and a thin-layer chromatogram (TLC) with immunostaining. Aliquots of sera and plasma from the patients were stored at −80°C prior to use.

2.2. Anti-ganglioside antibody assays

GM1, GM2, X1 and X2 were prepared from a bovine brain ganglioside mixture (Hirabayashi et al., 1988; Nakao et al., 1993). X1 and X2 were also synthesized as described elsewhere (Nakashima et al., 2011, 2012), and ELISA and TLC-immunostaining were performed. Initial dilution of serum samples was 1:500 for ELISA and 1:50 for TLC-immunostaining. The serum samples were serially diluted to find antibody titers by ELISA. Each ELISA was done in triplicates.

2.3. Complement deposition assays

The assays were performed as described elsewhere with a minor modification (Yuki et al., 2011). Sera from Patients 1, 2 and 3 below, the AMAN patient (Yuki et al., 1990) and 2 patients with MMN associated with IgM anti-GM1 antibodies were used. Phosphate-buffered saline containing 2% casein sodium was used for each dilution. To test whether IVIG blocks complement deposition by bound anti-GM1 antibodies, diluted normal sera was mixed with an equal volume of IVIG [Kenketu Glovenin®-I (Nihon Pharmaceutical, Tokyo, Japan)] or human serum albumin (Kenketsu Albumin 20 "KAKETSUKEN", KAKETSUKEN, Kumamoto, Japan). Each ELISA was done in quadruplicates.

3. Results

3.1. Synthesized X1 and X2 could be used for microtiter assay

TLC revealed high purity of the synthesized X1 and X2, and similar mobility in them, with natural X1 and X2 from bovine brains (Fig. 1B). TLC-immunostaining demonstrated that the IgM antibodies from the patient with ALS-like disorder bound to the synthesized X1 and X2, as well as to GM2, X1 and X2 isolated from the bovine brain ganglioside mixture. ELISA showed that the patient's IgM reacted with GM2 and the synthesized X1 and X2, but not with GM1, indicating that the synthesized X1 and X2 could be used as antigens on microtiter plates (Fig. 2A).

3.2. IgM anti-GM1 antibodies positive were not found in ALS or MND samples

Three serum samples from patients who were suspected of having ALS or MND contained IgG antibodies to GM1 on ELISA plates (Fig. 2A), whereas none of the samples had IgM anti-GM1 antibodies. IgG anti-GM1 antibody titers in Patients 1, 2 and 3 were 1:32,000, 1:2000 and 1:8000, respectively. Patient 2 also had high titers of IgG anti-X2 antibodies (1:2000). Serum IgG antibodies from Patient 1 bound strongly to GM1 from the bovine brain and the cauda equina on a TLC plate (Fig. 2B). Serum IgG antibodies from *Patients 2* and *3* also bound specifically to GM1, although the reactivity was weak. None of the other 652 sera had IgM and IgG antibodies to the gangliosides tested.

3.3. IgM anti-GM1 antibodies positive were found in MMN samples

Four serum samples from patients who were suspected of having MMN contained high titers of IgM anti-GM1 antibodies (1:8000, 1:4000, 1:4000 and 1:16,000). Two of the samples had high titers of IgG anti-GM1 antibodies (1:128,000 and 1: 64,000), and the latter sample had IgG anti-X2 antibodies (1: 64,000). Two patients who carried IgG ant-GM1 antibodies had been suspected of having MMN because of the presence of multifocal conduction blocks in the motor nerves. On reports of the antibody testing, however, N.Y. suggested the referring physicians to investigate the presence of antecedent infectious symptoms and to better define the onset and course of the illness. Guillain–Barré syndrome was eventually diagnosed by the physicians, although one of the patients was reported as having acute MMN (Sugie et al., 1998). None of the other 498 sera had IgM and IgG antibodies to the gangliosides tested.

3.4. IVIG inhibited complement deposition mediated by the anti-GM1 antibodies

Activated C3 component deposition mediated by the anti-GM1 antibodies was shown in the sera from Patients 1, 2 and 3 patients and the