

## SHORT REPORT

## Two species of antiganglioside antibodies in a patient with a pharyngeal-cervical-brachial variant of Guillain-Barré syndrome

Kouichi Mizoguchi, Asako Hase, Tomokazu Obi, Hiroaki Matsuoka, Masami Takatsu, Yoshirou Nishimura, Fumitoshi Irie, Yousuke Seyama, Yoshio Hirabayashi

### Abstract

**A patient with a pharyngeal-cervical-brachial variant of Guillain-Barré syndrome had anti-GT1a and anti-GD1a antibodies (IgG) in the serum. The activities of anti-GT1a antibodies were stronger than anti-GD1a antibodies and their activities declined later in the clinical course. These two different antibodies bound independently to each ganglioside in an absorption study with polystyrene beads coated with GT1a or GD1a.**

(*J Neurol Neurosurg Psychiatry* 1994;57:1121-1123)

Antiganglioside antibodies have been detected in patients with Guillain-Barré syndrome,<sup>1,2</sup> although it is not clear whether they have a specific role in the pathogenesis of Guillain-Barré syndrome. Here we report a patient with a pharyngeal-cervical-brachial variant of Guillain-Barré syndrome, who had two antibodies in his serum, one against GT1a, a minor ganglioside species, and the other against GD1a.

### Case report

A forty year old man was admitted for dysphagia on 25 June 1991. He had been treated with phenytoin and phenobarbitone for epilepsy since he was 18 years old. From 6 June, he experienced diarrhoea two or three times a day. He had difficulty in swallowing on 22 June. On the next day, he felt weakness in his neck and both upper limbs. The day before admission, weakness of the proximal regions of both lower limbs developed.

On admission, weakness of the bilateral facial muscle, dysphagia, and dysarthria were present. The strength of neck flexion and the upper limbs were 3/5 (MRC grade), and 4/5 in the lower limbs. Gripping strength was 7 kg on the right and 0 kg on the left. Deep reflexes were normal and pathological reflexes were not elicited. Sensory disturbance and ataxia were not seen. Routine laboratory examinations were normal, including complete blood count, blood chemistries, and immunological examinations. Protein concentration in CSF was 350 mg/l with normal cellularity. Stool culture was negative for

*Clostridium jejuni*. Motor nerve conduction velocities were normal in the right median, ulnar, and peroneal nerves, but compound muscle action potentials were 2.01 mV at the proximal and 4.39 mV at the distal portion of the ulnar nerve, and 0.366 mV and 0.834 mV in the peroneal nerve, respectively. F Waves of the right median and ulnar nerves had normal latencies, but could not be elicited in the right peroneal nerve.

Three days after admission, diplopia appeared with aggravated weakness of facial and limb muscles. He was treated with double filtration plasmapheresis on the 7th, 9th, 11th, 14th, and 16th days. The strength of all limbs, but not facial and neck muscles, improved to 4-5/5 with grip strength of 9.5 kg in the right and 6.0 kg in the left after plasmapheresis. Deep reflexes were slightly exaggerated. Protein concentration in CSF was normal on the 52nd day. Follow up motor nerve conduction velocity studies showed that compound action potentials of the ulnar and peroneal nerves were normalised by the 24th day. He was discharged 60 days after admission with almost full recovery except for the facial and neck muscles. The strength of his neck muscles had fully recovered by three months and his facial muscles by six months after discharge.

### Methods

ENZYMELINKED IMMUNOSORBENT ASSAY (ELISA)

Antigenic gangliosides, which included GM3, GM2, GM1a, GD1a, GD1b, GT1b, and GQ1b, were prepared from bovine brains by Q sepharose column chromatography according to the method of Hirabayashi *et al.*<sup>3</sup> GT1a was a gift from Dr S Ando (Tokyo Metropolitan Institute of Gerontology).

The ELISA was carried out by the method of Higashi *et al.*<sup>4</sup> The antigenic solution contained 20 pmol of each ganglioside. After blocking with 5% bovine serum albumin-phosphate buffered saline (BSA-PBS), triplicate samples of serum were diluted 1:100 with 0.25% BSA-PBS and incubated for two hours at room temperature. Goat anti-human IgM or IgG antibody conjugated to horse radish peroxidase (Jackson Immunoresearch Laboratories, Inc, West Grove, PA, USA) diluted 1:1000 with 0.25% BSA-PBS was

Department of  
Neurology, National  
Shizuoka Hospital  
24-1 Joto-cho,  
Shizuoka 420, Japan

K Mizoguchi  
A Hase  
T Obi  
H Matsuoka  
M Takatsu  
Y Nishimura

Laboratory for Glyco  
Cell Biology, Frontier  
Research Program,  
The Institute of  
Physical and  
Chemical Research  
(RIKEN), 2-1  
Hirosawa, Wako  
351-01, Japan  
F Irie  
Y Hirabayashi

Department of  
Physiological  
Chemistry and  
Nutrition, Faculty of  
Medicine, The  
University of Tokyo,  
Hongo, Bunkyo-ku,  
Tokyo, Japan  
F Irie  
Y Seyama

Correspondence to:  
Dr Kouichi Mizoguchi.

Received 7 September 1993  
and in final revised form  
1 March 1994.  
Accepted 8 March 1994

added as secondary antibody and incubated for two hours at room temperature. After further incubation with 50  $\mu$ l 0.1% *o*-phenylenediamine and 0.02% H<sub>2</sub>O<sub>2</sub> in 0.2M sodium citrate buffer for 15 minutes at room temperature, the enzyme reaction was stopped by adding 50  $\mu$ l of 1N HCl and measured with a microplate spectrophotometer at 492 nm.

#### ABSORPTION STUDY

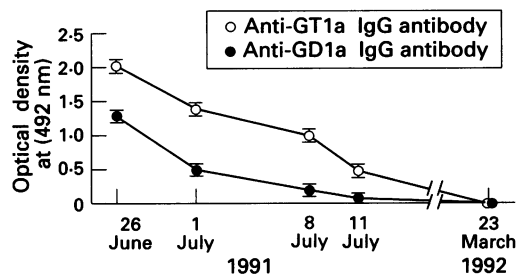
An absorption study was performed with GT1a or GD1a coated polystyrene beads as follows: a total of 2.5 mg of polystyrene beads (MW 50 000, Polysciences Inc) was added to 1 ml of chloroform-methanol solution (2:1 v/v), containing 1 mg of GT1a or GD1a. The solution was dried by rotary evaporator and the beads were suspended in 5 ml PBS. The patient's serum, diluted at 1:50 with 0.25% BSA-PBS, was mixed with the same volume of PBS containing GT1a or GD1a coated polystyrene beads. The solution was incubated at 37°C for 30 minutes, centrifuged at 3000 rpm for 15 minutes, and the supernatant was used as the primary antibody for ELISA. As a control, PBS was used instead of GD1a coated polystyrene beads.

#### Results

We detected anti-GT1a and anti-GD1a IgG antibodies in this patient's serum. Antibody activities against the other gangliosides used in this study could not be detected. Anti-GT1a antibodies reacted more strongly than anti-GD1a antibodies (figure). Both anti-GT1a and -GD1a antibodies showed their highest activities on the 2nd day after admission, declined with clinical course, and could not be detected after six months (figure).

In the absorption study, when the patient's serum was preincubated with GT1a coated polystyrene beads, anti-GT1a antibody was absorbed on to the beads, but anti-GD1a antibody activities did not decrease. On the other hand, the activities of anti-GT1a antibody did not decrease when the patient's serum was preincubated with GD1a coated polystyrene beads or only with PBS.

We also detected anti-GM1a IgG antibodies in two of seven typical patients with Guillain-Barré syndrome, anti-GQ1b and anti-GT1a IgG antibodies in all eight patients



Activities of anti-GT1a and -GD1a IgG antibodies of this patient. Open circles indicate the activity of anti-GT1a IgG antibody and closed circles that of anti-GD1a IgG antibody. The serum and primary antibodies were diluted 1:100.

with Miller-Fisher syndrome tested, and IgM antibodies against polysialogangliosides in a patient with macroglobulinaemia and sensory dominant neuropathy.<sup>7</sup> No IgG or IgM antibody activities against the eight gangliosides used in this study were detected in the serum of the other patients—namely, those with amyotrophic lateral sclerosis (n = 33), multiple sclerosis (n = 3), myasthenia gravis (n = 12), and other neurological diseases (n = 154), or normal controls at a dilution of 1:100.

#### Discussion

In 1986, Ropper reported three patients who, like ours, developed prominent weakness of the pharyngeal-cervical-brachial regions without either lower limb weakness or sensory disturbance, and labelled it "pharyngeal-cervical-brachial weakness resembling botulism or diphtheria".<sup>5</sup> Conduction block in the ulnar and peroneal nerves in our patient was compatible with postinfectious demyelinating polyneuropathy.

From our results it is suggested that the detected antibodies, anti-GT1a and anti-GD1a, participated in the pathological process of this patient, and that these two different antibodies independently reacted with GT1a and GD1a. Ilyas *et al* detected two kinds of anti-ganglioside antibodies in two patients with Guillain-Barré syndrome.<sup>1</sup> Their antibodies, however, reacted with the common structure of GD1a and GT1b, GalNAc-Gal-NeuAc-NeuAc. To our knowledge, there is no other report of a patient who has two antibodies reacting independently with a different ganglioside without sharing a common structure.

Anti-GD1a antibody was reported in patients with Guillain-Barré syndrome by Ilyas *et al*<sup>1</sup> and Yuki *et al*.<sup>2</sup> These antibodies were also detected in a patient with motor neuropathy with monoclonal IgM protein.<sup>7</sup> On the other hand, the existence of anti-GT1a IgG antibodies was reported in patients with Miller-Fisher syndrome, although such patients mostly have anti-GQ1b IgG antibodies.<sup>8</sup> GT1a was first detected in the human brain by Ando *et al*,<sup>9</sup> and later in the human spinal cord<sup>10</sup> and cauda equina.<sup>11</sup> GT1a is a minor ganglioside, forming 1.8% of the total gangliosides of human cerebral white matter, 2.2% of human cerebral grey matter, and 0.5% of the human spinal cord, respectively.<sup>12</sup>

We thank Dr S Ando (Tokyo Metropolitan Institute of Gerontology) for his gracious gift of GT1a.

- Ilyas AA, Willison HJ, Quarles RH, Jungalwala FB, Cornblath DR, Trapp BD, *et al*. Serum antibodies to gangliosides in Guillain-Barré syndrome. *Ann Neurol* 1988;23:440-7.
- Yuki N, Yoshino H, Sato S, Shinozawa K, Miyatake T. Severe acute axonal form of Guillain-Barré syndrome associated with IgG anti-GD1a antibodies. *Muscle Nerve* 1992;15:899-903.
- Hirabayashi Y, Nakao T, Matsumoto M. Improved method for large-scale purification of brain gangliosides by Q-Sepharose column chromatography. Immunochromatographic detection of C-series polysialogangliosides in adult bovine brains. *J Chromatogr* 1988;445:377-84.

- 4 Higashi H, Ikuta K, Ueda S, Kato S, Hirabayashi Y, Matsumoto M, Naiki M. Characterization of N-glycolylneuraminic acid containing glycosphingolipids from a Marek's disease lymphoma-derived chicken cell line, MSB1, as tumor-associated heterophile Hanganutziu-Deicher antigens. *J Biochem (Tokyo)* 1984;95:785-94.
- 5 Ropper AH. Unusual clinical variants and signs in Guillain-Barré syndrome. *Arch Neurol* 1986;43:1150-2.
- 6 Obi T, Kusunoki S, Takatsu M, Mizoguchi K, Nishimura Y. IgM M-protein in a patient with sensory dominant neuropathy binds preferentially to polysialogangliosides. *Acta Neurol Scand* 1992;86:215-8.
- 7 Bollensen E, Schipper HI, Steck AJ. Motor neuropathy with activity of monoclonal IgM antibody to GD1a ganglioside. *J Neurol* 1989;236:353-5.
- 8 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. *Neurol* 1993;43:1911-7.
- 9 Ando S, Yu RK. Isolation and characterization of a novel trisialo ganglioside, GT1a, from human brain. *J Biochem* 1977;252:6247-50.
- 10 Ueno K, Ando S, Yu RK. Gangliosides of human, cat, and rabbit spinal and cord myelin. *J Lipid Res* 1978;19:863-71.
- 11 Dawson G, Stefanson K. Gangliosides of human spinal cord: aberrant composition of cords from patients with amyotrophic lateral sclerosis. *J Neuroscience Res* 1984;12:213-20.