
Serum anti-GQ_{1b} IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: Clinical and immunohistochemical studies

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Article abstract—To determine the significance of serum anti-GQ_{1b} IgG antibody, we studied the disease spectrum associated with this antibody and GQ_{1b} epitope in the human nervous system. We examined sera from 19 patients with typical Miller Fisher syndrome (MFS), five patients with acute postinfectious ophthalmoplegia without ataxia (atypical MFS), six patients with Guillain-Barré syndrome (GBS) with ophthalmoplegia (GBS-OP[+]), and 23 patients with GBS without ophthalmoplegia (GBS-OP[−]). We also examined sera from 84 patients with other neurologic or non-neurologic disorders and from 16 normal control subjects. Eighteen of the 19 patients with typical MFS, all the patients with atypical MFS, and five of the six patients with GBS-OP(+) had increased anti-GQ_{1b} IgG activity in ELISA, but none of the patients in the other groups, including GBS-OP(−), had it. All the patients' sera that had anti-GQ_{1b} IgG antibody showed anti-GT_{1a} IgG activity. Results of absorption studies suggested that the same antibody reacted with GQ_{1b} and GT_{1a}. An anti-GQ_{1b} mouse monoclonal antibody immunostained the paranodal regions of the extramedullary portion of the human oculomotor, trochlear, and abducens nerves. Biochemical analysis showed that the human oculomotor nerve contained a larger amount of GQ_{1b} than did the ventral and dorsal roots of the spinal cord. We conclude that serum IgG antibody against GQ_{1b} is very closely associated with acute postinfectious ophthalmoplegia in MFS and GBS.

NEUROLOGY 1993;43:1911-1917

C. Miller Fisher described¹ a syndrome consisting of ophthalmoplegia, ataxia, and areflexia (Miller Fisher syndrome [MFS]). Although, on the basis of common clinical features, he regarded it as a variant of Guillain-Barré syndrome (GBS), the relation between MFS and GBS and the pathogenesis for the features unique to MFS are unknown.

Serum antiganglioside antibodies are present in GBS.²⁻⁵ Recently, we reported⁶ the presence of serum IgG antibody against ganglioside GQ_{1b} in patients with MFS in the acute phase and pointed out an immunologic feature common to MFS and GBS: the presence of antiganglioside antibody. Serum antiganglioside antibodies also are present in, and possibly involved in the pathogenesis of, motor neuron disease (MND) and multifocal motor neuropathy (MMN).⁷⁻¹¹

To evaluate the significance of serum anti-GQ_{1b} IgG antibody in detail, we investigated whether

this antibody is present in larger groups of patients that included those with atypical MFS and GBS with ophthalmoplegia. We also investigated the distribution of the epitope for anti-GQ_{1b} antibody immunohistochemically using a mouse monoclonal antibody (mAb).

Methods. *Examination of antiglycolipid antibody in patients' sera.* **Patients.** Serum samples were obtained from 19 patients with typical MFS, all of whom had the triad of the syndrome (ophthalmoplegia, ataxia, and hyporeflexia) but no major limb weakness or other signs suggestive of CNS involvement. We also examined five patients who experienced acute ophthalmoplegia after an infectious prodrome (four infected with common cold and one with diarrhea) who recovered without specific therapy. They all had a relatively mild bilateral abducens palsy; two had hyporeflexia, two a mild decrease of vibration sense in the lower extremities, and two a mild albuminocytologic dissociation in the cerebrospinal fluid, but

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Supported in part by a Grant-in-Aid for Scientific Research (04670485, 04250202) from the Ministry of Education, Science, and Culture of Japan and a grant from the Ministry of Health and Welfare of Japan.

Received October 13, 1992. Accepted for publication in final form February 16, 1993.

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Table. Clinical features of patient groups

	No. pts	Ophthalmoplegia		Ataxia	Hypo- or areflexia	Limb weakness	First serum examination*	
		Ext	Int				After prodrome	After onset
Typical MFS	19	+	- or +	+	+	- or ±	5-37	1-21
Atypical MFS	5	+	-	-	- or +	-	14-64	6-60
GBS-OP(+)	6	+	- or +	-, +, or ?	+	+	14-63	5-53
GBS-OP(-)	23	-	-	-, +, or ?	+	+	9-40	1-20

* Range in days.
 Ext External ophthalmoplegia.
 Int Internal ophthalmoplegia.
 MFS Miller Fisher syndrome.
 GBS-OP(+) Guillain-Barré syndrome (GBS) with ophthalmoplegia.
 GBS-OP(-) GBS without ophthalmoplegia.
 + Present.
 - Absent.
 ± Minimal.
 ? Unable to be assessed because of limb weakness.

none had ataxia or any other neurologic sign. This group was designated "atypical MFS."

Serum samples also were taken from six patients who had GBS and ophthalmoplegia (GBS-OP[+]). Twenty-three patients with GBS but without ophthalmoplegia (GBS-OP[-]) also were examined, three of whom showed ataxia that was considered to be due to loss of position sense. In the others, ataxia was absent or could not be assessed because of limb weakness. The clinical features of these patients are given in the table. Neurologists examined them in the acute phase. The first serum samples were taken in the acute phase, except for one patient with atypical MFS and one patient with GBS-OP(+), from whom samples were obtained 60 days (atypical MFS) and 53 days (GBS-OP[+]) after neurologic onset.

As disease controls, serum samples were taken from two patients with brainstem encephalitis, 24 with MS, five with MMN, 12 with chronic inflammatory demyelinating polyneuropathy (CIDP), and 23 with MND, as well as 18 patients with other immunologic disorders, nine of whom had systemic lupus erythematosus, seven polymyositis, and two mixed connective tissue disorder. Except for MND, the samples were taken in the acute, active, or relapse phases of the disorders. Samples also were taken from 16 normal control subjects. Both patients with brainstem encephalitis had lesions in the brainstem, as confirmed by MRI, showing abnormal ocular movement (conjugate gaze palsy in one, coarse horizontal gaze nystagmus in the other), ataxia, and hyporeflexia, as well as consciousness disturbance and dysarthria. They eventually recovered. Serologic examinations suggested herpes simplex virus infection in one of them, but the causative agent in the other is not known. Of the 24 patients with MS, nine had ataxia and four ophthalmoplegia or diplopia. All five patients with MMN had the serum anti-GM₁ IgM antibody. None of the patients with CIDP had ophthalmoplegia.

Enzyme-linked immunosorbent assay (ELISA). All the glycolipids except GQ_{1b} and asialo-GM₁ (GA₁) were purchased from Funakoshi (Tokyo, Japan). GQ_{1b} was bought from Dia-Iatron (Tokyo, Japan). GA₁ was prepared from the GM₁ of bovine brain in our laboratory. The titer of the serum anti-GQ_{1b} IgG antibody was determined by ELISA. Five hundred ng of purified GQ_{1b} in 50 μl of ethanol was added to individual wells of Linbro microtiter plates (Flow Laboratories, McLean, VA), after which the solution was dried by evaporation. Nonspecific protein-binding sites were saturated with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.4 (blocking solution). A 50-μl serum sample

diluted serially from 1:10 with blocking solution was added in duplicate to the antigen-coated and -uncoated wells, after which the plates were incubated overnight at 4 °C. The wells then were washed three times with the blocking solution and incubated for 2 hours at room temperature with 50 μl of peroxidase-conjugated goat anti-human IgG antibody (γ-chain specific; Cappel, West Chester, PA) diluted 1:500 with blocking solution. Next, they were washed three times then incubated with 200 μl of 40 mg/dl of o-phenylenediamine dihydrochloride and 0.006% H₂O₂ in phosphate-citrate buffer, pH 5.0, as the enzyme substrate. The reaction was stopped by the addition of 50 μl of 8N H₂SO₄. The color reaction was read at 492 nm with an ELISA reader (BioRad, Hercules, CA). Readings for the GQ_{1b}-uncoated wells were subtracted from those for the GQ_{1b}-coated wells. The titer for each patient was taken as the highest dilution factor at which the mean OD of the duplicate wells exceeded the cutoff value. This corresponded to the mean + 3 SD of the normal control sera at the dilution of 1:10. Taking into account the variations of the OD values in the different experiments, we determined the cutoff value for each assay by using serially diluted positive standard serum on the same plate. This value ranged from 0.215 to 0.350 in the anti-GQ_{1b} IgG antibody assay. Serum anti-GQ_{1b} IgM antibody and the IgM and IgG antibodies against other glycolipids (GM₁, GM₂, GM₃, GD_{1a}, GD_{1b}, GD₃, GT_{1b}, GA₁, galactocerebroside) also were examined by ELISA, as described earlier, at a serum dilution of 1:40. For the second antibody in the IgM class, we used peroxidase-conjugated goat antihuman IgM antibody (μ-chain specific; Cappel) diluted 1:200. The cutoff value also corresponded to the mean + 3 SD of the normal control sera for each glycolipid.

Thin-layer chromatography (TLC) and enzyme immunoassay. GM₁, GD_{1a}, GD_{1b}, GT_{1b}, GQ_{1b}, and GT_{1a} were the test antigens used. GT_{1a} was the gift of Dr. Susumu Ando, Tokyo Metropolitan Institute of Gerontology. A 500-ng sample of each purified ganglioside was loaded on a plastic-backed TLC plate (Macherey-Nagel, Düren, Germany) and developed with chloroform:methanol:0.2% CaCl₂ (45:55:10). The plate then was air-dried, dipped in a solution of 0.4% polyisobutylmethacrylate in *n*-hexane, and air-dried again. Nonspecific protein-binding sites were saturated with 10% normal goat serum in PBS. The different lanes on the plate were overlaid with sera from the patients or normal control subjects diluted with 10% normal goat serum in PBS: 1:40 for patients with anti-GQ_{1b} IgG antibody titers above 1:160 in ELISA and 1:20 for subjects with titers

below 1:80. The plate then was incubated overnight at 4 °C, after which it was washed with PBS and incubated for 2 hours at room temperature with peroxidase-conjugated goat antihuman IgG antibody diluted 1:200. Immunoreactants were made visible with PBS containing 50 mg/dl 3,3'-diaminobenzidine tetrahydrochloride and 0.01% H₂O₂.

Absorption study. Antiglycolipid antibodies were absorbed in the antigen-coated ELISA wells. Each well of the microtiter plate was coated with 500 ng of purified glycolipid. Nonspecific protein-binding sites were saturated as described earlier. Fifty μ l of sera diluted with 10% normal goat serum in PBS, in the same ratio used for the TLC-enzyme immunoassay, was added to both the antigen-coated and -uncoated wells, after which the plates were incubated overnight at 4 °C. The residual antiglycolipid activities of the supernatants were assayed by TLC-enzyme immunoassay and the activities absorbed in the wells were assayed by ELISA. We confirmed there were no significant differences in the protein concentrations of the materials absorbed by this method.

Immunohistochemical and biochemical studies. We used tissue specimens obtained from autopsied patients with no known neurologic disorders. These specimens were frozen within 18 hours of death and kept at -80 °C until used.

Immunohistochemical study. Human nerve tissues were studied immunohistochemically with a monoclonal antibody, mAb 7F5, that reacts with both GQ_{1b} and GT_{1a} and belongs to the IgG_{2a} subclass of the mouse.¹² This antibody was provided by Mecto (Tokyo, Japan). We examined all 12 cranial nerves and the ventral and dorsal roots of the lumbar spinal cord, as well as the femoral nerve, dorsal root ganglion (DRG), thoracic spinal cord, cerebellum, and brainstem. Specimens were frozen in isopentane cooled in liquid nitrogen. Sections of 10 μ m were cut on a cryostat at -25 °C, then mounted on gelatin-coated slide glasses, air dried, and fixed in cold acetone for 5 minutes, after which they were immunostained with Vectastain ABC kit (Vector Laboratories, Burlingame, CA). After incubating them with 1.5% normal horse serum, the sections were incubated overnight at 4 °C with mAb 7F5 diluted 1:400 (corresponding to 15 μ g of IgG_{2a} per ml), after which they were washed twice with PBS (10 minutes each), then incubated for 2 hours at room temperature with biotinylated horse antimouse IgG antibody diluted 1:200. After two washings with PBS, the sections were incubated for 1 hour at room temperature with the avidin-biotin complex. After two more PBS washes, the immunoreactants were made visible with 50 mg/dl of 3,3'-diaminobenzidine tetrahydrochloride and 0.01% H₂O₂ in PBS. Purified mouse IgG_{2a} (Chemicon, Temecula, CA) diluted to 15 μ g/ml was the control.

Ganglioside preparation. The total ganglioside fractions were prepared from the oculomotor nerve and the ventral and dorsal roots of the lumbar spinal cord. About 100 mg of each tissue was homogenized. The total lipid fractions were extracted sequentially with 3 ml each of chloroform:methanol:water at 2:1:0, 1:1:0, and 1:2:0.8 (by volume). The extract was evaporated serially on a rotary evaporator. Its residue was dissolved in 2 ml of chloroform:methanol (9:1), then applied to a Phenyl Sepharose column (Pharmacia, Uppsala, Sweden) (column volume, 2 ml). Neutral lipids and sulfatides were eluted with 10 ml each of chloroform:methanol (neutral lipids at 9:1 and sulfatides at 85:15). The total gangliosides were recovered with acidic phospholipids on elution with 10 ml of chloroform:methanol (1:1) and 20 ml of methanol.¹³ The

eluate obtained was evaporated in a rotary evaporator. The residue was given mild alkaline treatment with 0.5 M sodium hydroxide in methanol for 1 hour at 45 °C to degrade acidic phospholipids. After neutralization with 1N acetate in methanol, the sample was evaporated under nitrogen. The residue was dissolved in 500 μ l of chloroform:methanol:water (5:5:1) and applied to a gel chromatography column of Toyopearl HW-40 (Toso, Tokyo, Japan) (column volume, 10 ml) to remove salts. The first 3 ml of the eluate was discarded. The second 3 ml was collected and evaporated under nitrogen, after which the residue was dissolved in chloroform:methanol (1:1) at a concentration of 1 mg of original tissue per 4 μ l. The ganglioside patterns of samples were made visible with resorcinol-HCl reagent after chromatography on an HPTLC plate (Merck, Darmstadt, Germany) with chloroform:methanol:0.2% CaCl₂ (45:55:10).

TLC-enzyme immunoassay. TLC-enzyme immunoassays of the total ganglioside fractions from the oculomotor nerve and the ventral and dorsal roots of the spinal cord were done as described earlier. A sample corresponding to 2.5 mg wet weight of tissue was loaded on a plastic-backed TLC plate. After the plate had been developed, it was incubated with mAb 7F5 (1:200) or a patient's serum (1:40) for 1.5 hours at room temperature. The second antibody for mAb 7F5 was peroxidase-conjugated goat antimouse IgG antibody (1:200) (Cappel) and, for the patient's serum, peroxidase-conjugated goat antihuman IgG antibody (1:200).

Western blot analysis. Specimens were homogenized in ice-cold modified Laemmli's sample buffer containing 0.08 M Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, and 0.1 M dithiothreitol. After the samples had been immersed in boiling water for 1.5 minutes, insoluble precipitates were removed by centrifugation at 10,000 \times g and 4 °C for 15 minutes. The protein concentrations then were measured by the method of Lowry et al,¹⁴ as modified by Ross and Schatz,¹⁵ with BSA as the standard. Twenty μ g of protein per lane was separated by electrophoresis on a 15% SDS-polyacrylamide slab gel, then transferred electrophoretically to a nitrocellulose membrane.^{16,17} The blots were immunostained by enzyme-immunoassay with ProtBlot (Promega, Madison, WI). After incubation with 3% BSA for 6 hours at room temperature, the membrane was incubated overnight at 4 °C with the first antibody diluted with Tris-buffered saline (pH 8.0) containing 0.05% Tween 20. After being washed, the membrane was incubated for 2 hours at room temperature with alkaline phosphatase-conjugated antimouse IgG diluted 1:7,500. The immunoreactants were made visible with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as the enzyme substrates. MAb 7F5 and purified mouse IgG_{2a} (the control) were used as the first antibodies at 15 μ g of IgG_{2a} per ml.

Results. Examination of antiglycolipid antibody in patients' sera. Eighteen of the 19 patients with typical MFS, all five of those with atypical MFS, and five of the six with GBS-OP(+) had increased anti-GQ_{1b} IgG antibody titers ranging from 1:40 to 1:640 in ELISA (figure 1). In three of these patients, we could examine their sera as early as 1 or 2 days after neurologic onset, and they all had high titer of 1:320. None of the 23 patients with GBS-OP(-), the 84 disease control subjects, or the 16 normal control subjects had titers above 1:10.

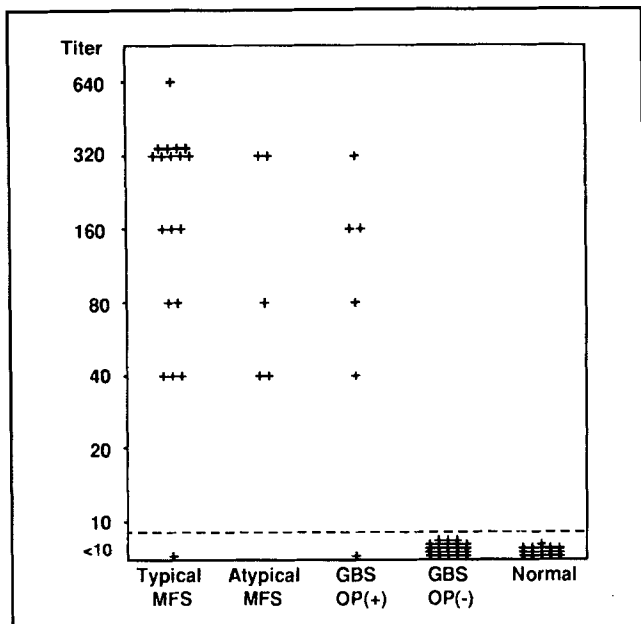


Figure 1. Anti-GQ_{1b} IgG antibody titers. Eighteen of the 19 patients with typical Miller Fisher syndrome (MFS), all five patients with atypical MFS, and five of the six patients with Guillain-Barré syndrome with ophthalmoplegia (GBS-OP[+]) show increased titers ranging from 1:40 to 1:640. The titers of patients with GBS without ophthalmoplegia (GBS-OP[-]) and those of the normal subjects are all less than 1:10.

In all 28 patients with anti-GQ_{1b} IgG antibody, anti-GT_{1a} IgG antibody also was detected in the TLC-enzyme immunoassay. The other antiglycolipid activities were detected in five of them: anti-GD_{1b} IgG activity in two (one with typical MFS, the other with GBS-OP[+]); anti-GD₃ IgG activity in one with GBS-OP(+); anti-GD_{1b} and relatively weak anti-GD₃ IgG activities in one with typical MFS (who also had IgM activities against GQ_{1b}, GM₁, and GD_{1b}); and anti-GQ_{1b} IgM activity in one with atypical MFS.

To investigate the cross-reactivity of the anti-GQ_{1b} and anti-GT_{1a} IgG antibodies, we did absorption studies on four patients: two with typical MFS, one with atypical MFS, and one with GBS-OP(+). Preliminary incubation with GQ_{1b} or GT_{1a} reduced the antibody activities against both GQ_{1b} and GT_{1a} in the sera of all the patients.

Serial studies of anti-GQ_{1b} IgG were done on 18 patients, five of whom were given prednisolone orally and three of whom received plasmapheresis therapy. The first serum samples had the highest anti-GQ_{1b} IgG activities, which decreased with time whether or not prednisolone or plasmapheresis treatment had been used. No patient showed a reincrease in anti-GQ_{1b} IgG activity except for one who showed a little reincrease in the activity without clinical exacerbation in association with the nonspecific rebound of the total serum IgG concentration after extensive plasmapheresis. Profiles of the anti-GQ_{1b} IgG activity and the clinical manifestations of one patient from whom sera could be

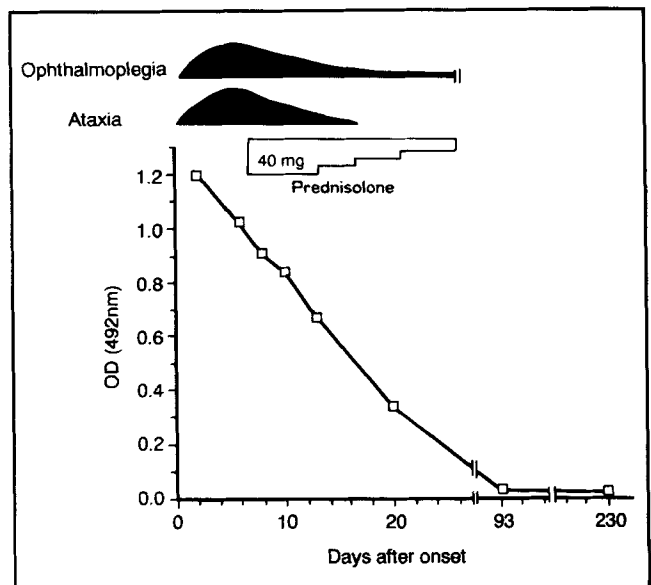


Figure 2. Profiles of the clinical manifestations and anti-GQ_{1b} IgG activity, at a serum dilution of 1:80, of a patient with typical MFS. Ophthalmoplegia represents the degree of diplopia in lateral gaze, and ataxia, unsteadiness of gait. The ophthalmoplegia was bilateral abducens palsy. Anti-GQ_{1b} IgG activity is present 2 days after neurologic onset but decreases during the progression phase before the administration of prednisolone.

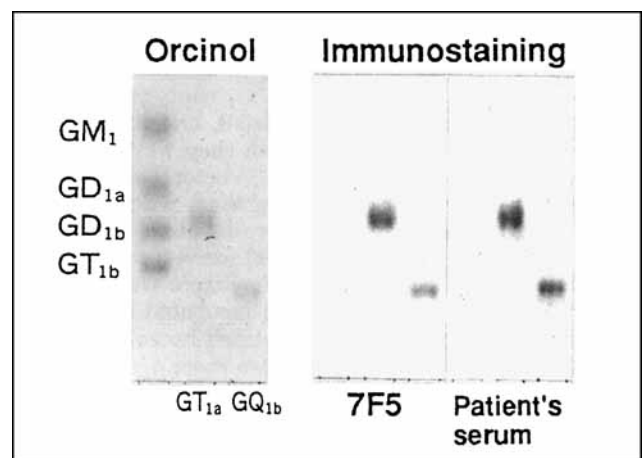
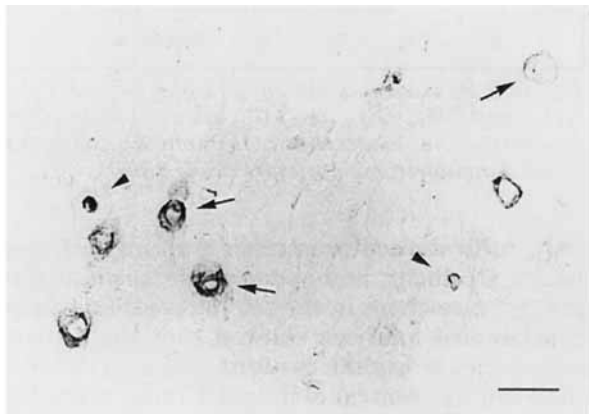


Figure 3. Binding characteristics of mAb 7F5. MAb 7F5 has the same specificity as the patient's sera. It reacts with GQ_{1b} and GT_{1a} but not with GM₁, GD_{1a}, GD_{1b}, or GT_{1b}.

obtained at short intervals from 2 days after neurologic onset are shown in figure 2. Although his clinical manifestations worsened during the first 6 days after onset, anti-GQ_{1b} IgG antibody activity was highest in the first sampling taken 2 days after onset, decreasing even in the phase when his clinical signs were exacerbated before prednisolone administration.

Immunohistochemical and biochemical studies. Binding characteristics of mAb 7F5 are shown in

Figure 4. Oculomotor nerve immunostained with an anti-GQ_{1b} monoclonal antibody, mAb 7F5. (A) Cross section. The many doughnut-shaped stainings appear to consist of unstained axoplasm surrounded by a stained portion. The entire surrounding portion is stained in some of them (arrows) and the inner part only in others (arrowheads). (Bar = 25 μm.) (B) Longitudinal section. The paranodal regions of Schwann cells mainly appear to be stained. (Bar = 10 μm.)



A



B

figure 3. This mAb recognizes GQ_{1b} and GT_{1a} but not GM₁, GD_{1a}, GD_{1b}, or GT_{1b}. Purified mouse IgG_{2a} used as the isotype control did not react with any of the gangliosides.

Unique immunostaining by mAb 7F5 was seen in the oculomotor, trochlear, and abducens nerves in both the proximal portion of the subarachnoid space and the distal portion adjacent to the external ocular muscles. Doughnut-shaped stainings were present in cross section (figure 4A). In longitudinal sections, the paranodal regions were immunostained (figure 4B). These stainings were lessened by prior treatment of a section with chloroform:methanol (1:1) for 1 minute. Similar stainings were not, or only rarely, present in the other peripheral nerve tissues. Some large DRG cells had granular stainings in their cytoplasm. In the CNS, the gray matter in the spinal cord and brainstem and the deep cerebellar nuclei generally had faint stains, but these were much weaker than the staining of the oculomotor, trochlear, and abducens nerves. No nerve fiber bundles in the spinal cord and brainstem, including the intramedullary portions of the oculomotor and abducens nerves, were stained.

Analysis of the total ganglioside fractions showed that the human oculomotor nerve had GQ_{1b}, but no GT_{1a} detectable in this condition (figure 5). Although GQ_{1b} was present in both the ventral and dorsal roots, its content was greater in the oculomotor nerve for the same wet weight of tissue. In the TLC-enzyme immunoassay of the total gan-

glioside fractions, both mAb 7F5 and the patient's serum immunostained only GQ_{1b} among the gangliosides. No specific bands were detected by the Western blot analysis.

Discussion. We confirmed the previously reported serum anti-GQ_{1b} IgG antibody in patients in the acute phase of typical MFS.⁶ Moreover, we found anti-GQ_{1b} IgG antibody in patients with atypical MFS and those with GBS-OP(+), whereas no patient with GBS-OP(-) had it. Although the grouping of these patients was arbitrary, the correlation with the clinical manifestations suggests that this antibody is most closely associated with acute ophthalmoplegia in MFS and GBS.

We detected anti-GT_{1a} IgG activity in all patients with anti-GQ_{1b} IgG activity. The absorption studies showed that the anti-GQ_{1b} and anti-GT_{1a} antibodies cross-reacted with each other's antigen, which suggested that the same IgG antibody bound to both antigens.

Serum anti-GQ_{1b} activities were present in patients with IgM paraproteinemia and sensory-dominant neuropathy,^{18,19} but presumably without ophthalmoplegia, the anti-GQ_{1b} activities possibly being derived from IgM M proteins binding to a broad spectrum of polysialogangliosides. In our series, the IgG antibodies against GQ_{1b} and GT_{1a} in patients with typical and atypical MFS and with GBS-OP(+) reacted with none of the other gangliosides examined or, at most, only with a very few

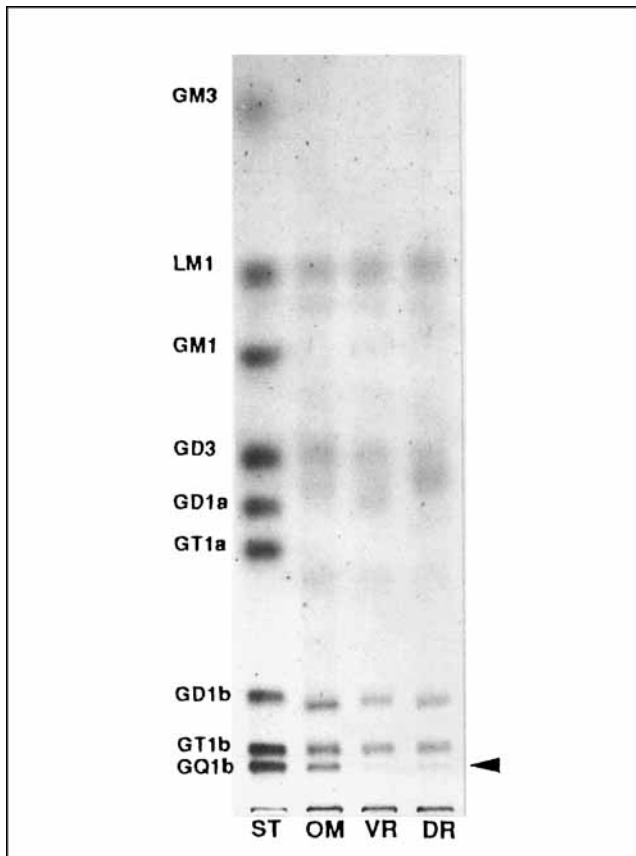


Figure 5. Ganglioside patterns. Ganglioside fractions corresponding to 4.0 mg wet weight of tissue were chromatographed on an HPTLC plate then stained with resorcinol. The oculomotor nerve (OM) has more GQ_{1b} than do the ventral root (VR) and dorsal root (DR) of the lumbar spinal cord for the same wet weight (arrowhead). ST = mixture of known standard gangliosides (500 ng each).

(GD_{1b}, GD₃, or GM₁) in four of the 28 patients, but never with GT_{1b} and GD_{1a}. The disialosyl residue attached to the nonreducing terminal of the gangliotetraose structure (figure 6, underlined) may be critical for the anti-GQ_{1b} antibody associated with ophthalmoplegia.

In the typical MFS, atypical MFS, and GBS-OP(+) groups, two patients (one with typical MFS, the other with GBS-OP(+)) did not have the anti-GQ_{1b} IgG antibody. Both of these anti-GQ_{1b}-negative patients had isolated complete unilateral abducens palsy, whereas the patients with anti-GQ_{1b} IgG antibody had bilateral ophthalmoplegia: 14 total external ophthalmoplegia, 13 bilateral abducens palsy with or without other impairment, and one bilateral palsy of adduction and infraduction. Although abducens palsy is the most common early extraocular sign in MFS and GBS, isolated complete unilateral abducens or oculomotor palsy, as in the two patients in our series, is rare.²⁰ The underlying mechanism of ophthalmoplegia in these two patients without anti-GQ_{1b} antibody may differ from that in patients with the antibody.

The immunohistochemical study with the anti-

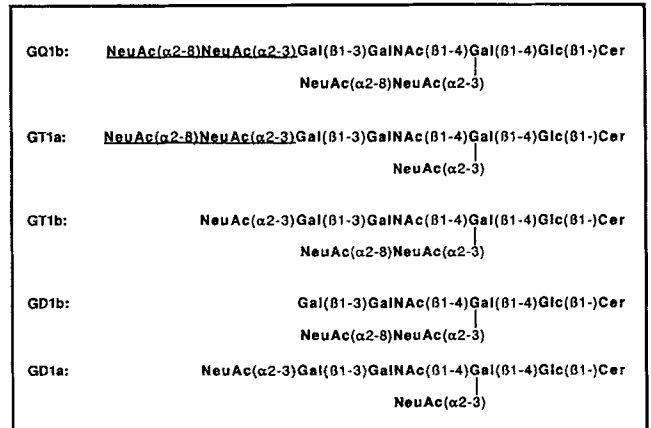


Figure 6. Structures of the gangliosides GQ_{1b}, GT_{1a}, GT_{1b}, GD_{1b}, and GD_{1a}. GQ_{1b} and GT_{1a} have a disialosyl residue in common that is attached to the nonreducing terminal of the gangliotetraose structure (underlined).

GQ_{1b} mAb showed prominent staining in the oculomotor, trochlear, and abducens nerves that was not present elsewhere in the nervous system examined. Biochemical analysis showed that the oculomotor nerve had a higher content of ganglioside GQ_{1b} than did the ventral and dorsal roots of the lumbar spinal cord for the same wet weight of tissue. Taking into account the negative results of Western blot analysis and the positive results of the TLC-enzyme immunoassay, we suspect that the mAb recognized ganglioside GQ_{1b} in the oculomotor nerve. The unique distribution of GQ_{1b} is compatible with the clinical association of anti-GQ_{1b} antibody with ophthalmoplegia.

The GQ_{1b} epitope was expressed mainly in the paranodal regions of the extramedullary portion of the three cranial nerves involved in ocular movement. The paranodal regions of Schwann cells have special features indicative of their involvement in impulse generation,^{21,22} and damage to these regions blocks impulse generation at the nodes of Ranvier.²³ These findings suggest the probable attack site of the anti-GQ_{1b} antibody and the cause of the conduction block. The extramedullary distribution of the attack site is compatible with the neuropathologic findings in a patient with GBS with ophthalmoplegia who had demyelinating lesions in the extramedullary portions of the oculomotor and abducens nerves.²⁴

Although anti-GQ_{1b} antibody might be elevated as a result of tissue destruction, we feel it is involved in pathogenesis because of the high titer as early as 1 or 2 days after neurologic onset. Close serial examination of one patient showed that the peak of anti-GQ_{1b} IgG activity preceded the maximum neurologic manifestations. In another patient, anti-GQ_{1b} IgG antibody reincreased transiently, without clinical exacerbation, in association with the nonspecific rebound of the total serum IgG concentration after extensive plasmapheresis, suggesting that the damage was not dependent on the intensity of the antibody activity alone. A time lag

between the peaks of antibody activity and the symptoms would be expected if the antibody is responsible for the initiation of damage.

Acknowledgments

We thank Dr. Takeshi Hayakawa, Dr. Tomoji Watanabe, Dr. Fumihiko Sato, Dr. Syuji Nishimura, Dr. Yutaka Awaya, Dr. Chikako Kimura, Dr. Hiroshi Abe, Dr. Keiko Kamakura, Dr. Kohei Ota, Dr. Hisayoshi Yoshida, Dr. Takamoto Kizu, Dr. Shigeo Murayama, Dr. Masanari Kunimoto, Dr. Kiyoharu Inoue, Dr. Fumiko Takano, Dr. Masanobu Uchikata, Dr. Ichiro Imafuku, Dr. Yoshitomo Takahashi, Dr. Etsuko Takeuchi, Dr. Toru Ishikawa, Dr. Kaneko Tojyo, Dr. Hiroyuki Shimada, Dr. Shigekazu Kobayashi, Dr. Motomu Tsubata, Dr. Keiichiro Nakano, Dr. Hideji Hashida, Dr. Keiko Ide, Dr. Yasuhisa Sakurai, Dr. Tadashi Komiya, Dr. Tai Miyazaki, Dr. Mitsuru Kawai, Dr. Masaki Kamei, Dr. Tomotaka Yamamoto, Dr. Masayuki Tanemoto, Dr. Tatsushi Toda, and Dr. Koichi Hashimoto for allowing us to study their patients. We also are deeply grateful to Dr. Hatsue Waki and Dr. Susumu Ando for providing technical advice and the ganglioside GT_{1a}.

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Neurology 1993;43;1911

DOI 10.1212/WNL.43.10.1911

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