

# Anti-GM<sub>1</sub> antibodies in patients with Guillain-Barré syndrome

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## Abstract

Anti-GM<sub>1</sub> antibodies were measured in 22 patients with the Guillain-Barré syndrome (GBS) and compared with anti-GM<sub>1</sub> antibody activity in patients with other neurological or immunological diseases and in normal subjects. Four out of 22 patients with GBS had raised IgM, IgG, or IgA anti-GM<sub>1</sub> antibody activities. All four patients were tetraparetic with only minimal or no sensory deficit. Three of the patients had highly raised antibody activity and showed severe residual deficits, while of the remaining patients with GBS, only one remained severely affected. One patient had anti-GM<sub>1</sub> antibodies specific for GM<sub>1</sub>, whereas the other three patients showed antibody activity with asialo-GM<sub>1</sub> or GD<sub>1b</sub>. The presence of anti-GM<sub>1</sub> antibodies may define a subgroup of patients with GBS who have a poor prognosis.

Increased titres of IgM anti-GM<sub>1</sub> antibodies are associated with lower motor neuron disease, sensorimotor neuropathy, or motor neuropathy.<sup>1</sup> Therapeutic reduction of antibody concentrations is associated with clin-

ical improvement, suggesting that the antibodies have a role in the disease.<sup>2-4</sup>

To determine whether anti-GM<sub>1</sub> antibodies are also increased in the Guillain-Barré syndrome (GBS) we measured antibody activities in 22 patients with the syndrome and compared them with anti-GM<sub>1</sub> antibody activity in patients with other neurological or immunological diseases and in normal subjects.

## Patients and methods

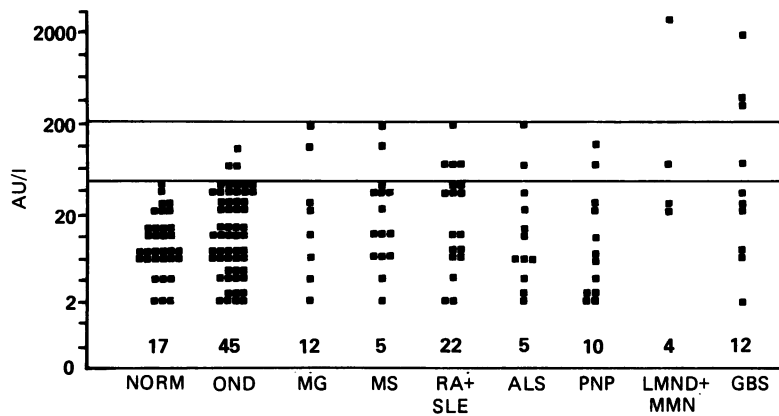
### PATIENTS

Included in the study were 22 patients with GBS (13 female, nine male, mean age 43 years) seen during the acute phase of the disease at the Department of Neurology of the University Hospital, Groningen. All patients fulfilled the criteria for GBS of the National Institute of Neurological and Communicative Disorders and Stroke.<sup>5</sup> Serum samples from patients with myasthenia gravis (20), multiple sclerosis (20), rheumatoid arthritis (20), systemic lupus erythematosus without neurological disease (20), amyotrophic lateral sclerosis (18), various types of neuropathies (22), lower motor neuron disease or multifocal motor neuropathy (8), other neurological diseases (stroke (20),

Table 1 Clinical and laboratory data on 22 patients with acute Guillain-Barré syndrome

Case No	Age (years)	PD	Paresis	Ventilation	Sensory deficit	MNCV (m/s)	EMG	Recovery (after 1 year)	Anti-GM <sub>1</sub>		
									IgM	IgG	IgA
1	10	GI	UE3 LE3	-	S- P-	M, 40.5	++	Bad	+	++	-
2	47	-	UE<3 LE<3	+	S- P+	M, 47	++	Bad	++	+	-
3	53	GI	UE<3 LE<3	+	S- P+	M, nm	++	Bad	+	++	++
4	28	UR	UE3 LE3	-	S- P+	M, 52	+	Good	-	+	+
5	16	UR	UE3 LE3	+	S+ P+	M, 52	+	Good	-	-	-
6	17	-	UE3 LE3	+	S+ P++	M, 32.5	++	Moderate	-	-	-
7	18	GI	UE3 LE<3	+	S+ P++	M, 43	-	Good	-	-	-
8	24	UR	UE4 LE3	-	S+ P+	M, 42	+	Good	-	-	-
9	28	UR	UE4 LE4	-	S+ P+	P, 34	-	Good	-	-	-
10	33	GI	UE<3 LE<3	+	S+ P++	M, nm	++	Bad	-	-	-
11	33	UR	UE4 LE4	-	S+ P+	M, 50	-	Good	-	-	-
12	36	UR	UE4 LE<3	-	S+ P++	P, 34	-	Good	-	-	-
13	43	GI	UE4 LE3	-	S+ P+	M, 58	-	Good	-	-	-
14	47	-	UE4 LE4	-	S+ P+	P, 40	-	Good	-	-	-
15	51	UR	UE4 LE4	-	S+ P++	M, 48	+	Good	-	-	-
16	59	-	UE5 LE4	-	S- P+	P, 44	+	Good	-	-	-
17	59	GI	UE<3 LE<3	+	S+ P++	M, 26	++	Moderate	-	-	-
18	61	-	UE<3 LE<3	+	S- P+	M, 47	nd	Dead	-	-	-
19	65	UR	UE4 LE4	-	S- P+	M, 25	+	Good	-	-	-
20	68	UR	UE<3 LE<3	+	S+ P++	M, 40	++	Good	-	-	-
21	70	UR	UE3 LE4	-	S+ P++	P, 32	+	Moderate	-	-	-
22	72	UR	UE3 LE<3	+	S+ P+	M, 20.5	+	Moderate	-	-	-

PD = prodromal disease: - = none, GI = gastrointestinal, UR = upper respiratory. Paresis: UE = upper extremity, LE = lower extremity, 5 = MRC (Medical Research Council) 5, 4 = MRC 4, 3 = MRC 3, <3 = MRC 0-2 (mean of three muscles). Ventilation: - = no additional ventilation, + = additional ventilation. Sensory deficit: S = superficial, - = normal, + = stocking/glove deficit, ++ = more, P = proprioceptive, - = normal, + = diminished proprioception, ++ = absent proprioception; MNCV = motor nerve conduction velocity: M = median nerve (normal  $\geq 50$  m/s); P = peroneal nerve (normal  $\geq 42$  m/s), nm = not measurable. EMG = electromyogram: - = no denervation, + = sporadic denervation potentials, ++ = severe denervation, nd = not done. Recovery (after one year): good = no residual motor signs/slight sensory signs and/or paraesthesia; moderate = residual motor and sensory signs/fully ambulant; bad = serious residual motor signs/braces or wheelchair; dead = died during acute phase. Anti-GM<sub>1</sub> antibodies: - = not raised, + = mildly raised (<1000 AU/l), ++ = highly raised.



Activities of IgM anti-GM<sub>1</sub> antibodies in arbitrary units per litre (AU/l).

NORM = normal subjects; OND = other neurological diseases; MG = myasthenia gravis; MS = multiple sclerosis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; ALS = amyotrophic lateral sclerosis; PNP = various types of polyneuropathies; LMND = lower motor neuron disease; MMN = multifocal motor neuropathy; GBS = Guillain-Barré syndrome. Numbers given along x axis represent numbers of patients with antibody activities of < 2 AU/l.

epilepsy (20), concussion (20), Alzheimer's disease (20), Parkinson's disease (20)), as well as normal subjects (50), served as controls.

#### ANTI-GM<sub>1</sub> ANTIBODY ASSAYS

Anti-GM<sub>1</sub> antibodies were measured by enzyme linked immunosorbent assay (ELISA). Microwells in a flat bottomed, 96 well ELISA plate (Hycult, Diagnostic Systems, Peyrelevalde, France) were coated with 100  $\mu$ l methanol containing 5  $\mu$ g/ml GM<sub>1</sub> (Sigma, St Louis, Missouri, United States). In other microwells only methanol was added to act as control. The methanol evaporated overnight. Wells were saturated with 100  $\mu$ l ELISA solution containing 1% bovine serum albumin (BSA), in phosphate buffered saline (0.15M NaCl, 0.01M NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4) for four hours. 100  $\mu$ l of each patient's serum diluted 1:50 in ELISA solution was added in duplicate and the plates incubated overnight. All incubations and washes were carried out at 4°C. The plates were then washed five times in ELISA solution, and peroxidase-conjugated rabbit antibodies to human IgM, IgG, or IgA (Dakopatts, Glostrup, Denmark) diluted 1:1000 in ELISA solution were added. Antibody binding was detected spectrophotometrically at 492 nm as previously described.<sup>1</sup>

The optical density (OD) was compared with the OD obtained from the serum of a patient with raised IgM anti-GM<sub>1</sub> antibodies (case HU<sup>2</sup>) or with that of a patient with raised IgG and IgA anti-GM<sub>1</sub> antibodies (case 3, table 1). Undiluted serum from these positive controls (used as standards) was set at 100 000 AU/l (arbitrary units per litre). Standard curves were obtained by using eight dilutions of the positive control in each experiment. With a log-logit transformation<sup>6</sup> computer program each patient's serum GM<sub>1</sub> antibody activity was determined by plotting the OD on a standard curve and the activity of antibodies was thus calculated in AU/l. Calculated values in wells coated with BSA were subtracted from those in experimental wells coated with GM<sub>1</sub>. For patients with high initial anti-GM<sub>1</sub> antibody

readings serum samples were diluted until an OD corresponding to the linear part of the standard curve was obtained.

To determine the fine specificities of the anti-GM<sub>1</sub> antibodies serum samples were also tested for antibody binding to asialo-GM<sub>1</sub> and GD<sub>1b</sub> (Bio-Carb, Lund, Sweden), which share a terminal Gal( $\beta$ 1-3)GalNAc determinant with GM<sub>1</sub>. Standard curves were obtained from serum samples of patients with high antibody activity to asialo-GM<sub>1</sub> and GD<sub>1b</sub> (case HU<sup>2</sup>) for IgM antibodies to asialo-GM<sub>1</sub> and GD<sub>1b</sub>, case 1 (table 1) for IgG and IgA antibodies to asialo-GM<sub>1</sub>, case 3 (table 1) for IgG and IgA antibodies to GD<sub>1b</sub>. AU/l were set in the standard curves at a level where the OD corresponded to the OD in the standard curve for IgM anti-GM<sub>1</sub> antibodies.

#### Results

In normal individuals IgM anti-GM<sub>1</sub> antibody activity ranged from 0 to 34 AU/l (figure). In patients with neurological or immunological diseases 7% (range 0%–12%) had IgM anti-GM<sub>1</sub> antibodies in the range of 34 to 200 AU/l. This range was defined as borderline—not specific for one disease or syndrome. Only activities higher than 200 AU/l for IgM anti-GM<sub>1</sub> antibodies were considered to be raised. In the control groups one patient had increased activity of IgM anti-GM<sub>1</sub> antibodies (3450 AU/l). This patient had a multifocal motor neuropathy. Activities of IgG and IgA anti-GM<sub>1</sub> antibodies in normal controls were 0 AU/l and 0–2 AU/l respectively. In normal controls and in patients with GBS without anti-GM<sub>1</sub> antibodies activities of IgM, IgG, and IgA antibodies to asialo-GM<sub>1</sub> were 0–130 AU/l, 0–80 AU/l, and 0–100 AU/l respectively; the ranges of IgM, IgG, and IgA antibody activities to GD<sub>1b</sub> were 0–10 AU/l, 0–25 AU/l, and 0–10 AU/l respectively.

The clinical and laboratory data obtained for the 22 patients with GBS are shown in table 1. Increased activities of anti-GM<sub>1</sub> antibodies were present in four patients with GBS. In case 1 both IgM and IgG anti-GM<sub>1</sub> antibodies were raised. Case 2 showed predominantly raised IgM and mildly raised IgG anti-GM<sub>1</sub> antibodies. Case 3 showed predominantly raised IgG and mildly raised IgM anti-GM<sub>1</sub> antibodies. IgG and IgA anti-GM<sub>1</sub> antibodies were mildly raised in case 4. All four patients with increased anti-GM<sub>1</sub> antibodies had severe tetraparesis with only minimal or no sensory deficits. The patients in cases 1, 2, and 3, who had raised anti-GM<sub>1</sub> antibody activities, remained severely disabled. The patient in case 4, who had mildly raised anti-GM<sub>1</sub> antibodies, completely recovered. In contrast, only one out of 18 patients with GBS without anti-GM<sub>1</sub> antibodies had severe tetraparesis with minimal sensory deficits (case 18), and only one remained severely disabled (case 10).

Antibodies in cases 1 and 2 also bound to the gangliosides GD<sub>1b</sub> and asialo-GM<sub>1</sub> (table 2) and may have been specific for the Gal(B1-3)GalNAc epitope. In case 3, however, the antibodies were specific for GM<sub>1</sub>. The

Table 2 Fine specificities and course of raised activities of IgM, IgG, and IgA antibodies to GM<sub>1</sub>, asialo-GM<sub>1</sub> (aGM<sub>1</sub>), and GD<sub>1b</sub> (in AU/l, — = not raised)

Day	IgM			IgG			IgA		
	GM <sub>1</sub>	aGM <sub>1</sub>	GD <sub>1b</sub>	GM <sub>1</sub>	aGM <sub>1</sub>	GD <sub>1b</sub>	GM <sub>1</sub>	aGM <sub>1</sub>	GD <sub>1b</sub>
12	350	900	250	1800	2000	500	—	—	—
30	170	40	12	700	900	300	—	—	—
99	50	30	—	240	250	30	—	—	—
156	—	—	—	140	200	—	—	—	—
					Case 1				
3	1300	1480	950	130	270	250	—	—	—
17	520	690	490	—	—	—	—	—	—
30	220	250	160	—	—	—	—	—	—
35	180	250	130	—	—	—	—	—	—
101	23	70	25	—	—	—	—	—	—
472	—	—	—	—	—	—	—	—	—
					Case 2				
7*	110	—	—	7000	—	200	5000	—	100
10*	210	—	—	9000	—	200	4000	—	100
13*	200	—	—	9000	—	140	3000	—	150
17*	90	—	—	3500	—	140	2000	—	110
20*	90	—	—	2600	—	80	1000	—	50
22	120	—	—	2300	—	60	1500	—	240
34	20	—	—	1000	—	—	150	—	—
111	—	—	—	300	—	—	—	—	—
					Case 3				
5	—	—	—	600	—	550	55	—	200
17	—	—	—	200	—	300	3	—	100
32	—	—	—	30	—	200	—	—	—
103	—	—	—	—	—	—	—	—	—
159	—	—	—	—	—	—	—	—	—

\*Plasmapheresis.

antibodies in case 4 also bound to GD<sub>1b</sub> but not to asialo-GM<sub>1</sub>.

The IgM, IgG, and IgA antibody activities to GM<sub>1</sub>, asialo-GM<sub>1</sub> and GD<sub>1b</sub> were highest at the onset of the disease and decreased with time (table 2).

### Discussion

Patients with GBS antibody binding to glycolipids, including gangliosides, has been reported.<sup>7-11</sup> Ilyas *et al* reported antibodies to several gangliosides, but not to GM<sub>1</sub> in five out of 26 patients with GBS, and antibody titres decreased with clinical improvement.<sup>8</sup> Svennerholm *et al* detected antiganglioside antibodies in 39 out of 50 patients with GBS, including one with anti-GM<sub>1</sub> antibodies, but no correlation was found with the severity or course of the disease.<sup>9</sup> Kusunoki *et al* reported antibodies to gangliosides in eight out of 11 cases of GBS, four with IgM anti-GM<sub>1</sub> antibodies.<sup>10</sup> Yuki *et al* recently reported two cases of an axonal form of GBS with anti-GM<sub>1</sub> antibodies. The illness in both patients was preceded by campylobacter enteritis.

We detected increased activity of anti-GM<sub>1</sub> antibodies in four out of 22 patients with GBS. These patients predominantly had motor neuropathy with severe denervation and the three patients with the highest activities remained severely disabled. The patient in case 3, however, also had markedly slowed motor nerve conduction velocities. This patient was the only one with anticampylobacter antibodies, indicating that anti-GM<sub>1</sub> antibodies are not specific for this organism. It is not known whether the anti-GM<sub>1</sub> antibodies define a distinct syndrome or whether they occur in some cases of otherwise typical or severe GBS. They could contribute to the disease by binding to the surface of neurons or

to the nodes of Ranvier, as has previously been suggested.<sup>12,13</sup>

In cases 1, 3, and 4 the anti-GM<sub>1</sub> antibodies were predominantly of the IgG and IgA isotypes, suggesting that the anti-GM<sub>1</sub> response in these cases was driven by T cells.<sup>13</sup> Since gangliosides by themselves do not typically induce a T cell response, the anti-GM<sub>1</sub> antibodies might be induced by complexes that contain both GM<sub>1</sub> and a T cell antigen. The response could be stimulated by bacteria or viruses that bear GM<sub>1</sub> or a cross reactive antigen, by complexes composed of toxins or other proteins from foreign organisms that bind to GM<sub>1</sub> as a receptor, or by neural complexes that contain GM<sub>1</sub> associated with a protein recognised by T cells.<sup>14,15</sup> In patients with campylobacter, the enterotoxin which binds to GM<sub>1</sub><sup>16</sup> might cause disease or induce antibodies to the GM<sub>1</sub>-toxin complex. The presence of antibodies to several neural glycolipids in GBS supports a model in which the neural tissue itself is the source of the antigenic stimulus, but the breakdown of tissue alone is unlikely to be responsible for the antibodies, as raised titres are not found in other types of neuropathies or inflammatory diseases as the antibodies occur early in the course of the disease. A neurotropic virus or an activated latent virus might incorporate neural glycolipids into its coat and induce an autoimmune response<sup>15</sup> or the antibodies could be generated in the course of an ongoing T cell response to a neural antigen associated with GM<sub>1</sub>. Investigations of the mechanisms responsible for induction of anti-GM<sub>1</sub> antibodies in GBS might thereby provide clues to the identity of the T cell antigen.

The study suggests that patients with acute GBS who have highly raised anti-GM<sub>1</sub> antibody activities constitute a subgroup with motor neuropathy predominantly and substan-

tial axonal damage. Detection of anti-GM<sub>1</sub> antibodies in patients with GBS therefore may be of prognostic value. Further elucidation of the pathogenic role of these autoantibodies in GBS may help in developing more specific and effective treatment.

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