Acta Neurol Scand 2002: 106: 54–57
Printed in UK. All rights reserved

ACTA NEUROLOGICA
SCANDINAVICA
ISSN 0001-6314

Anti-GM1 ganglioside antibodies in Parkinson's disease

Zappia M, Crescibene L, Bosco D, Arabia G, Nicoletti G, Bagalà A, Bastone L, Napoli ID, Caracciolo M, Bonavita S, Di Costanzo A, Gambardella A, Quattrone A. Anti-GM1 ganglioside antibodies in Parkinson's disease.
Acta Neurol Scand 2002: 106: 54–57. © Blackwell Munksgaard 2002.

Objectives – To determine whether anti-GM1 antibodies are increased in Parkinson's disease (PD). *Methods* – Serum immunoglobulin M (IgM) and IgG anti-GM1 antibodies were detected by enzyme-linked immunosorbent assay (ELISA) in 147 patients with PD and in 186 age-matched normal control subjects. Sera were assayed at initial dilution of 1:800 for IgM and 1:200 for IgG and were considered positive at absorbance values exceeding the value of 0.05 for IgM and 0.1 for IgG. *Results* – Forty patients with PD (27.2%) had sera positive for IgM anti-GM1 antibodies, whereas only five normal controls (2.7%) resulted positive ($P < 0.0001$). Most of patients (75%) with positive sera had a tremor-dominant form of PD. Only two patients with PD (1.4%) and none of normal controls had sera positive for IgG anti-GM1 antibodies. *Conclusion* – A consistent portion of parkinsonians, mainly with a tremor-dominant form of PD, may have increased circulating IgM anti-GM1 antibodies.

M. Zappia[1,3], L. Crescibene[3], D. Bosco[1], G. Arabia[1], G. Nicoletti[3], A. Bagalà[3], L. Bastone[3], I. D. Napoli[3], M. Caracciolo[3], S. Bonavita[2], A. Di Costanzo[2], A. Gambardella[1,3], A. Quattrone[1,3]

[1]Institute of Neurology, University of Catanzaro, Catanzaro; [2]Institute of Neurology, 2nd University of Naples, Naples; [3]Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone (CS), Italy

Key words: Parkinson's disease; anti-GM1 antibodies; GM1 ganglioside

Prof. Aldo Quattrone, Clinica Neurologica, Policlinico Universitario Mater Domini, Via T. Campanella, 88100 Catanzaro, Italy
Tel.: ++39-0961-775322
Fax: ++39-0961-777775
e-mail: quattrone.unicz@interbusiness.it

Accepted for publication November 28, 2001

High titer serum antibodies to GM1 ganglioside are commonly found in patients with lower motor neurone syndromes (1), multifocal motor neuropathy (2), and Guillain–Barré syndrome (3). It has been suggested that the antibodies could have a role in the pathogenesis of these diseases, because a reduction of antibody titers by immunosuppression is followed by clinical improvement in some cases (4). Increased circulating anti-GM1 antibodies have been also reported in multiple sclerosis (5), especially in the primary progressive type, in which they may serve as a marker of axonal damage. In Parkinson's disease (PD), an earlier study (6) reported high titers of anti-GM1 antibodies only in parkinsonian demented patients, but those results were not subsequently confirmed by other studies (2, 7). These studies (2, 6, 7), however, involved only a few patients with PD, making it difficult to draw meaningful conclusions on this issue. Nevertheless, the ascertainment of antibodies to GM1 ganglioside in PD may be of interest, especially because GM1 ganglioside is involved in the function of damaged nigrostriatal dopaminergic neurones (8–11).

In the present study, we examined the antibody response to GM1 ganglioside in a large group of patients with PD and in healthy subjects, to determine whether anti-GM1 antibodies are increased in PD.

Subjects and methods

We studied 147 patients with PD (82 men and 65 women; mean age ± SD, 65.8 ± 8.7 years), diagnosed according to the United Kingdom PD Society Brain Bank criteria (12), and 186 normal control subjects (111 men and 75 women; mean age ± SD, 67.1 ± 8.9 years). Patients with PD were consecutively selected among the outpatients treated with L-dopa and attending the Institute of Neurology of the University of Catanzaro from January 1999 to June 2000. Control subjects were drawn randomly during the same time period among the unselected sample of subjects that were

part of a study on aging. Both patients and control subjects received a complete neurological examination with a general cognitive evaluation by the Mini Mental State Examination (MMSE), in order to exclude neurological diseases (except of PD in patients), especially neuromuscular disorders. Each subject gave a fully informed consent for the study.

Serum anti-GM1 antibodies (IgM and IgG) of patients and controls were measured by enzyme-linked immunosorbent assay (ELISA) according to standard procedures (13, 14). Briefly, purified bovine GM1 ganglioside (Sigma, St Louis, MO, USA) at concentration of 1 μg/ml in ethanol was added to microwell plates (Labsystem, Helsinki, Finland) until evaporation at room temperature. Wells were saturated for 2 h with blocking solution pH 7.4: for IgM procedure, the blocking solution was 100 μl phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma, St Louis, MO, USA), whereas for IgG procedure the blocking solution consisted of 300 μl PBS containing 10% foetal calf serum (14) (FCS; Hyclone Laboratories, Logan, UT, USA). After washing three times with washing solution (PBS plus 1% BSA for IgM; PBS plus 10% FCS for IgG), the plates were incubated overnight at 4°C with 50 μl of patients' sera at initial dilution of 1:800 for IgM and 1:200 for IgG. After washing the wells five times in washing solution, IgM and IgG reactivity was detected by adding 100 μl peroxidase-conjugated rabbit antihuman IgM or IgG (Sigma, St Louis, MO, USA) diluted in blocking solution 1000-fold or 2000-fold, respectively, and incubating plates at room temperature for 1 h. Again, the wells were washed five times before adding 50 μl of o-phenylenediamine (OPD) solution (Abbott Laboratories, Chicago, IL, USA). The reaction was stopped after 17 min with 50 μl of sulfuric acid 1 N. The absorbance of the reaction product was measured at 492 nm. Results were expressed as the difference between the absorbance obtained from GM1-coated wells and the absorbance obtained from BSA or FCS-coated wells. Serum was considered positive when this difference exceeded the value of 0.05 for IgM and the value of 0.1 for IgG; these cut-off values were based on initial control studies, considering for the tested initial serum dilutions the mean values plus 3 SD of absorbance values of healthy subjects. Sera showing absorbance values above these cut-off levels were further titrated by twofold dilution until negative.

Statistical analysis testing the differences of anti-GM1 antibodies between patients and controls was carried out with the chi-square test, whereas demographic and clinical data were compared according to specific data distributions, using the unpaired *t*-test, the Mann–Whitney *U*-test, and the chi-square test when appropriate.

Results

Demographic data (age and gender) of patients and normal subjects were similar. The clinical characteristics of patients with PD are shown in Table 1: most patients had a moderate disease and about 60% of patients presented with a tremor-dominant form of PD. All patients had a good clinical response to L-dopa, and 40% of patients exhibited drug-induced dyskinesias.

Figure 1 shows the serum absorbance values of each subject tested for IgM and IgG anti-GM1 antibodies at initial serum dilutions 1:800 and 1:200, respectively. Concerning IgM antibodies (Fig. 1A), 40 of 147 (27.2%) patients with PD had sera with positive absorbance values (i.e. above the cut-off level of 0.05), whereas only 5 of 186 (2.7%) normal controls were positive ($P < 0.0001$). Moreover, among the subjects who were positive at serum dilution 1:800, 12 patients with PD were also positive with titer up to 1:1600, one patient was positive up to 1:3200 and another patient up to 1:6400, whereas none of normal subjects were positive to further titration. Concerning IgG anti-GM1 antibodies (Fig. 1B), only two patients with PD (1.4%) and none of control subjects had absorbance values above the cut-off level at initial serum dilution 1:200 (difference not significant).

Comparing the clinical characteristics of patients who had positive or negative absorbance values of anti-GM1 IgM antibodies (Table 2), no differences were evident for age, gender, cognitive status, duration and severity of PD, L-dopa dosage and percentage of dyskinetic patients. The only clinical difference was a more frequent tremor-dominant form of PD in patients with positive IgM values (75%) with respect to patients who were negative, in which tremor-dominant and akinetic-rigid forms were almost equally distributed.

Table 1 Clinical characteristics of 147 patients with Parkinson's disease[a]

Hoehn–Yahr (stage)	2.7 ± 1
Mini mental state (score)	23 ± 5.8
Duration of disease (months)	82 ± 57.9
Daily L-dopa dose (mg)	509.3 ± 265
Akinetic-rigid form (no. of patients)	59
Tremor-dominant form (no. of patients)	88
L-Dopa-induced dyskinesias (no. of patients)	59

[a] Plus–minus values are mean ± SD.

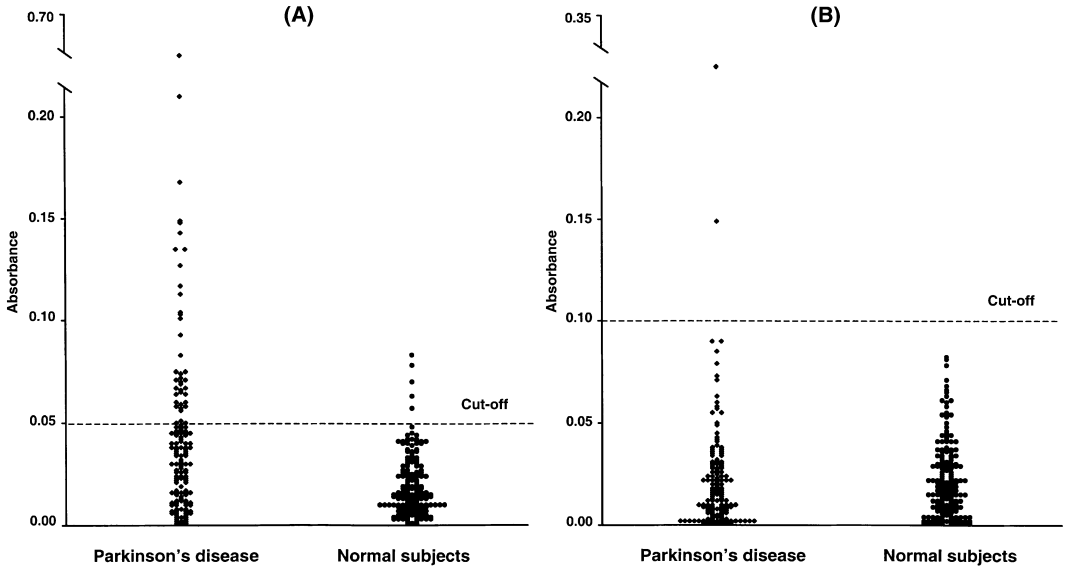

Figure 1. Absorbance values in ELISA assays of IgM (A) and IgG (B) anti-GM1 antibodies in the sera of 147 patients with Parkinson's disease and 186 age-matched normal control subjects. Sera were diluted at 1:800 for IgM assay and at 1:200 for IgG assay.

Table 2 Clinical characteristics of patients with Parkinson's disease resulting positive or negative to IgM anti-GM1 antibodies assay at initial serum dilution of 1:800[a]

	IgM positive ($n = 40$)	IgM negative ($n = 107$)	*P*-value
Age (years)	64.2 ± 8.9	66.3 ± 8.6	NS
Gender (men/women)	22/18	60/47	NS
Hoehn–Yahr (stage)	2.7 ± 0.9	2.6 ± 1	NS
Mini mental state (score)	23.6 ± 5.3	22.7 ± 6	NS
Duration of disease (months)	82.5 ± 54.6	81.7 ± 59.4	NS
Daily L-Dopa dose (mg)	482.2 ± 286.1	514.5 ± 257.4	NS
Form of PD (tremor/akinetic-rigid)	30/10	58/49	< 0.05
L-Dopa-induced dyskinesias (yes/no)	16/24	43/64	NS

[a] Plus–minus values are mean ± SD. Patients were considered positive when the serum absorbance value was above the cut-off level of 0.05.
NS = not significant.

Discussion

Our study demonstrates that more than one-quarter of patients with PD had sera with increased IgM anti-GM1 antibodies, and most of these patients presented with a tremor-dominant form of PD. Conversely, IgG anti-GM1 antibodies were not consistently increased as compared with normal controls. Previous studies, at variance with our results, reported normal levels of anti-GM1 antibodies in parkinsonian patients (2, 7). These studies, however, involved only a few patients with PD, being specifically designed to address anti-GM1 antibodies in other diseases. Thus, their results could not be compared with our data deriving from a large population of parkinsonian subjects.

The present findings, however, should be interpreted with caution, because the significance of increased circulating anti-GM1 antibodies in non-inflammatory diseases remains to be fully understood. Indeed, high titers of anti-GM1 antibodies have been reported in various conditions. Only in some immune-mediated motor nerve disorders, such as multifocal motor neuropathy (1, 2) and a motor axonal variant of Guillain–Barré syndrome (3), has a pathogenic role of anti-GM1 antibodies been recognized. In other diseases not considered primarily inflammatory, but in which anti-GM1 antibodies were found moderately increased [such as amyotrophic lateral sclerosis (15), multiple sclerosis (5), Alzheimer's disease (6), epilepsy (16) and PD, as demonstrated in the present study], it is unclear whether the anti-GM1 antibodies have a role in the associated disease or are only an associated abnormality. On the other hand, we cannot exclude that the presence of increased anti-GM1 antibodies in PD could be related to some inflammatory process, as recent work from human and animal studies has provided evidence of an inflammatory process also in PD, expressed as proliferation of activated microglia in the substantia nigra and elevation of cytotoxic cytokines (17).

The occurence of anti-GM1 antibodies in PD suggests that GM1 ganglioside may be involved in the function of nigrostriatal dopaminergic neurons. Indeed, it has been reported that GM1 could have

a neurotrophic-like activity in animal models of PD by stimulating the rescue of damaged dopaminergic neurons (8), by restoring dopamine striatal levels (9), and by increasing the activity of tyrosine hydroxylase in nigral dopaminergic neurons (10). In accordance with these observations (8–10), a recent placebo-controlled study showed that GM1 ganglioside administration was effective in improving motor function in patients with PD (11). Taking these evidences into account, we can hypothesize that the production of increased anti-GM1 antibodies in PD could be an autoreactive response determining an altered function of GM1 ganglioside in dopaminergic nigrostriatal neurons. This autoreactive process, however, should concern only a minority of parkinsonians, because we found increased IgM anti-GM1 antibodies in only 27% of our patients. Most of these patients had a tremor-dominant form of PD suggesting that the production of increased IgM anti-GM1 antibodies could be specific for a peculiar subpopulation of parkinsonians. We cannot exclude, however, that the presence of anti-GM1 antibodies in patients with PD could be an epiphenomena related to neuronal loss, such as that observed in other neurodegenerative disorders (6, 15). Further studies are needed to clarify and to confirm the present findings.

References

1. Pestronk A, Chaudhry V, Feldman EL et al. Lower motor neuron syndromes defined by patterns of weakness, nerve conduction abnormalities, and high titers of antiglycolipid antibodies. Ann Neurol 1990;**27**:316–26.
2. Sadiq SA, Thomas FP, Kilidireas K et al. The spectrum of neurologic disease associated with anti-GM1 antibodies. Neurology 1990;**40**:1067–72.
3. Kornberg AJ, Pestronk A, Bieser K et al. The clinical correlates of high-titer IgG anti-GM1 antibodies. Ann Neurol 1994;**35**:234–7.
4. Pestronk A, Cornblath DR, Ilyas AA et al. A treatable multifocal motor neuropathy with antibodies to GM1 ganglioside. Ann Neurol 1988;**24**:73–8.
5. Sadatipour BT, Greer JM, Pender MP. Increased circulating antiganglioside antibodies in primary and secondary progressive multiple sclerosis. Ann Neurol 1998;**44**: 980–3.
6. Chapman J, Sela BA, Wertman E, Michaelson DM. Antibodies to ganglioside GM1 in patients with Alzheimer's disease. Neurosci Lett 1988;**86**:235–40.
7. Weller M, Stevens A, Sommer N, Dichgans J, Kappler B, Wiethölter H. Ganglioside antibodies: a lack of diagnostic specificity and clinical utility? J Neurol 1992;**239**: 455–9.
8. Schneider JS, Pope A, Simpson K, Taggart J, Smith MG, DiStefano L. Recovery from experimental parkinsonism in primates with GM1 ganglioside treatment. Science 1992;**256**:843–6.
9. Schneider JS, Kean A, DiStefano L. GM1 ganglioside rescues substantia nigra pars compacta neurons and increases dopamine synthesis in residual nigrostriatal dopaminergic neurons in MPTP-treated mice. J Neurosci Res 1995;**42**:117–23.
10. Kastner A, Herrero MT, Hirsch EC et al. Decreased tyrosine hydroxylase content in the dopaminergic neurons of MPTP-intoxicated monkeys: effect of levodopa and GM1 ganglioside therapy. Ann Neurol 1994;**36**: 206–14.
11. Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, Tatarian GT. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology 1998;**50**:1630–6.
12. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;**55**:181–4.
13. van Schaik IN, Bossuyt PMM, Brand A, Vermeulen M. Diagnostic value of GM1 antibodies in motor neuron disorders and neuropathies: a meta-analysis. Neurology 1995;**45**:1570–577.
14. Bernardi G, Terrani C, Frigerio S et al. Antiganglioside GM1 antibodies by ELISA technique: standardization problems. Eur J Clin Chem Clin Biochem 1995;**33**: A183.
15. Pestronk A, Adams RN, Clawson L et al. Serum antibodies to GM1 ganglioside in amyotrophic lateral sclerosis. Neurology 1988;**38**:1457–61.
16. Bartolomei F, Boucrat J, Barrie M et al. Cryptogenic partial epilepsies with anti-GM1 antibodies: a new form of immune-mediated epilepsy? Epilepsia 1996;**37**:922–6.
17. Hirsch EC. Glial cells and Parkinson's disease. J Neurol 2000;**247 (Suppl. 2)**:II/58–II/62.