Increased Circulating Antiganglioside Antibodies in Primary and Secondary Progressive Multiple Sclerosis

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Abstract

Plasma samples from 70 patients with multiple sclerosis (MS), 41 patients with other neurological diseases (OND), and 38 healthy subjects were examined for anti-bodies against gangliosides GM1, GM3, GD1a, GD1b, and GD3 using enzyme-linked immunosorbent assays. The percentages of subjects with increased anti-GM3 responses were significantly higher in the primary progressive MS (56.3%) and secondary progressive MS (42.9%) groups than in the relapsing-remitting MS (2.9%), healthy subject (2.6%), and OND (14.6%) groups. Elevated antiganglioside antibodies may be secondary to axonal damage or may be a cause of axonal damage and accumulating disability in progressive MS. In either case, they may serve as a marker of axonal damage in MS.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Typically MS initially has a relapsing-remitting (RR) course; however, often this relapsing-remitting course eventually changes to a progressive one (secondary progressive [SP] MS). In 10 to 20% of cases of MS, the disease has a progressive course from onset (primary progressive [PP] MS). The cause of MS is unclear, but there is increasing evidence that it is an autoimmune disease.1,2 Because primary demyelination is a characteristic pathological feature of MS, myelin components, particularly myelin proteins, are considered to be the likely target antigens.1,2 Recently we have shown that patients with RRMS or SPMS, but not PPMS, have increased peripheral blood T-cell proliferative responses to a region of myelin proteolipid protein (PLP184-209) that is encephalitogenic in mice.3 The lack of an increased T-cell response to this region of PLP in PPMS raises the possibility that the main target antigens in PPMS are not myelin antigens, but axonal antigens.4 An immune attack directed primarily at axonal antigens could explain the progressive course of this form of MS, as the capacity for CNS axonal regeneration is much more limited than the capacity for CNS remyelination, which is likely to contribute to the recovery from attacks of RRMS. The transition from RRMS to SPMS could involve the spreading of the immune response from myelin to axonal antigens.4

Gangliosides constitute an important group of axonal antigens and are also minor constituents of myelin. A number of studies have found increased amounts of antiganglioside antibodies in the sera or cerebrospinal fluid (CSF) of patients with MS compared with healthy subjects.5-12 However, the significance of this is unclear, because in most studies the antiganglioside levels in MS patients were not compared with those in patients with OND6,9,12 or, if they were compared, were not significantly different.7,11 The only study that has compared antiganglioside antibody levels among the RR, SP, and PP forms of MS is that of Acarin and colleagues.12 They found elevated serum antibodies against GM1, asialoGM1, and GD1a significantly more frequently in PPMS than in RRMS or SPMS; however, the number of patients with PPMS was small (n = 8) and patients with OND were not studied.

In the present study, we examined the antibody responses to five different gangliosides in a larger group of MS patients and in patients with OND, as well as healthy subjects, to determine whether antiganglioside responses are increased in patients with progressive MS compared with patients with RRMS and with controls.

Subjects and Methods

Plasma

After informed consent had been obtained, peripheral blood was collected from 70 patients with clinically definite MS or laboratory-supported definite MS according to the criteria of
Poser and associates,13 41 patients with OND and 38 healthy subjects. Of the patients with MS, 33 had RRMS, 21 had SPMS, and 16 had PPMS, as defined by the criteria of Lublin and Reingold.14 The blood was diluted with an equal volume of tissue culture medium containing heparin (10 units/ml), and separated on a Ficoll gradient, with the plasma (upper fraction) being collected for antibody testing.

Enzyme-Linked Immunosorbent Assay
Bovine gangliosides GM1, GM3, GD1a, GD1b, and GD3 were purchased from Sigma. They were coated onto enzyme-linked immunosorbent assay (ELISA) plates at a concentration of 500 ng/well in methanol. Control wells were coated with methanol alone. The methanol was evaporated at room temperature and the plates blocked by the addition of phosphate-buffered saline (PBS) containing 5% skim milk powder for 5 hours at room temperature. The plates were washed four times with PBS, and plasma samples diluted 1/10 in PBS-skim milk were added to the wells and incubated overnight at 4°C. After five washes with PBS, alkaline phosphatase-conjugated anti-human immunoglobulin (Ig) (polyvalent or IgA-, IgD-, IgG-, or IgM-specific) diluted in PBS-skim milk was added to the plates and incubated for 2 hours at room temperature. Plates were washed six times with PBS, and p-nitrophenyl phosphate as substrate was added to the wells. The absorbance was read at 405 nm in an ELISA plate reader 2 hours later. Initial studies using plasma, serially diluted 1/2 to 1/100, from 20 healthy subjects showed that all samples gave specific absorbance readings of less than 0.10 unit at a 1/10 dilution. Therefore, a 1/10 dilution was used for testing all plasma samples.

Statistical Analysis
The specific absorbance of the test wells was determined by subtracting the average absorbance of triplicate wells containing no antigen and the average absorbance of triplicate wells containing no primary antibody from the average absorbance of the triplicate test wells. The mean specific absorbances for each subject group were compared using analysis of variance (ANOVA). If the p value by ANOVA was below 0.05, each pair of groups was compared using Student's t test. We also used \( \chi^2 \) analysis to compare the percentages of subjects with a specific absorbance above 0.10 unit (an arbitrary cut-off level that was greater than the mean +3 SD of the responses of healthy subjects for each of the gangliosides). Comparisons of pairs of groups were made with \( \chi^2 \) analysis only if the p value for the 5 \( \times \) 2 contingency table analysis was below 0.05.

Results
Figure 1 shows the data points for each subject and each ganglioside. Figure 2A shows the mean antiganglioside antibody levels in the plasma of PPMS patients, SPMS patients, RRMS patients, healthy subjects, and OND patients. When the mean responses of the five groups of subjects were compared by ANOVA, the p value was less than 0.05 for GM1, GM3, and GD3. The pairs of subject groups were then compared using Student's t test for these gangliosides. For GM1, there were significant differences between PPMS patients and healthy subjects (p = 0.026), and between SPMS patients and healthy subjects (p = 0.014). For GM3, the mean specific absorbance of the PPMS patients was significantly higher than those of RRMS patients (p = 0.003), healthy subjects (p = 0.002), and OND patients (p = 0.029). The mean specific absorbance of the SPMS patients was also significantly higher than those of RRMS patients (p = 0.005) and healthy subjects (p = 0.002), but not from that of the OND group. For GD3, the only significant difference was between patients with SPMS and healthy subjects (p = 0.022).
Fig 1. Specific absorbance values for each subject and each ganglioside. The horizontal line at 0.10 unit represents the arbitrary cut-off level, which was greater than the mean +3 SD of the responses of healthy subjects for each of the gangliosides. The patients with OND had the following diagnoses: epilepsy \((n = 10)\), Parkinson's disease \((n = 8)\), Wilson's disease \((n = 1)\), cerebrovascular disease \((n = 5)\), intracranial aneurysm \((n = 1)\), acoustic neuroma \((n = 1)\), neurofibromatosis type 1 \((n = 1)\), hydrocephalus \((n = 1)\), neurosarcoidosis \((n = 1)\), acute disseminated encephalomyelitis \((n = 1)\), transverse myelitis \((n = 1)\), motor neuron disease \((n = 2)\), multifocal motor neuropathy \((n = 1)\), hereditary sensory neuropathy \((n = 1)\), Guillain-Barré syndrome \((n = 2)\), C-8 nerve root lesion \((n = 1)\), nonspecified peripheral neuropathy \((n = 2)\), and proximal myopathy \((n = 1)\).

Fig 2. (A) Mean (± SE) specific absorbances in ELISA assays of antiganglioside antibodies in the plasma of primary progressive multiple sclerosis (MS) patients, secondary progressive MS patients, relapsing-remitting MS patients, healthy subjects, and patients with other neurological diseases. (B) The percentages of individuals in each subject group with a specific absorbance greater than 0.10 unit.
The percentages of individuals in each group with a specific absorbance greater than 0.10 unit were also determined and compared by χ² analysis (see Fig 2B). The p values for this analysis show the same pattern of significantly increased responses to GM1 and GM3 as were found above, except that for GM3 the percentage of subjects with an increased response in the SPMS group was also significantly higher than in the OND group (p = 0.03). For GD3, there were significant (p < 0.05) differences between PPMS patients and healthy subjects, between SPMS patients and healthy subjects, and between SPMS patients and OND patients. For GD1b, the percentage of subjects with an increased response in the PPMS group was significantly higher than in the group of healthy subjects (p = 0.049). In total, 62.5% of PPMS patients, 61.9% of SPMS patients, 29.4% of RRMS patients, 15.8% of healthy subjects, and 29.3% of OND patients had an increased response (specific absorbance > 0.10 unit) to one or more gangliosides. Anti-GM3 antibodies were predominantly of the IgM isotype (data not shown).

Discussion

Primary demyelination is a characteristic feature of MS lesions, and it is likely that clinical recovery from at-tacks of RRMS is at least partly due to remyelination by oligodendrocytes. However, axonal damage and loss also occur in MS15,16 and may contribute to persistent and progressively increasing disability.17 Antiganglioside antibodies could possibly play a role in axonal damage. Convincing evidence that immune responses against gangliosides can be pathogenic has been provided by the induction of experimental sensory ataxic neuropathy in rabbits by immunization with GD1b.18

Our results indicate that patients with PPMS or SPMS have increased circulating antiganglioside antibodies compared with controls and patients with RRMS. This pattern of increased antiganglioside antibody responses in progressive MS compared with RRMS is not a general one for antibody responses in MS, as we have found no such difference for antibodies against PLP peptides and myelin basic protein peptides (Greer JM, Pender MP, unpublished observations). Acarin and co-workers12 found increased serum antiganglioside, especially anti-GD1a, antibodies in a small group of patients with PPMS compared with patients with RRMS, but they did not find any differences between SPMS and RRMS. In the present study, the strongest responses were directed against GM3, a minor component of the human CNS.19 Stevens and associates10 found increased IgM anti-GM3 antibodies in the CSF, but not the sera, of patients with chronic progressive MS.

The increased antiganglioside antibodies in progressive MS compared with RRMS may be secondary to axonal damage or may be a cause of axonal damage and accumulating disability in progressive MS. In either case, they may serve as a marker of axonal damage in MS. As glycolipid antigens can interact with T-cell receptors after binding to the major histocompatibility complex-like CD1 cell surface glycoproteins,20 it is likely that T cells are also involved in antiganglioside immune responses.

This work was supported by the National Health and Medical Research Council of Australia.

References