

A characteristic ganglioside antibody pattern in the CSF of patients with amyotrophic lateral sclerosis

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Abstract

Paired cerebrospinal fluid and serum samples of patients with amyotrophic lateral sclerosis (n = 35) revealed no consistent abnormalities of CSF cell count, CSF albumin, CSF IgG, CSF IgM, IgG or IgM index, or oligoclonal immunoglobulin band formation in the CSF. Determination of IgG and IgM CSF and serum antibodies to gangliosides GM1, GM2, GM3, AGM1, GD1a, GD1b, and GT1b showed a characteristic pattern which allowed the differentiation of amyotrophic lateral sclerosis from controls and from patients with other neurological disorders including multiple sclerosis. Specifically, patients with the disease had elevated CSF IgM antibodies to all gangliosides except AGM1. The lack of correlation between the CSF findings and corresponding serum antibodies suggests a chronic, compartmental, intrathecal immune response of low activity in amyotrophic lateral sclerosis. Whether this immune response is primary and of pathogenetic significance, or an epiphenomenon of neuronal degeneration, remains to be investigated.

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Amyotrophic lateral sclerosis is characterised by a progressive loss of upper and lower motor neurons of unknown aetiology.¹ Cerebrospinal fluid studies are scarce and have produced no consistent abnormalities in patients with this disease.^{2,3} Several authors have postulated that a disturbance of immunoregulation may play a role in the degeneration of motor neurons in these cases.⁴⁻⁷ The detection of polyclonal serum antibodies to GM1 ganglioside in such patients^{8,9} has reinforced the search for immunological events in the pathogenesis of the disease. As the pathological changes are largely confined to the central nervous system and because there is no significant blood-brain barrier disturbance, studies on the CSF are more likely to establish immune dysfunction than investigation of the systemic immune response. This study reports an analysis of CSF and serum IgG and IgM antibodies to seven different gangliosides in 35 patients with amyotrophic lateral sclerosis and examines their correlation with routine CSF parameters.

Materials and methods

Thirty five patients with amyotrophic lateral sclerosis, 16 men and 19 women, mean age 53.5 (2.4) years, range 25-79 years at diagnosis, without a family history of the illness, were included in this study. The diagnosis was clinical and based on the presence of both upper and lower motor neuron symptoms and signs. Eight patients had prominent bulbar signs, 31 had definite spasticity. For inclusion in this study, the onset of symptoms had to be insidious and not started before the age of 20. The clinical course had to be progressive. Specific attention was given to alternative diagnoses of cervical spondylosis, neoplastic lesions of cervical spinal cord and brain stem, polymyositis, peripheral neuropathy, progressive muscle atrophy, Charcot-Marie-Tooth disease, spinocerebellar degeneration and familial spastic paraplegia. CSF and serum samples were obtained after informed consent, centrifuged and stored at -70°C.

CSF parameters and ganglioside antibodies were estimated as previously described.¹⁰⁻¹³ Briefly, micro-enzyme linked immunosorbent assay (ELISA) plates (M129A, Dynatech, Denkendorf, Germany) were coated with purified ganglioside antigen, GM1, GM2, GM3, AGM1, GD1a, GD1b, or GT1b (150 ng per well, Sigma, St Louis, MO, United States) diluted in methanol. Antigen purity was confirmed by thin layer chromatography. Protein binding sites on the well that might cause non-specific binding of immunoglobulins were blocked by incubation with bovine serum albumin (0.1%) in phosphate-buffered saline for 24 hours before the incubation of CSF or serum samples. The CSF samples were matched to an IgG concentration of 5 mg/l. Serum was diluted 1:50 in phosphate-buffered saline. No detergents were used throughout the assay. IgG or IgM ganglioside reactive antibodies were labelled by anti-human IgG (Sigma A-5403) or anti-human IgM (Sigma A-3914) antisera conjugated to alkaline phosphatase diluted 1:1000 in phosphate-buffered saline. Monoclonality of positive samples was excluded by differential assays for κ or λ light chains as previously described.¹²

Seven internal reference sera obtained from healthy volunteers or seven CSF samples from patients with suspected, but eventually disproven, neurological disease served as a control for interassay variability. The interassay correlation calculated for nine runs was 0.91. The ratio of the optical density of a

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Table 1 Mean normalised optical density readings (SD) for ganglioside antibodies in CSF (adjusted to 5 mg/l IgG) and serum (diluted 1:50) of patients with amyotrophic lateral sclerosis and controls with other neurological diseases.

ALS CSF	OND CSF	ALS serum	OND serum	Controls serum
GM1-IgG	1.09 (0.38)	1.00 (0.15)	1.08 (0.28)	1.00 (0.14)
GM1-IgM	1.17 (0.17)*	1.06 (0.15)	1.09 (0.34)	1.00 (0.28)
GM2-IgG	0.94 (0.14)	1.04 (0.11)	1.16 (0.21)*	1.00 (0.16)
GM2-IgM	1.22 (0.25)	1.00 (0.14)	1.12 (0.36)	1.00 (0.28)
GM3-IgG	0.91 (0.18)	1.00 (0.11)	1.11 (0.27)*	1.00 (0.19)
GM3-IgM	1.30 (0.37)*	1.00 (0.11)	1.13 (0.33)	1.00 (0.25)
AGM1-IgG	0.94 (0.17)	0.99 (0.14)	1.21 (0.34)*	1.00 (0.20)
AGM1-IgM	1.08 (0.16)	0.99 (0.06)	1.10 (0.34)	1.00 (0.21)
GD1a-IgG	1.05 (0.17)	1.01 (0.12)	1.06 (0.18)	1.00 (0.15)
GD1a-IgM	1.22 (0.28)*	0.98 (0.08)	1.00 (0.31)	1.00 (0.27)
GD1b-IgG	0.95 (0.16)	1.00 (0.12)	1.06 (0.18)*	1.00 (0.13)
GD1b-IgM	1.57 (0.49)*	1.00 (0.06)	1.13 (0.39)	1.00 (0.26)
GT1b-IgG	1.18 (0.25)	1.00 (0.13)	1.20 (0.20)	1.00 (0.13)
GT1b-IgM	1.47 (0.40)*	1.00 (0.10)	1.05 (0.32)	1.00 (0.26)

ALS = amyotrophic lateral sclerosis; OND = other neurological diseases.

*Significantly ($p < 0.03$) elevated readings in patients with amyotrophic lateral sclerosis compared with those with other neurological diseases.

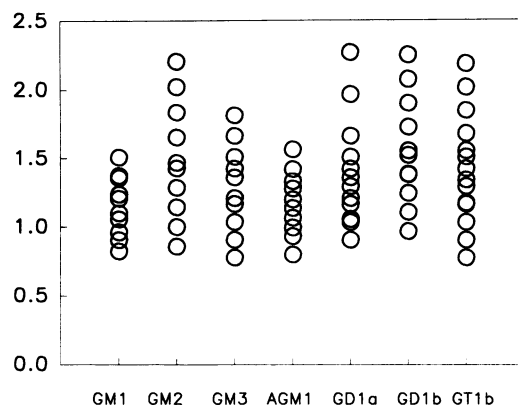
Table 2 Incidence of pathological CSF and serum ganglioside antibodies (normalised optical density readings beyond the third percentile) in patients with amyotrophic lateral sclerosis.

	CSF IgG		IgM		Serum IgG		IgM	
	n	%	n	%	n	%	n	%
GM1	3	9	5	14	5	14	4	11
GM2	1	3	10	29	5	14	3	9
GM3	2	6	21	60	3	9	5	14
AGM1	0	0	9	26	5	14	6	17
GD1a	3	9	13	37	2	6	1	3
GD1b	1	3	27	77	5	14	5	14
GT1b	10	29	24	69	13	37	2	6

patient sample over the mean optical density of the seven internal standards was calculated, thus arbitrarily assigning the value of one to this mean. These normalised optical density results obtained in amyotrophic lateral sclerosis were then compared with a control group of patients with other neurological diseases ($n = 24$) such as tension headache or normal pressure hydrocephalus. Patients with neuroinflammatory and neuroimmunological disorders were excluded. The means of the actual optical density readings in the group with other neurological diseases at 30 minutes were between 0.072 and 0.079 for the seven CSF IgM ganglioside antibodies, between 0.138 and 0.191 for CSF IgG, between 0.294 and 0.462 for serum IgM, and between 0.265 and 0.394 for serum IgG.

The results in amyotrophic lateral sclerosis were also compared with two groups of previously characterised¹³ patients with multiple

Figure 1 Individual normalised optical density readings for CSF IgM ganglioside antibodies in patients with (left) amyotrophic lateral sclerosis and (right) multiple sclerosis.



sclerosis of chronic progressive ($n = 13$) and relapsing remitting type ($n = 35$). The statistical analysis included testing for normal distribution, Student's t test, linear correlation, discriminant analysis, and analysis of variance.

Results

The routine laboratory investigations of CSF and serum samples from amyotrophic lateral sclerosis did not yield consistent abnormalities. None of the patients had a paraprotein on serum immune electrophoresis. Patients with the disease did not have higher total serum IgG or IgM levels than controls. There were also no significant differences between patients and controls in CSF cell counts, CSF albumin, CSF IgG, CSF IgM, or IgG or IgM index. Seven patients had elevated CSF IgG, one had elevated CSF albumin, six had mild pleocytosis. Two had CSF IgG oligoclonal immunoglobulin bands detected. CSF IgG and IgG index in these patients were normal.

The results of CSF and serum ganglioside antibody determination in amyotrophic lateral sclerosis and the incidence of pathological normalised optical density readings in these patients are provided in tables 1 and 2. The controls with other neurological diseases were not different from the internal controls. CSF IgG, but not age or gender, had an influence on ganglioside antibodies in those with other neurological diseases, before adjustment. The Pearson coefficients were 0.14 (GM1), 0.56 (GM2), 0.37 (GM3), 0.23 (AGM1), 0.48 (GD1a), 0.47 (GD1b) and 0.35 (GT1b). Among the seven ganglioside antibodies examined, there was considerable intercorrelation in the amyotrophic lateral sclerosis samples (Pearson coefficient 0.3–0.6), suggesting that most of the sera contain either antibodies with multiple ganglioside reactivity or multiple antibodies against different gangliosides, a suggestion that was confirmed by twin layer chromatography. The degree of normalised optical density elevation among the patients with the disease was fairly heterogeneous (figs 1 and 2). The two patients with CSF oligoclonal bands did not differ in their ganglioside

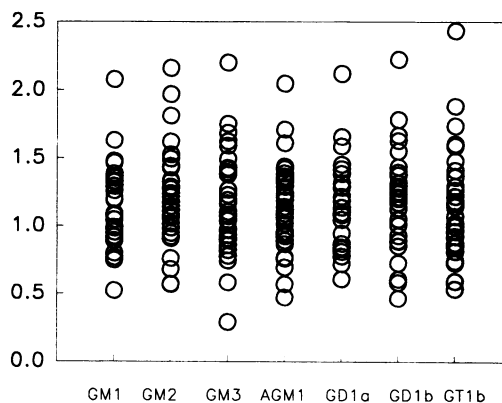
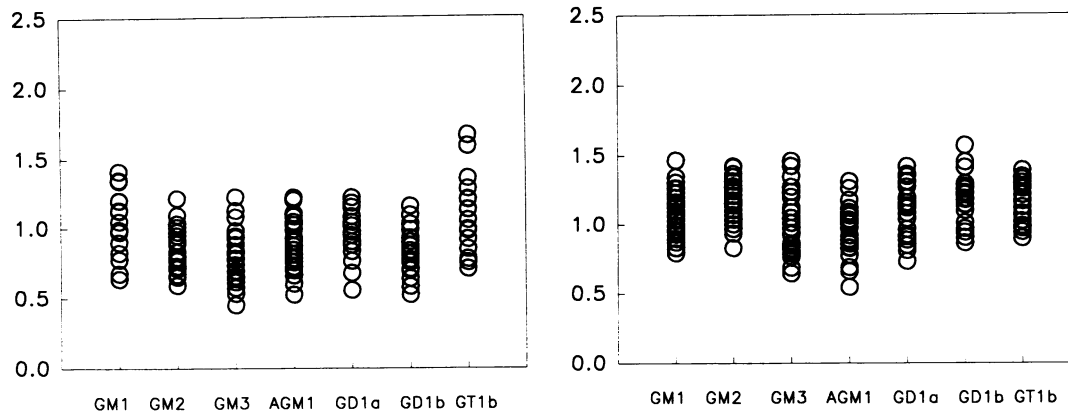


Figure 2 Individual normalised optical density readings for CSF IgG ganglioside antibodies in patients with (left) amyotrophic lateral sclerosis and (right) multiple sclerosis.



antibody pattern from the other patients with the illness.

A comparison of serum ganglioside antibodies between patients with the disease and controls or those with other neurological disease showed elevated IgG to GM2, GM3, AGM1 and GT1b in the disease but no difference in IgM. Serum reactivity in amyotrophic lateral sclerosis differed from either type of multiple sclerosis only in their higher IgG anti-AGM1 optical density readings.

CSF IgM to all gangliosides except AGM1 was elevated in the patients compared with those with other neurological diseases. CSF IgM to GM2, GM3, GD1a, GD1b, and GT1b was also higher than in chronic progressive multiple sclerosis. On the other hand, patients with chronic progressive multiple sclerosis had higher CSF IgG optical density readings to GM2, GM3, GD1a, and GD1b than patients with amyotrophic lateral sclerosis. CSF IgG antibodies to GM2, GM3, GD1a, and GD1b, as well as IgM antibodies to GD1a, GD1b, and GT1b were lower than in relapsing remitting multiple sclerosis. There was no correlation between CSF or serum ganglioside antibodies in amyotrophic lateral or multiple sclerosis.

Discriminant analysis performed on normalised CSF optical density readings to all seven gangliosides allowed correct reclassification of 75% of the patients with the disease. When variables were selected by stepwise entry depending on Wilk's λ reduction, the same classification results were obtained using IgG antibodies to GM2 and GM3, which are low in amyotrophic lateral sclerosis, in association with IgM antibodies to GD1a and GD1b, which are high. None of the controls and 3% of the patients with multiple sclerosis were falsely classified as having amyotrophic lateral sclerosis.

The same procedure was then applied to serum ganglioside antibodies: this analysis of 35 serum samples from patients with amyotrophic lateral sclerosis, according to their pattern of ganglioside reactivity, allowed correct classification in 83%. Six per cent of the controls with other neurological diseases were mistakenly diagnosed as having the disease (false positives). No single patient with multiple sclerosis received a diagnosis of amyotrophic lateral sclerosis. When only IgG antibodies to GM1, GM2, and AGM1, as

well as IgM antibodies to AGM1 were entered into the analysis, patients with the disease were still correctly identified in 62%, but 14 controls were now misclassified as having the illness. Analysis of variance detected no effect of age, gender or distribution of clinical signs on CSF or serum antibodies in the disease.

Discussion

The growing number of experimental and clinical studies on altered immune function in amyotrophic lateral sclerosis has not led to the identification of a consistent abnormality,⁷ except for the few cases associated with plasma cell dyscrasia.^{2,14} The detection of immune complexes in serum³⁻⁵ and nervous tissue¹⁵ as well as of IgG deposits in motor neurons¹⁶ and lymphocytic infiltrates¹⁷ and major histocompatibility complex class II antigen expression in nervous tissue¹⁸ may well be epiphenomenal to neuronal degeneration and does not prove an autoimmune aetiology.

We could not confirm elevated serum IgG or IgM levels or quantitative intrathecal IgG or IgM synthesis or blood-brain barrier breakdown as characteristic features of the disease in our patients.³ Previous efforts to identify anti-neuronal protein antibodies in such patients were not successful.¹⁹ Polyclonal or monoclonal serum antibodies to GM1, which were once thought to be associated with the disease,^{8,9} have now been recognised to be probably more tightly linked to a lower motor neuron syndrome distinct from amyotrophic lateral sclerosis.^{12,20-24} Moreover, the spectrum of neurological disease with similar levels of serum anti-GM1 antibodies is broad.^{12,23}

To our knowledge, the present work is the first comprehensive study of ganglioside antibodies in the CSF of patients with amyotrophic lateral sclerosis. We have identified different patterns of ganglioside antibody reactivity in serum and CSF, both of which are nevertheless characteristic of the disease. This pattern may be the only currently available biochemical test for the diagnosis of amyotrophic lateral sclerosis. The normalised serum optical density readings obtained in this study are lower than those found in patients with multifocal motor neur-

opathy.^{12,23} The lack of correlation between our serum and CSF data suggests that there is a specific compartmental intrathecal immune reaction of IgM type in the disease. As conventional isoelectric focusing for CSF oligoclonal bands is useful for IgG analysis but does not readily detect IgM,²⁵ it is not surprising that oligoclonal bands were seen in only two of our patients. Even sophisticated techniques of IgM oligoclonal band analysis fail to yield positive results in the disease.^{26,27} The lack of elevation of total CSF IgM or the IgM index appears to prove that the immune activation detected in this study cannot be very strong. Nevertheless, we have identified a phenomenon which distinguishes amyotrophic lateral sclerosis from other neurological diseases and normal controls which warrants further investigation. Rather than claiming a primary pathogenetic role of ganglioside antibodies, we suggest that our findings may be related to an alteration of the ganglioside composition in the brain²⁸ and spinal cord²⁹ of patients with the disease.

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