Oligoclonal immunoglobulins and plasma cells in spinal fluid of patients with multiple sclerosis

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Summary and conclusions

A new modification of polyacrylamide gel electrophoresis (PAGE) was applied to cerebrospinal fluid proteins from patients with multiple sclerosis (MS). The same spinal fluids were also examined by a cytological technique. Over 90% of patients with clinically definite or early probable or latent MS showed abnormal PAGE patterns in the form of oligoclonal gammaglobulin bands. Reactive (atypical, large) lymphocytes or typical plasma cells were found in some patients. In all such cases an oligoclonal pattern was present.

The finding of oligoclonal bands provides valuable supporting evidence for the diagnosis of MS in the less definite clinical categories.

Introduction

It is well known that the cerebrospinal fluid (CSF) may be abnormal in multiple sclerosis (MS) and that alterations in the properties of the γ -globulins are characteristic though not diagnostic of the disease. Traditionally this change has been recognised by the presence of a paretic colloidal gold curve, though this abnormality was found in only about 17% of patients.¹ Measurement of γ -globulin by immunoprecipitation increased the yield of positive results to about 70%.¹

A further advance was made by Lowenthal² and Kutt *et al*,³ who applied the technique of gel electrophoresis, and abnormalities have been reported in up to 94% of cases.⁴ Others have been less successful,¹ and it has been shown that concentration of the CSF—which was performed in all previous electrophoretic studies of MS—may of itself alter the electrophoretic pattern.⁵ We have therefore modified the technique so that a satisfactory examination may now be performed on 0.5 ml of unconcentrated CSF. We report the prevalence of abnormalities in different groups of patients with MS classified according to the certainty of diagnosis. Since plasma cells have been found in CSF from patients with MS⁶ we have correlated the chemical findings with the differential cytology.

Methods

We modified Ornstein and Davis's⁷ original acrylamide gel method by omitting the stacking gel.⁸ As a result all the oligoclonal γ -globulin bands were present in the resolving gel. The 7% resolving gel was polymerised with the ammonium persulphate technique. Two gels were run for each CSF sample. One was loaded with 100 μ g total

protein and the other with 200 μ g. The first gel was stained with 0.5 g Coomassie Blue/l and the second with 10 g naphthalene black/l. Gels were destained with 7% acetic acid. The gels were scanned in a Joyce Loebel densitometer. The immunoglobulins were identified (1) by displaying a higher relative affinity for Coomassie Blue than for naphthalene black (since they are basic proteins), and (2) by direct immunofixation of the gels with anti-IgG serum.⁹ The figure gives the results of electrophoresis of CSF from a normal subject and from a patient with MS. The striking difference is in the γ region, which shows a homogenous zone in the normal CSF but a series of diffuse, though clearly recognisable bands in the CSF from the patient with MS—an oligoclonal pattern. It is important to distinguish the diffuse bands of the oligoclonal pattern from the sharp bands produced by haptoglobins resulting either from haemolysis in a specimen contaminated by blood or from a high total protein concentration.

The total protein concentration was measured by nephelometry.¹⁰ The amount of IgG was determined by using the technique for radial immunodiffusion with Hyland low-level plates. Lange's colloidal gold test was performed.¹⁰ Cells were centrifuged from 0.3 ml CSF with a Shandon cytocentrifuge.¹⁰

Coomassie-stained gels from two CSF samples. Top: Normal subject Bottom: Patient with MS. Origin is on right. Gel length 14 cm.

Patients

We selected 172 patients at random whose provisional diagnosis on admission was MS and on whom polyacrylamide gel electrophoresis (PAGE) of the CSF had been performed; 145 remained after exclusion of those whose CSF studies were technically unsatisfactory (including those with traumatic punctures) and those for whom no definite diagnosis was made. There were 31 patients with other diseases—namely, optic neuritis (4 cases) (shown separately in the table), cerebrovascular disease (6), cerebral atrophy (5), Guillain-Barré syndrome (2), and idiopathic ocular palsy (2) and one case each of idiopathic epilepsy, chronic brain trauma in a boxer, multisystem degeneration, Charcot-Marie-Tooth disease, spinal angioma, prolapsed intervertebral disc, trigeminal neuralgia, costoclavicular syndrome, aqueduct stenosis, polymyalgia, congenital syphilis, and depression. The other 114 patients were thought to have MS with varying degrees of certainty and were classified according to the system of McDonald and Halliday.¹¹

Results

The table gives the numbers of patients with abnormalities in amount of total protein, γ -globulin as a percentage of total protein, Lange colloidal gold curve, and cytology and an oligoclonal pattern.

As expected^{1,12} fewer than 15% of patients with clinically definite or early probable or latent MS had a protein concentration exceeding 0.6 g/l. The highest concentration recorded was 0.82 g/l.

The IgG concentration was 13% or more in three-quarters of the patients with clinically definite MS and two-thirds of those with early probable or latent disease. None of the patients with optic neuritis had a raised IgG concentration, but five of the 27 patients with other diseases had abnormal values.

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CSF findings in different categories of MS and other diseases. Figures are numbers of patients

	Total protein		IgG by immunoprecipitation		Colloidal gold curve			Pleocytosis ($>5 \times 10^6/l$)		Atypical lymphocytes or plasma cells or both	Oligoclonal pattern		Total
	>0.6 g/l	≤ 0.6 g/l	$\geq 13\%$	$<13\%$	1st zone	Mid-zone	Normal	Present	Absent		Present	Absent	
Category of MS:													
Clinically definite	2	30	24	8	16	4	12	9	23	9	30	2	32
Early probable or latent	8	50	38	20	22	5	31	23	35	7	52	6	58
Progressive probable	1		1				1		1		1		1
Progressive possible	3	5	6	2	4		4	3	5	3	7	1	8
Suspected	1	14	6	9	3		12	4	11	2	9	6	15
Optic neuritis	1	3		4	1		3		4		4		4
Other diseases	2	25	5	22		3	24		27	1	5	22	27

The Lange curve was regarded as abnormal if at least two consecutive tubes were graded 2 or more. A paretic curve showed the main change in the first three tubes. The curve was paretic in 16 out of 32 clinically definite cases of MS and 22 out of 58 early probable or latent cases. The only patient with a disease other than MS who had a paretic Lange curve had optic neuritis.

Pleocytosis (over 5×10^6 cells/l) was present in nine patients with clinically definite MS and 23 patients with early probable or latent disease. Atypical lymphocytes or plasma cells were found in 21 patients with any category of MS but in only one of the 31 patients with other diseases; the latter was a patient with the Guillain-Barré syndrome whose total cell count was normal. These cells were present in nine out of 32 patients with clinically definite disease and seven out of 58 patients with early probable or latent disease. In eight out of 22 patients these abnormal cells were found in the absence of pleocytosis.

With PAGE oligoclonal patterns were found in 30 patients with clinically definite MS and in 52 of those with early probable or latent disease. The overall incidence in the patients with MS (excluding the suspected group) was 90 out of 99. All four patients with optic neuritis and five of the 27 with other diseases had oligoclonal patterns. These are discussed in more detail below.

Discussion

The incidence of abnormal CSF findings increases with clinical confidence in diagnosis. The typical patient with clinically definite MS has an oligoclonal pattern (94% of cases), a raised IgG concentration (75%), a paretic Lange curve (50%), and plasma cells or reactive lymphocytes (28%). He is less likely to have an increased total protein concentration (6%). Interestingly over half of the patients classified as "suspected" MS (a single episode suggestive of the disease without evidence of any lesion or with evidence of a single lesion only¹¹) had an oligoclonal pattern. We regard this as strong supporting evidence for the diagnosis but do not at present change the diagnostic category on the basis of the CSF findings. It will be important to follow up these patients to see whether new lesions develop.

We emphasise that oligoclonal patterns are *not specific* to MS, having been found in various acute and chronic inflammatory disorders of the central and peripheral nervous systems. We have found oligoclonal patterns in optic neuritis (four cases, three recurrent) and the Guillain-Barré syndrome (one case). Others have also reported oligoclonal patterns in neurosyphilis, subacute sclerosing panencephalitis, and chronic bacterial and other infections of the CNS.¹ We have also found an oligoclonal pattern in Charcot-Marie-Tooth disease of the demyelinating type (one case), idiopathic epilepsy (one), recurrent brain trauma in a boxer (one), and multisystem degeneration (one). An oligoclonal pattern has been reported occasionally in the last three conditions.¹³ Although an oligoclonal pattern may be found in a wide range of disorders, most can usually be distinguished readily from MS on clinical grounds. The findings of an oligoclonal pattern in patients with isolated optic neuritis is interesting. There is evidence that this clinical syndrome represents more than one disease¹⁴ but that it is most commonly a manifestation of MS.¹⁵ Sandberg-Wollheim¹⁶ reported that the

risk of developing MS after optic neuritis is not greater in patients with an oligoclonal pattern.

There has been no systematic study of the relation between electrophoretic patterns and the differential cytology of CSF in patients with MS. Since CSF lymphocytes from individual patients may synthesise oligoclonal IgG^{17,18} it is particularly interesting that all our 22 patients with atypical lymphocytes or plasma cells showed oligoclonal bands. Since not all of these patients had a pleocytosis, differential cytology is clearly valuable in assessing patients suspected of having MS.

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ONE HUNDRED YEARS AGO The committee for encouraging the use of horse-flesh as an article of food have issued a return showing that the number of horses, asses, and mules slaughtered in Paris for consumption in 1878 was 11 319, or 700 more than in the previous year. The continued increase in the use of horse-flesh is, they say, a proof that the prejudice against it is being gradually overcome. A prize of 1200 francs was awarded by M Decroix to the founder of the first shop for the sale of horse-flesh in London, opened in May last. That venture, during the four months it was carried on, did not, however, meet with all the desired success, the chief reason for which was (the committee say) that the director was quite ignorant of the English language. The committee now offer a medal of honour to any English butcher who shall take up the trade and continue it for three months at least. But, till English cooks are more skilled in concealing their raw material, and till English prejudices among the working classes are less intense against even Australian beef, there is little hope of a demand for horse-flesh. (*British Medical Journal*, 1879.)