

Relation between benign course of multiple sclerosis and low-grade humoral immune response in cerebrospinal fluid

LENA STENDAHL-BRODIN AND HANS LINK

From the Departments of Ophthalmology and Neurology, University Hospital, Linköping, Sweden

SUMMARY The prognosis in multiple sclerosis (MS) is related to the presence of an abnormal humoral immune response within the central nervous system: 14/17 MS patients (82%) without oligoclonal CSF IgG displayed no or slight disability after a mean duration of MS of 17 years, while 53% of 88 patients with oligoclonal CSF IgG had a benign course after a mean duration of 13 years ($p < 0.05$). A benign course also was more often accompanied by a normal CSF IgG index. MS patients without oligoclonal CSF IgG had elevated CSF/serum ratios of albumin in 6%, and of the complement factors C3 in 0% and C4 in 6%, as against 20%, 27% and 37%, respectively, in MS patients with oligoclonal CSF IgG.

Protein migrating as bands in the gamma globulin region on electrophoresis of cerebrospinal fluid (CSF) but not serum from multiple sclerosis (MS) patients was suggested by Laterre¹ to represent an oligoclonal reaction with IgG synthesis within the central nervous system (CNS). The IgG character of electrophoretically separable gamma globulin bands was soon proved,² as was the ability of MS CSF lymphocytes to synthesize oligoclonal IgG *in vitro*.³ The oligoclonal character of CSF IgG in MS has been further documented by demonstration of a restricted heterogeneity of γ chains,² an abnormally high kappa to lambda light chain ratio⁴ and predominance of γ_1 chains.⁵

About 90% of MS patients display oligoclonal IgG when CSF is studied by electrophoresis on agar gel^{2,6,7} on agarose gel⁸ or by isoelectric focusing.^{9,10} The number and mobility of oligoclonal IgG bands in the individual MS patient has been shown to be constant during the course of the disease although the bands may be more or less strong.¹² We have not encountered a single case in whom the bands have vanished once they have appeared. This observation requires stan-

dardization of CSF electrophoresis regarding amount of IgG applied.⁸ Tourtellotte and Booe¹³ have shown recently that treatment with ACTH or prednisone reduces the CSF IgG levels but does not eradicate the oligoclonal IgG band pattern.

There is, however, a substantial although small group of patients with clinically definite MS in whom oligoclonal CSF IgG can not be demonstrated. It is not known whether such MS patients differ regarding the course of disease or frequency of other CSF abnormalities when compared with patients with oligoclonal CSF IgG.

Materials and Methods

One hundred and five consecutive patients (65 females) with MS were studied. Eighty-eight of them had oligoclonal IgG demonstrable in CSF by agarose gel electrophoresis. The diagnosis of MS was made according to the clinical criteria of Müller.¹¹ None of the patients was treated with corticosteroids or cytotoxic immunosuppressive drugs for at least six months prior to the investigation.

The patients were classified into two groups regarding disability. Patients who had no or slight disability were able to manage on their own in every respect, while those who had moderate or severe disability were dependent on a variable

Address for reprint requests: Dr H Link, Department of Neurology, University Hospital, S-581 83 Linköping, Sweden.

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degree of assistance, and their professional and social lives were disturbed.

15 ml of CSF was obtained by lumbar puncture. A serum specimen was taken simultaneously. CSF leukocytes were counted and differentiated by phase contrast microscopy. The erythrocyte count in CSF did not exceed $100 \times 10^6/l$. IgG and albumin were determined simultaneously in previously unfrozen, unconcentrated CSF and in serum, mostly within 24 hours after lumbar puncture, and the CSF IgG index (see below) was determined. A portion of the CSF was concentrated by ultrafiltration in collodion bags (Sartorius Membranfilter, Göttingen, W. Germany) at 4°C to an IgG concentration of about 3 g/l and then analysed by agarose gel electrophoresis in parallel with serum. The remaining unconcentrated CSF was stored at -20°C and then investigated simultaneously regarding IgA, kappa and lambda light chains, and complement factors C3 (beta 1C/beta 1A globulin) and C4 (beta 1E globulin) together with the corresponding serum.

Immunochemistry

Determinations of albumin, IgG, IgA, and kappa and lambda light chains were carried out on unconcentrated CSF and on serum by an automatic immunoprecipitation technique utilizing nephelometric analyses of antigen-antibody complexes in a continuous flow system (Auto-Analyser II, Technicon Corp., Inc., Tarrytown, N.Y.). The complement factors C3 and C4 were determined by single radial immunodiffusion¹⁴ modified as described.¹⁵ Antisera against albumin, IgG, IgA, kappa and lambda Bence Jones protein were purchased from Dakopatts (Copenhagen, Denmark), antiserum against C3 from Organon Technica (Oss, Holland) and C4 from Behringwerke (Marburg-Lahn, W. Germany).

The CSF IgG index¹⁶ equal to the ratio (CSF IgG/serum IgG): (CSF albumin/serum albumin) which takes into account the influence of serum IgG as well as of blood brain barrier disturbances was calculated; an index value above 0.70 which constitutes the upper normal limit in our laboratory is considered to indicate IgG synthesis within the CNS. The IgA concentration in CSF was similarly presented by the CSF IgA index; the upper normal value is 0.62.

The kappa/lambda ratio⁴ was determined. The 95% confidence limits in our laboratory are 0.7 to 1.7 for CSF and 0.7 to 1.3 for serum.

Agarose gel (Behringwerke) electrophoresis was carried out as described previously.⁸ The occurrence of one or more homogeneous bands

in the gamma globulin region in addition to those normally seen was considered abnormal.

Results

Relation between oligoclonal CSF IgG and clinical variables

Subgrouping of the MS patients according to presence or absence of oligoclonal CSF IgG revealed only a slight difference regarding age at onset, while the mean duration of the disease was somewhat longer in the group without oligoclonal CSF IgG (table 1). Fourteen of the 17 patients (82%) without oligoclonal CSF IgG displayed no or slight disability, however, after a mean duration of disease of 17 years, in contrast to 53% of the patients with oligoclonal CSF IgG after a mean duration of 13 years ($p < 0.05$; χ^2 with Yates' correction for small numbers).

Table 1 Relation between oligoclonal CSF IgG and clinical findings in multiple sclerosis

CSF abnormality	Oligoclonal CSF IgG	
	Present (n = 88)	Absent (n = 17)
Age at onset (mean)	30 years	28 years
Duration of disease (mean)	13 years	17 years
Slight or no disability 61 patients	47 (53%)	14 (82%)
Moderate to severe disability 44 patients	41 (47%)	3 (18%)

Relation between oligoclonal CSF IgG and other CSF abnormalities

The patients with oligoclonal CSF IgG displayed significantly higher frequencies of elevated CSF IgG index values ($\chi^2 = 22.81$; $p < 0.001$), elevated kappa/lambda ratios ($p < 0.05$) and elevated CSF/serum C4 ratios ($p < 0.05$) (table 2). In contrast, abnormal blood brain barrier as determined by the CSF: serum albumin ratio, elevated CSF IgA index values, and elevated CSF: serum C3 ratios were found at similar low frequencies irrespective of the presence of oligoclonal CSF IgG.

Relation between CSF IgG index and clinical variables

Table 3 shows that patients with a malignant course of MS only rarely displayed a normal CSF IgG index, in contrast to patients with the most benign course ($p < 0.001$). Conversely, the highest CSF IgG index values were observed somewhat more frequently among the patients with a malignant course. Two of the 19 patients, however, with the most benign course displayed CSF IgG index values above 1.2.

Table 2 CSF findings in 105 multiple sclerosis patients subgrouped according to presence of oligoclonal IgG in CSF

CSF abnormality	Oligoclonal CSF IgG	
	Present (n = 88)	Absent (n = 17)
Mononuclear pleocytosis (>5 cells per mm ³)	27/83 (33%)	2/16 (13%)
Elevated CSF-IgG index (>0.70)	73 (83%)	4 (24%)
Elevated CSF-IgA index (>0.62)	5/68 (7%)	0/14
Abnormal CSF kappa/lambda ratio (>1.7)	37/82 (45%)	2/16 (13%)
Elevated CSF/serum albumin ratio	16/79 (20%)	1/16 (6%)
Elevated CSF/serum C ₃ ratio (>4.1)	22/81 (27%)	0/16
Elevated CSF/serum C ₄ ratio (>10.5)	29/78 (37%)	1/16 (6%)

Table 3 Relation between course of multiple sclerosis and CSF IgG index

	Number of cases	CSF IgG index		
		<0.7	0.7-1.2	>1.2
Whole material	105	28 (27%)	49 (46%)	28 (27%)
Malignant course*	17	2 (12%)	8 (47%)	7 (41%)
Benign course†	44	15 (34%)	22 (50%)	7 (16%)
Benign course‡	19	9 (47%)	8 (42%)	2 (11%)

* Moderate to severe disability within 5 years after onset.

† Slight or no disability after 10 years.

‡ Slight or no disability after 20 years.

Discussion

For the demonstration of oligoclonal IgG, electrophoresis of CSF and serum taken simultaneously is usually carried out on a suitable supporting medium, usually agar gel or agarose gel; more recently, commercially available ready-prepared agarose gel plates have been used.^{10, 17} A useful alternative for demonstration of oligoclonal IgG is isoelectric focusing, which can also be performed on commercially available ready-prepared thin-layer polyacrylamide gel plates.¹⁸

Although technical factors may sometimes determine the presence or absence of oligoclonal IgG demonstrable on electrophoresis of CSF, it has been shown that oligoclonal IgG, from its onset of appearance, remained rather constant regarding number and position of electrophoretically separable bands in consecutive CSF specimens from the individual MS patient during the course of disease, at least when agar gel electrophoresis was used as supporting medium.¹² In contrast, the CSF IgG expressed as percentage of the total protein fluctuated in the individual MS patient between abnormally high values indi-

cating IgG synthesis within the CNS, and normal values.

CSF and serum obtained on more than one occasion, covering an observation time of usually more than 3 months, were studied in most of our 17 MS patients who did not display oligoclonal CSF IgG, with the same results. An absent, or less pronounced humoral immune response within the CNS may be anticipated in these patients. This is further strengthened by our finding in this patient group of significantly lower frequencies of abnormal CSF IgG index values and CSF kappa:lambda ratios. However, our data indicate that these two CSF abnormalities may occur in single cases of MS with normal CSF electrophoresis.

The present results indicate that MS patients without oligoclonal IgG in CSF demonstrable by agarose gel electrophoresis, although representing a minority of the patients with this disease, had a better prognosis than patients with oligoclonal CSF IgG. Thus, the grade of the humoral immune response within the CNS seems to be coupled to the prognosis in MS. Similar observations have previously been made when the IgG level in CSF expressed as the percentage of the total protein was considered.¹⁹ MS patients therefore with the most malignant course (severe disability within 10 years) had elevated CSF IgG levels more frequently than patients with a benign course (no disability after 10 or more years). Although determination of the IgG:total protein ratio of CSF is less reliable compared to the CSF IgG index for demonstration of IgG synthesis within the CNS,²⁰ our present findings using the CSF IgG index confirmed the relation between benign course of MS and normal CSF IgG levels, and vice versa.

Electrophoresis of CSF for demonstration of oligoclonal IgG and determination of the CSF IgG index is carried out in neurological patients with suspected MS. Our results indicate that these CSF studies may also be of value for the prognostic evaluation of patients with clinically definite MS.

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Notices

12th World Congress of Neurology,
20–25 September 1981, Kyoto, Japan.

Congress Programme

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Details may be obtained from the Pain Congress Secretariat, University of Edinburgh, Centre for Industrial Consultancy and Liaison, 16 George Square, Edinburgh EH8 9LD, Scotland, UK.

International Symposium on Gilles de la Tourette syndrome

This will be held in New York City 28–29 May 1981.

Further information may be obtained from: Dr Arnold Friedhoff, Director, Millhauser Laboratories, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA.

Migraine Symposium

International Congress of Neurology, Kyoto, Japan, 22 September 1981.

Further information may be obtained from: Dr F Clifford Rose, Princess Margaret Migraine Clinic, Charing Cross Hospital, London W6 8RF.

Correction

In the review of "Muscular Dystrophy and other Inherited Diseases of Skeletal Muscles in Animals" edited by JP Harris (*Journal of Neurology, Neurosurgery, and Psychiatry* 1980; **43**:859) it was incorrectly stated that Alexander Sandow, to whom the volume was dedicated, had died a year before the Symposium was held. In fact, Dr Sandow was a co-organiser of the Conference, helped to bring it to a successful conclusion, and died three months later.

Correction

The authors of the paper "Relation between benign course of multiple sclerosis and low-grade humoral immune response in cerebro-spinal fluid", Dr Stendahl-Brodin and Dr Link (Vol 43 p 102) wish to draw attention to an error in their calculations. The significance of one result was grossly overrated. The following results are obtained when correctly applying Fisher's exact test. Fourteen of the 17 patients (82%) without oligoclonal CSF IgG displayed no or slight disability after a mean duration of disease of 17 years, in contrast to 53% of the patients with oligoclonal CSF IgG after a mean duration of 13 years ($p < 0.05$). The patients with oligoclonal CSF IgG displayed significantly higher frequencies of elevated CSF IgG index values ($p < 0.001$), elevated kappa/lambda ratios ($p < 0.05$), elevated CSF/serum C3 ratios ($p < 0.05$) and elevated CSF/serum C4 ratios ($p < 0.05$) (table 2). In contrast, abnormal blood brain barrier as determined by the CSF/serum albumin ratio, and elevated CSF IgA index values were found at similar low frequencies irrespective of the presence of oligoclonal CSF IgG.

Table 3 shows that patients with a malignant course of MS only infrequently displayed a normal CSF IgG index, in contrast to patients with the most benign course ($p < 0.05$).