Patterns of blood-brain barrier impairment and clinical features in multiple sclerosis

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Abstract

Seventy four patients with clinically definite multiple sclerosis were studied by using polyacrylamide gel electrophoresis of cerebrospinal fluid to assess blood-brain barrier function. Blood-brain barrier impairment was associated with recent clinical relapses of multiple sclerosis and worsened across a spectrum from the relapsing-remitting type of multiple sclerosis to secondary and primary progressive disease. The association between blood-brain barrier impairment and primary progressive disease is particularly interesting in the light of recent evidence that focal gadolinium enhancement on MRI is relatively unusual in patients with this disease.

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Focal disruption of the blood-brain barrier is a well established feature of the lesions of multiple sclerosis. Broman showed that trypan blue, which is usually excluded by the blood-brain barrier escapes from cerebral vessels in the vicinity of plaques after death.¹ More recent histopathological studies have illustrated the occurrence of a protein rich exudate from veins and capillaries in acute plaques and have emphasised that the ring of leakage extends deeply into normally myelinated white matter around the edge of plaques.^{2,3} These pathological observations are in keeping with evidence from neuroimaging, from both contrast CT scanning⁴ and gadolinium enhanced MRI,⁵ that increased permeability of the blood-brain barrier to contrast agents is a consistent early feature of new lesions in multiple sclerosis.

Several lines of evidence suggest that this impairment of the blood-brain barrier may play a part in the pathogenesis both of the remitting and of the persistent neurological disability caused by multiple sclerosis. Firstly, the use of high dose intravenous steroids, which has been shown to accelerate recovery from relapse, is associated with an appreciable reduction in both contrast CT scan and gadolinium MRI enhancement of recent lesions.^{6,7,8} This effect, with concomitant reduction of oedema and of serum protein leakage, may be related to the short term therapeutic effect of steroids. Secondly, the study of experimental allergic encephalo-

myelitis,⁹ and limited clinico pathological correlation in multiple sclerosis,¹⁰ suggest that the focal blood-brain barrier impairment detected by gadolinium MRI in these conditions is accompanied by perivascular inflammation. Work in vitro with oligodendrocyte and other glial cell lines has shown the selective vulnerability of oligodendrocytes to damage by exposure to cytokines¹¹ and serum proteins, for example complement.¹² The combination of perivascular inflammation and associated focal breakdown in the blood-brain barrier might be expected to cause focal demyelination and tissue damage of the kind seen in the established lesions of multiple sclerosis.

Study of the CSF, which intercommunicates freely with brain extracellular fluid,¹³ offers another approach to the investigation of blood-brain barrier disturbance in multiple sclerosis. Albumin concentrations in CSF are raised in only a fifth of patients with multiple sclerosis,¹⁴ and measurement of albumin or total protein is a relatively insensitive gauge of blood-brain barrier impairment. Polyacrylamide gel electrophoresis of CSF is a well established technique, used routinely in many laboratories, principally for detecting oligoclonal bands.^{15,16} It is also a sensitive tool in the assessment of blood-brain barrier function for detecting subtle changes in the distinctive protein profile of normal CSF resulting from a relatively minor influx of serum across a damaged blood-brain barrier.^{15,17,18-19}

Using this approach Takeoka *et al* showed that, among patients with multiple sclerosis and raised total protein in CSF, qualitative electrophoretic abnormalities suggestive of blood-brain barrier disturbance were detectable in 95% of patients; in those patients with multiple sclerosis whose total protein was normal, 56% nevertheless displayed qualitative abnormalities.¹⁹ No distinctions were made in this work between different clinical subtypes of multiple sclerosis, nor between patients in relapse and those in remission. Walker *et al* found that blood-brain barrier impairment, defined by abnormalities, on polyacrylamide electrophoresis, was more common among patients with primary and secondary progressive multiple sclerosis than among patients with relapsing-remitting disease.²⁰

The work reported here extends these findings by describing the relations between two grades of blood-brain barrier disturbance

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(mild and moderate), as assessed by polyacrylamide gel electrophoresis, and a number of clinical features of multiple sclerosis: clinical type (relapsing-remitting, primary and secondary progressive), recency of relapse, and degree of disability. Our principal aims were to examine the pattern of blood-brain barrier impairment in relapse and remission and to discover whether analysis of CSF can distinguish the clinical types of multiple sclerosis.

Methods

CLINICAL

The study population consisted of consecutive patients with clinically definite multiple sclerosis who underwent lumbar puncture in the course of the routine clinical investigation of their symptoms between 1986 and 1988. All were inpatients at the National Hospital for Neurology and Neurosurgery, and full neurological examinations were documented in every case. In many cases Kurtzke disability scores²² had been recorded at the time of the examination.

Four categories of information were obtained retrospectively from the clinical notes. (i) Clinical type of disease: relapsing-remitting disease was defined as disease with relapses followed by substantial or complete remissions; secondary progressive disease was defined as disease that was initially relapsing-remitting, but in which a progressive worsening had supervened, with or without continuing superimposed relapses; primary progressive disease was defined as disease progressive from onset, without clinical evidence of relapse, but with evidence for new lesions appearing at least one month from onset.²¹ Patients' disease types were classified from the clinical notes by one of the authors (BM), and then checked independently by another (DB) who was blind to the laboratory findings. (ii) Date of last relapse: relapse was defined as the development of a neurological disturbance, or worsening of a pre-existing disturbance, lasting for at least 24 hours. Patients within two months of the onset of a relapse were arbitrarily taken to be "in relapse." (iii) Date of lumbar puncture, and therefore proximity to relapse. (iv) Kurtzke disability score²² at the time of the lumbar puncture. Most patients had been scored during their admission, and in these cases the assessment was checked by one of the authors; the remaining patients were scored retrospectively: where the notes did not allow confident scoring of disability the patients were excluded from further study.

BLOOD-BRAIN BARRIER IMPAIRMENT

Total protein in CSF was measured by using benzethonium chloride, as previously described.²³ Albumin and IgG concentrations were determined by electroimmunoassay.²⁴ A volume of CSF containing a standard protein loading of 100 µg was then electrophoresed on 6 mm rod polyacrylamide gels, made up

to 7%T, 2.5%C, as previously described.²⁵ After electrophoresis, gels were stained with Coomassie brilliant blue and destained with 7% acetic acid. The gels were then scanned with a microdensitometer (Joyce Loeble Chromoscan 3) to measure the staining density of the following protein bands: group components (Gc1 and Gc2), haptoglobin type 2-1 monomer (Hp 2-1), transferrin (Trf), and asialotransferrin protein (also known as "tau" protein). The distance travelled by albumin along the gel was taken as one unit; the relative distances travelled by the bands of interest, at which they were scanned by the microdensitometer, were 0.32 (Hp 2-1); 0.53 (Trf); 0.76 (Gc2); and 0.82 (Gc1). The microdensitometer generated a printout displaying automated measurements of the peak absorbance of the protein bands, both above protein free background ("height") and above the immediately preceding region of gel ("size"). All gels were also inspected by eye to ensure that the densitometric measurements were of the correct protein bands.

CSF samples containing more than 100 red blood cells/mm³ or with haptoglobin-haemoglobin complexes in the 'γ' region, were excluded from the study because of the effect of contamination by blood on the density and position of the Hp 2-1 band. The greater of the two height values of the group components was taken as Gc height for the purposes of the definitions below.

Three categories of blood-brain barrier function were defined: (i) normal: CSF samples in which the ratio of Hp 2-1 size to Trf height was <0.01 and in which the ratio of Gc height to tau height was <1; (ii) mild impairment: samples in which the ratio of Hp 2-1 size to Trf height was >0.01, but in which the ratio of Gc height to tau height was <1; (iii) moderate impairment: samples in which the ratio of Gc height to tau height was >1. These distinctions are based upon a previous study of over 1000 specimens¹⁹ and are in basic agreement with the data of Felgenhauer.²⁶ We chose to distinguish only two categories of blood-brain barrier impairment, mild and moderate, as extreme disruption of the blood-brain barrier with CSF total protein of more than 1 g/L is rarely seen in multiple sclerosis and is indeed sufficiently unusual to cast some doubt upon the diagnosis.²⁷

STATISTICS

Relations between blood-brain barrier function and clinical type of disease were analysed with Spearman's rank correlation. The χ^2 test and Fisher's exact test were used to examine the relation between blood-brain barrier function and recency of relapse. The Kruskal-Wallis test for non-parametric data was used to analyse differences in age, disability, disease duration, albumin quotients, and IgG indices between the three clinical types of disease; a corrected Mann-Whitney test was used to probe pairwise differences between the types.

Table 1 Clinical details of the study patients.

	Disease type		
	Primary progressive	Relapsing-remitting	Secondary progressive
Total No	(22)	(26)	(26)
No of men	11	11	13
Mean age	50	37	45
Mean duration of disease (years)	7	6	14
Mean disability (Kurtzke score)	4.5	2.0	4.0

Results

PATIENT DETAILS

Seventy four patients were included, 35 men and 39 women. Numbers, sex ratios, ages, disease durations, and disability scores in the three clinical groups are summarised in table 1. There were significant differences between the three groups with respect to age, disease duration, and disability ($p < 0.01$). Patients with relapsing-remitting disease were on average younger than patients in the other two groups. Patients with secondary progressive disease had a longer duration of disease than patients in the other two groups. Patients in the primary and secondary progressive groups were significantly more disabled than those in the relapsing-remitting group. The sex ratios did not differ significantly between groups. A total of 67 of the patients (91%) had oligoclonal bands of IgG in their CSF, but not in their serum, on isoelectric focusing.

BLOOD-BRAIN BARRIER IMPAIRMENT AND CLINICAL TYPES

Table 2 summarises the results for blood-brain barrier function in the three clinical types of multiple sclerosis. There was a significant tendency for function to worsen from relapsing-remitting through secondary progressive to primary progressive disease ($r = 0.38$, $p = 0.003$). This trend was more pronounced when patients in relapse in the relapsing-remitting and secondary progressive groups were excluded from the analysis ($r = 0.52$, $p = 0.0001$; table 2).

Neither albumin quotients (CSF albumin/serum albumin) nor IgG indices (CSF IgG/serum IgG)/(CSF albumin/serum albumin) distinguished the three groups, including or excluding patients in relapse. The albumin quotient was significantly higher in patients with moderate blood-brain dysfunction (mean 1.2×10^{-2}) than in patients with normal function (mean 0.57×10^{-2} ; $p < 0.05$).

Table 2 Blood brain barrier function and disease types. Values are numbers (percentages)

Blood-brain barrier function	Disease type		
	Relapsing-remitting	Secondary progressive	Primary progressive
<i>Including patients in relapse</i>			
Normal	21 (81)	18 (69)	7 (32)
Mild impairment	4 (15)	4 (15)	11 (50)
Moderate impairment	1 (4)	4 (15)	4 (18)
Total	26 (100)	26 (100)	22 (100)
<i>Excluding patients in relapse</i>			
Normal	14 (100)	18 (78)	7 (32)
Mild impairment		4 (17)	11 (50)
Moderate impairment		1 (4)	4 (18)
Total	14 (100)	23 (100)	22 (100)

The quotients among patients with moderate and mild (mean 0.64×10^{-2}) dysfunction were not significantly different.

BLOOD-BRAIN BARRIER IMPAIRMENT AND RECENCY OF RELAPSE

Among patients susceptible to relapse, in the relapsing-remitting and secondary progressive groups, impairment was more common in relapse than in remission ($p < 0.0001$). Indeed, in the relapsing-remitting group, only patients in relapse had evidence of blood-brain barrier impairment.

BLOOD-BRAIN BARRIER IMPAIRMENT AND DISABILITY

There was a trend towards an association between increasing blood-brain barrier impairment and increasing disability but this did not attain significance.

Discussion

Our main findings are that the state of the blood-brain barrier, as judged by CSF electrophoresis of cerebrospinal fluid, differed significantly between the clinical types of multiple sclerosis and worsened in relapse. As would be expected, our patients with primary and secondary progressive disease were more disabled and older than patients with the relapsing-remitting type. Disease duration was longer among patients with secondary progressive multiple sclerosis than in the other two groups.

The association between recent relapse and blood-brain barrier impairment, is in keeping with a number of reports that other CSF variables reflect the occurrence of recent relapse: thus, following a relapse, there is a trend towards a higher CSF leucocyte count,²⁰ an increased likelihood of finding free light chains of immunoglobulin,²⁸ and an increased incidence of oligoclonal IgM.²⁹

The association between primary progressive disease and blood-brain barrier impairment is consistent with a previous result from this laboratory²⁰ but is perhaps unexpected in the context of recent MRI findings. In Thompson *et al*'s patients, new lesions were less often seen on MRI, and less frequently enhanced with gadolinium, in primary than in secondary progressive disease, despite similar levels of disability and rates of disease progression.³⁰ There are several possible explanations for this discrepancy. It may be that the lesions underlying the blood-brain barrier impairment which we have detected in primary progressive multiple sclerosis are smaller, or more diffuse; undergo blood-brain barrier disturbance of briefer duration; or are more often spinal than those of secondary progressive multiple sclerosis. Alternatively, a predilection for involvement of the spinal cord in primary progressive disease might give rise to an unrepresentative effect on the protein composition of lumbar CSF. As Thompson *et al* suggest, comparative pathological study of patients with primary and sec-

ondary progressive multiple sclerosis may help to resolve this issue.³⁰

Several variables may be interacting to produce the trend we have reported here for blood-brain barrier impairment to worsen across a spectrum from relapsing-remitting to secondary and primary progressive disease: age, disease duration, disability, immobility (which may be associated with a rise in CSF total protein³¹), and clinical type. However, our data suggest that the primary association is between blood-brain barrier impairment and clinical type of disease. This emerges particularly clearly from a comparison of the primary and secondary progressive groups: age and degree of disability were similar in these two groups, and disease duration was greater in the secondary progressive group, yet blood-brain barrier impairment was significantly greater among the primary progressive patients than among the secondary progressive patients.

Our results must be evaluated in relation to the disadvantages of a relatively small and partly retrospective study and the interpretation of the electrophoretic findings used to assess blood-brain barrier integrity. These will be discussed in turn.

Retrospective assessment of clinical notes may lead to imprecision in the quantification of disability, the assignment of clinical type and the timing of relapses. However, provided, as in this study, the assessor is blind to the results with which the clinical features are to be correlated, retrospective analysis should not introduce any systematic bias, although it may of course obscure important findings, by contaminating the results with excessive "noise".

The principle underlying our method of assessment of the blood-brain barrier is that, as the barrier is breached the CSF, which has a distinctive protein profile, comes to resemble serum more closely than is usual.^{15 17-19 26} In the early stages of blood-brain barrier impairment the approximation of the CSF protein profile to that of serum can occur while total protein is still within the normal range.^{17 19} The failure of the albumin quotient to distinguish clinical groups that were clearly distinct on CSF electrophoresis is in keeping with this observation.

Mild blood-brain barrier impairment is characterised by the appearance of haptoglobin species (Hp type 2-1 monomer and types 2-1 and 2-2 polymers) which are normally largely excluded by the intact barrier.^{17 19 26 32 33} One disadvantage of our method for detecting mild impairment, which depends on measurement of the amount of Hp 2-1 monomer, is that the Hp 2-1 species can only be found in the CSF of individuals with the 2-1 haptoglobin genotype; this is the commonest of the haptoglobin genotypes in European populations, but "mild" impairment can thus only be detected in about half of individuals.³⁴ Although this will result in an underestimate of the prevalence of mild blood-brain barrier impairment in all groups of patients, we are not aware of any evidence that there is an

association between clinical type of multiple sclerosis and haptoglobin haplotype; thus we would expect any inaccuracy in the estimate of the frequency of mild impairment to be uniform across groups. A second consideration is that no published large scale electrophoretic studies show that concentrations of Hp 2-1 monomer are consistently low in normal CSF, although the available evidence from smaller studies suggests this.^{17 33}

Is impairment of the blood-brain barrier the only possible explanation of the association between the presence in CSF of the Hp 2-1 monomer and the clinical features described in this paper? We believe that it is the most likely explanation, and it is in keeping with the finding that moderate blood-brain barrier impairment is associated with the same clinical features; alternative possibilities include an increase in serum levels of Hp 2-1 monomer,³⁵ and a genetic association between the Hp 2-1 genotype and disease type in multiple sclerosis. We hope to analyse these possibilities further in future work.

The detection of "moderate" impairment of the blood-brain barrier is less controversial and depends upon the fact that a protein specific to CSF ("tau" protein, a desialated derivative of transferrin) is progressively "diluted out" as serum proteins enter the CSF across an impaired barrier.¹⁵ A ratio of group components to tau protein greater than 1 is generally associated with a raised total concentration of CSF protein: this is born out in the present results by the raised albumin quotient in patients with moderate blood-brain barrier impairment.

Thus this study of associations between the state of the blood-brain barrier, as assessed from the CSF, and clinical features in multiple sclerosis suggests that clinical relapse and progressive, especially primary progressive, disease are associated with breakdown of the barrier. These findings may be of pathogenetic importance with respect to the access of myelinotoxic factors to the central nervous system, particularly in patients with primary progressive disease, in whom disability tends to be greatest. They provide further rationale for treatment aimed at stabilising the blood-brain barrier. A prospective study is underway to explore these findings further, and to establish whether evidence of blood-brain barrier impairment from the CSF predicts a response to treatment with intravenous steroids. Neuropathological study of tissue from patients with differing disease types may help to clarify the anatomical basis of these findings.

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