Changes in Cerebrospinal Fluid IgG and Apolipoprotein E Indices in Patients with Multiple Sclerosis during Demyelination and Remyelination

Nader Riffat,1 Robert H. Christenson,2 Benjamin B. Gelman,3 and Lawrence M. Silverman1

Approximately 85% of patients with multiple sclerosis (MS) can be diagnosed by using magnetic resonance imaging and laboratory tests such as determination of the cerebrospinal fluid (CSF) IgG index and electrophoresis to detect oligoclonal banding. However, these tests results are abnormal in MS patients whether they are in clinical remission or acute exacerbation. Because apolipoprotein E (apo E) is synthesized in the central and peripheral nervous system, particularly during remyelination, we propose that apo E might be a reliable marker of the remyelination that accompanies clinical remission in MS patients. We studied 33 patients with MS, 22 in remission and 11 in exacerbation, and 26 controls of comparable ages. The apo E index, calculated from the concentrations of apo E and albumin in CSF and serum, allowed us to discriminate between MS patients in remission and MS patients in exacerbation (P < 0.001); the IgG index failed to show similar differences. However, combining the apo E and IgG indices gave maximum discrimination between controls, MS patients in remission, and those in exacerbation. This study suggests that apo E measurements should be included in the laboratory evaluation of MS patients.

Additional Keyphrases: albumin · immunoturbidimetry

The diagnosis of multiple sclerosis (MS) presents particular challenges to both clinicians and laboratorians. Besides the specific clinical presentation of neurological symptoms and radiographic evidence of demyelination, laboratory data consistent with an immunological disturbance are necessary for the diagnosis of "definite MS" (1). Other patients classified as "probable MS" present with similar neurological problems but do not meet all the criteria for definite disease, because their symptoms may be secondary to MS or may represent some other neurological disorder. Furthermore, managing MS patients requires an objective assessment of disease activity, which is not possible by radiographic techniques or the laboratory tests mentioned above.

The most common immunological tests used for diagnosing MS include electrophoresis of cerebrospinal fluid (CSF) to detect oligoclonal banding (2), and calculation of the CSF IgG Index (3) and of the central nervous system IgG synthesis rate (4). None of these tests is specific for the diagnosis of MS, and results can be abnormal in other demyelinating disorders (5). Moreover, these tests do not reflect disease activity, reportedly giving abnormal results during both the exacerbation and remission phases of MS (6).

Myelin basic protein (MBP) was originally reported to be abnormal only during the exacerbation phase of MS (7), and thus it was a candidate for use in assessing disease activity. Unfortunately, technical difficulties and lack of diagnostic specificity have kept this test from being clinically reliable (8). Apolipoprotein E (apo E) has an important role in the remyelination process after experimental demyelination in animals (9). Increases in apo E therefore may reflect the remyelination process after an acute demyelinating episode of MS. To quantify apo E for this study, we used recently described immunoturbidimetric assays (10, 11), and we examined its usefulness as an indicator of disease activity in MS patients.

Materials and Methods

Patients with definite MS were selected according to established criteria (1). Patients with MS in remission were recruited and selected if, before they entered the study: (a) they had had a clinical diagnosis of MS for at least a year and a Kurtzke Disability Score between 3 and 7 (12); (b) they had not suffered an acute relapse within three months; (c) they had not been on immunosuppressive therapy within three months; and (d) they had not had a progressive deterioration in their neurological status within 12 months before entering the study, as demonstrated by an increase of at least 1, but not more than 3, Kurtzke units.

Paired specimens of serum and CSF were obtained from 33 patients with definite MS, 22 in remission and 11 in exacerbation, ages 28 to 55 y. The diagnosis of MS was based on clinical and radiographic findings. In addition, all patients had an increased IgG Index (>0.7) and (or) oligoclonal protein bands in their CSF. We used as controls paired specimens from 26 patients of comparable ages, who underwent lumbar puncture as part of a diagnostic evaluation of infectious, neoplastic, or other systemic disorders. None of the control subjects had cytological or clinical evidence of neurologic dysfunction, and none had been given prophylactic brain irradiation or neurotoxic chemotherapy. Serum and CSF samples from patients with MS in acute exacerbation, obtained before any treatment, were stored at −20 °C until analysis.

We determined the concentrations of apo E in serum and CSF and of albumin in CSF by immunoturbidimetry, using antisera and calibrator for apo E from Daiichi Pure Chemicals, Ltd., Tokyo, Japan, and antisera and calibrator for albumin from Atlantic Antibodies, Scarborough, ME 04074, as previously described (10, 11, 13). Serum albumin was quantified colorimetrically by the brom cresol green dye-binding method (14). The concentrations of IgG in serum and CSF were determined by nephelometry, with antisera and calibrators from Beckman Instruments, Inc., Brea, CA 92621 (15).

We calculated the CSF IgG Index, as previously described (16), to correct for the contribution of serum IgG to the CSF pool and to normalize for individual variation in the IgG

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3 Nonstandard abbreviations: MS, multiple sclerosis; IgG, immunoglobulin G; CSF, cerebrospinal fluid; MPB, myelin basic protein; and apo, apolipoprotein.
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serum concentration. Because we are interested in the altered metabolism of central nervous system-derived apo E, we applied the same strategy and used an analogous formula to calculate an apo E Index (based on a relative molecular mass of apo E = 34 000):

\[
\text{CSF apo E Index} = \frac{[\text{apo E}]_{\text{CSF}} \times [\text{albumin}]_{\text{CSF}}}{[\text{apo E}]_{\text{serum}} \times [\text{albumin}]_{\text{serum}}}
\]

Where \([\text{apo E}]_{\text{CSF}}\) is the mass concentration of apo E in CSF, \([\text{apo E}]_{\text{serum}}\) is the mass concentration of apo E in serum, \([\text{albumin}]_{\text{CSF}}\) is the mass concentration of albumin in CSF, and \([\text{albumin}]_{\text{serum}}\) is the mass concentration of albumin in serum.

For statistical comparisons between the means of the patients' and the control groups for all variables measured, we used the two-tail unpaired Student's t-test. The level of significance we chose for rejection of the null hypothesis was \(P < 0.01\).

**Results**

Table 1 lists the measured concentrations of apo E, IgG, and albumin in serum and CSF and the apo E and IgG Indices. The mean CSF apo E Index of MS patients in remission was fourfold that of controls or of MS patients in exacerbation. The mean CSF IgG Index of MS patients, both those in remission and those in exacerbation, was two- to threefold that of controls. As previously reported (6), we saw no significant differences between the IgG Index values for the MS patients in remission and values for those in exacerbation.

The scattergrams of the individual values for CSF apo E Index show that there is overlap between apo E Index values of 8 and 13, within which fell four controls, 8 MS patients in remission, and one patient in exacerbation (Figure 1). The scattergrams of the individual values for CSF IgG Index demonstrate that four MS patients in remission had a value below 0.53, the mean value for the controls. The scattergrams for CSF apo E and IgG Indices also show the most overlap between apo E Index values for controls and MS patients in exacerbation and between IgG Index values for MS patients in exacerbation and remission.

Sixteen of 20 controls had apo E Index values <9 and IgG Index values <0.7; 10 of 11 MS patients in exacerbation had an apo E Index <9 and IgG Index values >0.7; and 16 of 22 MS patients in remission had an apo E Index >9 and an IgG Index value >0.7 (Figure 2).

**Discussion**

Here we have described our studies of patients with MS in acute exacerbation, patients with MS in remission, and controls with no neurological abnormalities. We measured albumin and IgG in serum and CSF to calculate the IgG Index. Together with electrophoresis of CSF to demonstrate oligoclonal banding patterns, this Index provides evidence for immune dysfunction and comprises the laboratory component of the standard workup for MS (17). As has been previously reported, our results show that the IgG Index— together with oligoclonal banding—discriminates between MS patients and controls but not between MS patients in remission and those in exacerbation (6). Myelin basic protein was initially reported to be abnormal only in patients during exacerbation (7). Subsequent attempts to measure MBP reliably and therefore to correlate MBP with disease activity have been largely unsuccessful, owing to technical complications. The highly basic nature of MBP and difficulty in producing antisera specific for the amino acid region indicative of disease activity have made these assays clinically unreliable (8). At present, no commercial assay kit is FDA approved, nor is any appropriate commercial antiserum available that is specific for this amino acid sequence.

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**Table 1. Results (Mean ± SD) for Apo E, IgG, and Albumin in Multiple Sclerosis Patients and Controls**

<table>
<thead>
<tr>
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<th>MS patients</th>
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<td></td>
<td>Controls</td>
<td>Remission</td>
<td>Exacerbation</td>
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<td></td>
<td>(n = 26)</td>
<td>(n = 22)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>CSF apo E Index</td>
<td>5.2 ± 2.8</td>
<td>20.6 ± 13.5</td>
<td>5.3 ± 2.9</td>
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<tr>
<td>CSF IgG Index</td>
<td>0.53 ± 0.12c</td>
<td>1.14 ± 0.69d</td>
<td>1.62 ± 0.78d</td>
</tr>
<tr>
<td>CSF apo E, mg/L</td>
<td>1.4 ± 0.5</td>
<td>1.7 ± 0.8</td>
<td>1.5 ± 0.6</td>
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<tr>
<td>CSF IgG, mg/L</td>
<td>26 ± 13*</td>
<td>76 ± 132</td>
<td>91 ± 77f</td>
</tr>
<tr>
<td>CSF albumin, mg/L</td>
<td>255 ± 124</td>
<td>170 ± 83</td>
<td>258 ± 96</td>
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<tr>
<td>Serum apo E, mg/L</td>
<td>54.8 ± 24.4p</td>
<td>29.9 ± 12.5h</td>
<td>52.2 ± 17.6p</td>
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<tr>
<td>Serum IgG, g/L</td>
<td>10.7 ± 3.15</td>
<td>11.45 ± 5.03</td>
<td>11.19 ± 8.36</td>
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<tr>
<td>Serum albumin, g/L</td>
<td>40.4 ± 8.8</td>
<td>46.7 ± 2.6</td>
<td>42.7 ± 7.0</td>
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*vs †, † vs ‡

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**Fig. 1. Scattergram of cerebrospinal apo E and IgG Indices in controls and multiple sclerosis patients in remission or exacerbation**

**Fig. 2. Comparison of cerebrospinal fluid apo E and IgG Indices of controls and multiple sclerosis patients in remission or exacerbation**
We have elsewhere reported increases in CSF apo E Index values in MS patients in remission (18). In this same report, we suggested that the origin of the increased CSF apo E might be secondary to remyelination, similar to the mechanism that reportedly resulted in increased apo E synthesis after Wallerian degeneration in rats (19). In addition, we suggested that apo E may function in the transport of myelin lipid. Here, we demonstrate that MS patients during exacerbation consistently show a normal apo E Index, while those in clinical remission have a significantly increased Index. One possible explanation of the increased Index might lie in the decreased apo E concentrations in serum observed in MS patients in remission as compared with controls or patients in exacerbation (slightly less than a 50% decrease). Thus, assay of apo E in serum may provide an assessment of remyelination without a lumbar puncture. However, the increased apo E Index observed in MS patients in remission is fourfold greater than the apo E Index in either the control or exacerbation groups. Thus, a decrease in apo E concentrations in serum cannot totally account for the increased apo E Index. Most likely, there is a concomitant increase in synthesis of apo E in the central nervous system secondary to the remyelinating process.

While the initial diagnosis of MS poses a substantial clinical problem, advances in electrophysiological testing and, particularly, the improvements in magnetic resonance imaging have eased this diagnosis. When these are used together with the IgG Index and electrophoresis of CSF for oligoclonal banding, 85% of MS cases can now be detected. Nevertheless, none of the above procedures reflects disease activity, which is a serious clinical consideration in the management of this disease. For example, while new treatment protocols are currently being evaluated, there is no reliable laboratory test to indicate efficacy. Without such a tool, the clinician must depend upon historical and clinical data in managing patients. As Figure 1 makes clear, the apo E Index provides such a tool by reliably differentiating MS patients in remission from those in exacerbation. These observations lead us to suggest that the apo E Index be included in the standard laboratory workup of patients with MS. Together with the IgG Index, the apo E Index allows patients to be conveniently classified as shown in Figure 2. This graphically demonstrates that the combination of tests will clearly differentiate three groups of patients: (a) patients without neurological diseases, (b) MS patients in remission, and (c) MS patients in exacerbation. This representation is more effective than Figure 1 in resolving these three groups, demonstrating the usefulness of combining the IgG and apo E Indices. These data do not include patients with other demyelinating conditions, and this needs further investigation. In addition, longitudinal studies in MS patients might reinforce our observations correlating the apo E Index with disease activity.

As a final consideration, the technical aspects of the apo E Index are similar to those for the IgG Index. As laboratory methods for immunonephelometry and immunoturbidimetry have become more reliable, the precision and accuracy of the IgG Index have improved. Because the same instrumentation is used to generate the components of the apo E Index, we would expect quality-control factors to be similar, and our experience has shown this to be the case.

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References