Cerebrospinal Fluid and Plasma Apolipoproteins in Patients with Multiple Sclerosis*

BENJAMIN B. GELMAN, Ph.D., M.D.,†‡
NADER RIFAI, Ph.D.,
ROBERT H. CHRISTENSON, Ph.D.,
and LAURENCE M. SILVERMAN, Ph.D.

†Department of Pathology and Laboratory Medicine,
University of North Carolina at Chapel Hill,
Chapel Hill, NC 27514
and
Durham Veterans Administration Hospital & Duke University Medical Center,
Durham, NC 27705

ABSTRACT

Apolipoprotein (apo) E is synthesized by cells of the central and peripheral nervous systems, and its synthesis and secretion in the peripheral nervous system of animals are greatly stimulated following Wallerian degeneration. It has been suggested that apo E functions in the metabolism of myelin lipids, but its physiologic role in nervous tissue has not been elucidated. To determine if apo E might play a role in demyelinating neuropathy, the concentrations were examined of apop E and A-1 in the cerebrospinal fluid (CSF) and plasma of patients with multiple sclerosis during clinical remission, and in patients with no neurologic disease. Serum and CSF albumin concentrations were measured to account for the possible influences of serum apo E concentration and/or altered blood-brain barrier permeability on the CSF apo E pool. A CSF index for apo E and apo A-1 was calculated in the same manner presently used for calculation of immunoglobulin G (IgG) production in the nervous system of patients with multiple sclerosis (MS). The results showed that the concentrations of apo E, apo A-1, and albumin in the CSF of the MS patients were not significantly altered. The concentration of apo E in the serum, however, was significantly (p < 0.001) decreased by 44 percent in the MS patients. The difference was relatively specific for serum apo E because the serum apo A1 and albumin concentrations were unchanged. The calculated CSF apo E index in the MS patients was increased by a factor of four over the control patients; however, the decreased serum apo E concentration, by decreasing the denominator of the equation, accounted for most of the increase in the calculated CSF apo E index in the MS patients. Our

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data, therefore, do not support the suggestion that the CSF apo E concentration or the calculated index would be useful diagnostically for measuring demyelination or remyelination in MS patients. They do, however, indicate that the serum apo E concentration is altered in certain MS patients. The latter finding is potentially important clinically because serum apo E is a potent suppressor of lymphocyte-mediated immune function and might be related to the altered suppressor T lymphocyte function observed in the peripheral blood MS of patients.

Introduction

Apolipoprotein (apo) E is one of eight major apolipoproteins which function in the transport, metabolism, and cellular recognition of serum lipoproteins. This protein has also been shown to be a potent suppressor of lymphocyte activation in vitro. Recent studies have demonstrated that apo E is synthesized in cells of the central (CNS) and peripheral (PNS) nervous systems of many species, but its function in the nervous system has not been elucidated. Immuno-histochemical studies in rats showed that apo E antigenicity is localized within astroglial cells in the CNS, and in unmyelinating Schwann cells and macrophages in the PNS, but not within neurons. Apo E is synthesized and secreted in greatly elevated amounts by rat peripheral nerve following Wallerian degeneration and during selective demyelination and remyelination in the absence of axonopathy. All of these reports suggest that apo E has a vital function in neural lipid metabolism, perhaps during the normal and pathologic turnover of myelin cholesterol, but no evidence has yet appeared demonstrating altered metabolism of neural apo E in any human neurologic disease state.

One possible approach to the study of neural apo E in patients is to examine the concentration of apo E in human cerebrospinal fluid (CSF). Cerebrospinal fluid is a convenient and accessible product of the human brain which has already proved to be useful in the detection of active demyelination in the CNS. Apo E is one of the principal apolipoprotein constituents of normal CSF, being present at a concentration that is far greater than would be expected by diffusion of a filtered plasma protein through the blood-brain barrier (BBB). This suggests that the pool of apo E in human CSF is at least partially synthesized within the nervous system and may be a reasonably representative patient sample of CNS-derived apo E. If CNS apo E is important in the metabolism of myelin lipids in humans, it is reasoned that its concentration in the CSF might be altered in a group of patients with multiple sclerosis (MS), a disease characterized by primary degeneration of myelin and marked alteration in myelin cholesterol and lipid metabolism. To test this hypothesis, the concentrations were measured of apo E, apo A-1 (another major apolipoprotein present in human CSF), and albumin in the CSF and serum of 22 patients with MS during clinical remission, and in 28 patients with no neurologic disease.

Methods

Patients

All 22 patients who had MS were between the ages of 28 and 55 years and were diagnosed by established clinical criteria. Specimens were obtained from all these patients during periods of...
clinical remission. The control specimens (n = 28) were obtained from non-neurologic patients of comparable ages who underwent lumbar puncture as part of a diagnostic evaluation of possible infectious, neoplastic, or other systemic disorder. None of the control subjects had cytologic (CSF), or clinical evidence of neurologic dysfunction, and none had been given prophylactic brain irradiation, neurotoxic chemotherapy, or immunosuppressive agents. Cerebrospinal fluid obtained by lumbar puncture and serum obtained by venupuncture were collected within 24 hours of each other and stored frozen at −35°C prior to assays.

**ASSAYS**

Serum and CSF apolipoproteins A-I and E were determined by immunoturbidimetric assays using antisera and calibrator for apo E, * antisera for apo A-I, † and calibrator for apo AI. Serum albumin was measured colorimetrically by the bromocresol green method. Cerebrospinal fluid albumin was assayed by immunoturbidimetric assay using antisera and calibrator.

**EXPRESSION OF RESULTS**

Since higher serum apolipoprotein concentration and/or breakdown of the blood-brain barrier could both lead to apparent increases in the CSF apolipoprotein concentration owing to contamination by serum-derived apolipoproteins, it was considered extremely important to correct for any influence these variables might introduce in our analysis. This same consideration is encountered in the measurement of immunoglobulin G (IgG) production in the CNS in the diagnosis of MS. To correct for the contribution of serum IgG to the CSF pool, a CSF IgG index is calculated which corrects for variation in the BBB by measuring the CSF concentration of albumin (a serum-derived protein) and by normalizing for individual variation in serum IgG concentration. Thus, it is accepted that CSF IgG index is a reflection of IgG production in the CNS (versus leakage of serum IgG through the BBB). This same strategy was used to calculate apo E and apo A-I indexes, since the present authors were interested in altered metabolism of CNS-derived apo E. The formula for this calculation is directly analogous to that described for the CSF IgG index and is given in equation 1 where 

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

**STATISTICS**

Statistical comparisons between the means of the patient and control groups for all parameters measured were performed using the Student’s t test. The level of significance chosen for rejection of the null hypothesis was \( p < 0.01 \).

**Results**

The values obtained for assays of the apolipoprotein and albumin concentrations in CSF and serum are presented in Table I. The concentration of apo E in normal CSF was about three percent of
TABLE I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MS Patients (n = 22)</th>
<th>Controls (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF apo E concentration</td>
<td>0.165 ± 0.061</td>
<td>0.142 ± 0.046</td>
</tr>
<tr>
<td>CSF apo A-1 concentration</td>
<td>0.280 ± 0.164</td>
<td>0.453 ± 0.308</td>
</tr>
<tr>
<td>CSF albumin concentration</td>
<td>16.9 ± 8.3</td>
<td>26.1 ± 12.4</td>
</tr>
<tr>
<td>Serum apo E concentration</td>
<td>3.02 ± 1.27†</td>
<td>5.37 ± 2.39</td>
</tr>
<tr>
<td>Serum apo A-1 concentration</td>
<td>140 ± 22</td>
<td>137 ± 39</td>
</tr>
<tr>
<td>Serum albumin concentration</td>
<td>4668 ± 262</td>
<td>4075 ± 857</td>
</tr>
<tr>
<td>CSF apo E§ index</td>
<td>20.6 ± 13.5‡</td>
<td>5.13 ± 2.74</td>
</tr>
<tr>
<td>CSF apo A-1§ index</td>
<td>0.59 ± 0.27</td>
<td>0.49 ± 0.15</td>
</tr>
</tbody>
</table>

*Apolipoprotein E, apolipoprotein A-1, and albumin concentrations in cerebrospinal fluid and serum, and calculated cerebrospinal fluid apolipoprotein indexes in multiple sclerosis patients and nonneurologic controls (mean ± standard deviation).

†p < 0.001
‡p < 0.0001
§see equation 1 in methods for calculation of index values.

The rationale for this study was based upon recent reports which demonstrate that apo E is synthesized by cells of the CNS and PNS in animals and might play a role in the normal and pathologic changes in the metabolism of myelin lipid,1-5,8,9,12,13,14,18,27,28,29,33 Other than data showing the presence of apo E mRNA in fetal human brain tissue,6 no evidence has been presented demonstrating the presence of apo E synthesis in the human nervous system or of altered neural apo E metabolism in any human neurological disease state. This question was approached by measuring the apo E concentration in the CSF of patients with MS. Patients with MS were selected as a prototypal disease population because the pathologic changes are characterized by primary degeneration of myelin, a marked alteration of myelin cholesterol and lipid metabolism, and a relative sparing of axonal degeneration.1-5,15,25,32 Selected patients were in clinical remission, when remyelination would be most likely to occur, because animal experiments suggest that apo E is synthesized during remyelination8 and de novo myelination.12 To ensure that the CSF apo E measured is produced within the CNS, serum values and albumin concentrations were also measured in both fluids to account for variations in serum apolipoprotein concentration and/or altered permeability of the BBB, similar to previously established methods for biochemical analysis of CSF in MS patients.7,11,17 Our results show that the mean CSF apo E concentration in the
MS patients was not different from a neurologically normal control group. Furthermore, our values for CSF apo E and A1 concentrations in these patients are nearly identical to previously-reported values obtained using an electroimmunoassay. The calculated CSF apo E index, however, was increased by a factor of four in the MS patients over the controls. The reason for this apparent discrepancy lies in the fact that serum apo E concentration was unexpectedly decreased in the MS patients; none of the other components of the apo E index were significantly different in the MS patients. The decrease in the denominator of the calculated CSF index in the MS patients tends to make the index value increase, even though there was no actual increase in the CSF concentration of this protein. Thus, our data calculation should not be taken as confirmation of our main hypothesis that the group of patients with a prototypal demyelinating neuropathy exhibit an altered CSF apo E concentration. The present authors believe the calculation is pertinent, however, because it is analogous to the calculated CSF IgG index which is useful diagnostically. Furthermore, in patients with normal serum concentrations of apo E, the CSF index is probably a valid reflection of neurally-synthesized apo E. The CSF apo E index, therefore, affords clinical investigators with a laboratory method which will be useful in future studies on the role of apo E in human neurologic dysfunction.

To interpret the meaning of the 44 percent decrease in the serum apo E concentration in the MS patients, it is necessary to consider the known functions of this protein in the serum. Apolipoprotein E is one of eight major apolipoproteins which are important components of the serum lipoproteins and functions in the transport, metabolism, and cellular recognition of circulating lipids. This led to the suggestion that the function of this protein in the nervous system relates to metabolism of myelin lipid. However, one of the lesser-known properties of apo E is that it is a potent suppressor of lymphocyte-mediated immune responses in vitro. Delipidated apo E and apo E-containing lipoproteins can bind to receptors on the surface of peripheral blood lymphocytes and then block mitogen-induced membrane phospholipid metabolism in the lymphocytes. Since the demyelination in MS is believed to be mediated immunologically, this immunosuppressive property of serum apo E might be related to the well-described abnormalities of peripheral blood lymphocytes in patients with MS. Functional studies of peripheral blood lymphocytes in MS patients have shown repeatedly that T suppressor cell functions are defective in active MS but are restored to normal during remission. Therefore, the decreased serum apo E concentrations observed in our MS patients during remission might be associated with a decrease in apo E-mediated lymphocyte suppression leading to the restoration of normal lymphocyte function observed during remission. Thus, the lowered serum apo E concentrations observed by us in MS patients during remission suggest that the serum apo E concentration could have an important function in regulating the altered immune status of MS patients. In support of this suggestion, Shore et al recently reported that the serum concentration of apo E-containing lipoproteins in rats with experimental allergic encephalomyelitis (EAE) is greatly increased. Experimental allergic encephalomyelitis is considered to be a model of acute MS because the demyelination involves sensitization of lymphocytes against myelin components, and the inflammatory demyelination which ensues is pathologically similar to MS.
Thus, serum apo E may participate in the altered immunologic function associated with immune-mediated demyelination. Modulation of serum lipoproteins might, therefore, be a rational therapeutic strategy to alter the immune-mediated events occurring in patients with MS.

In summary, the data of this study show that the CSF concentrations of apo E and apo A1 were not altered in a group of patients with a prototypal demyelinating disease during remission. There was a substantial increase in the calculated CSF apo E index in the MS patients, but most of this change was caused by a significant decrease in the serum concentration of this protein. This unexpected decrease in the serum concentration of apo E might be related to the ability of this protein to modulate peripheral blood lymphocyte activation during the waxing and waning of immune-mediated demyelination in MS. Further investigation of CSF and serum apo E concentrations are needed to elucidate the possible role of this apolipoprotein in the pathogenesis and/or diagnosis of neurologic diseases in humans.

References


