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Components in multiple sclerosis cerebrospinal fluid that are detected by radioimmunoassay for myelin basic protein

(myelin proteins/demyelination)

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ABSTRACT **Components in cerebrospinal fluid that are antigenically related to myelin basic protein have been identified by a technique described recently [Barbarese, E., Braun, P. E. & Carson, J. H. (1977) *Proc. Natl. Acad. Sci. USA* 74, 3360–3364] involving separating the cerebrospinal fluid proteins by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and measuring the individual components by radioimmunoassay for myelin basic protein. Samples of cerebrospinal fluid from 48 different patients (23 with definite multiple sclerosis, 4 with suspected multiple sclerosis, and 21 with other neurological diseases) were examined by this technique. The results indicate that cerebrospinal fluid can contain at least three separate components that are detected by radioimmunoassay for myelin basic protein. On the basis of their apparent molecular weights, the three components were identified as follows: component I, intact myelin basic protein; component II, proteolytic fragments of myelin basic protein; and component III, a protein of unknown origin with an apparent molecular weight of 50,000. Most samples of cerebrospinal fluid (45 of 48) from patients with multiple sclerosis and from patients with other neurological diseases contained components I and II. Component III was detected in all of the samples from patients with definite multiple sclerosis, in three of four samples from patients with suspected multiple sclerosis, and in none of the samples from patients with other neurological diseases. Some implications of these findings are discussed.**

Multiple sclerosis (MS) is a disease that causes localized demyelination in the central nervous system. There have been several reports (1–3) that cerebrospinal fluid (CSF) from patients with MS contains material that is detected by radioimmunoassay for myelin basic protein (MBP). However, it is not clear what this material represents or whether conventional radioimmunoassay techniques can detect all of the MBP-related material present in CSF. In the work presented here, a technique described by Barbarese *et al.* (4) has been used to identify the components in CSF that are detected by radioimmunoassay for MBP. The sample is first incubated with sodium dodecyl sulfate in order to disrupt any noncovalent complexes. The dissociated proteins are separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis which displays the individual proteins in a linear array according to their molecular weights (5). The gel is sliced and the protein in each slice is eluted and subjected to radioimmunoassay. In this way, MBP is measured separately from CSF components, such as lipids, nucleic acids, macroglobulins, endogenous antibodies, and other macromolecules that might bind to it and interfere with the assay; proteolytic fragments of MBP are measured separately from the intact molecule, and proteins other than MBP that crossreact in the assay can be identified. Samples of CSF from 23 patients with definite MS, 4 patients with suspected MS, and 21 patients with other neurological diseases were examined by this technique and the various MBP-related components present in each sample were tabulated.

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MATERIALS AND METHODS

Cerebrospinal Fluid. Samples of CSF were obtained from either T. A. McPherson of the W. W. Cross Cancer Institute, Edmonton, AB, or W. Sheremata of the Montreal Neurological Institute, Montreal, PQ. The 23 "definite MS" samples were from patients with MS as defined by the criteria of Schumacher *et al.* (6). The four "suspected MS" samples were from patients which met some but not all of these criteria. The 21 "non-MS" samples were from 10 patients with intervertebral disc disease, 7 with encephalitis, 2 with headache, 1 with polyneuropathy, and 1 with hyperthyroidism. Ten of the "definite MS" samples and 10 of the "non-MS" samples were analyzed with prior knowledge of the diagnosis. The rest of the samples were analyzed without this prior knowledge.

Radioimmunoassay on Polyacrylamide Gels. The method has been described in detail (4). Aliquots of CSF (0.5 ml, 100–200 μg of protein) were lyophilized, dissolved in 1% sodium dodecyl sulfate, and boiled for 90 sec. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was performed as described by Weber and Osborne (5). Molecular weight standards were: lactoperoxidase, 78,000; bovine serum albumin, 68,000; pepsin, 35,000; trypsin, 24,000; and cytochrome *c*, 12,400. The gels were cut into 3-mm slices and the protein in each slice was eluted and analyzed by radioimmunoassay performed with antiserum raised against purified human MBP (1). The effective range of the assay was 5 ng of MBP, which gave 2% inhibition, to 100 ng of MBP, which gave >95% inhibition.

RESULTS

Fig. 1 shows the densitometric and radioimmunoassay profiles for gels of a typical MS and a typical non-MS CSF. The densitometric profiles of the two gels were qualitatively similar although differences in the heights of some of the peaks were apparent. The radioimmunoassay profiles of the two gels showed several inhibiting components. On both gels there was a major component of inhibiting activity in slices 18–23, which is designated component I. This component has an apparent molecular weight of 18,500, which is the molecular weight of MBP. Both gels also contained several smaller components of inhibiting activity in slices 24–28, which are collectively designated component II. These components have apparent molecular weights less than 15,000 suggesting that they represent proteolytic fragments of MBP. On the gel of MS CSF there was

Abbreviations: MS, multiple sclerosis; CSF, cerebrospinal fluid; MBP, myelin basic protein.

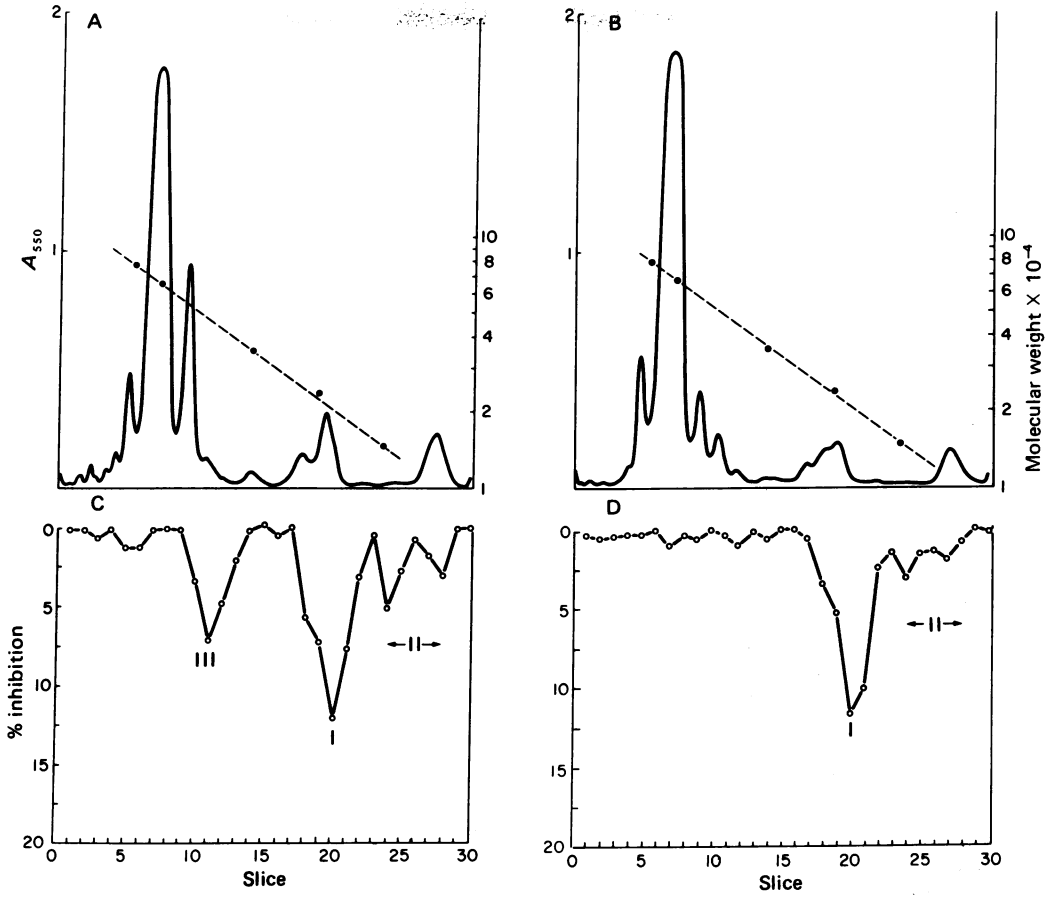

FIG. 1. (*Upper*) Proteins separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (8 mA per gel, 8 hr). (*A*) Typical MS CSF. (*B*) Typical non-MS CSF. (*Lower*) Radioimmunoassay profiles. (*C*) Of gel shown in *A*. (*D*) Of gel shown in *B*. The positions of molecular weight standards electrophoresed at the same time as the CSF proteins are indicated by solid circles.

a third component of inhibiting activity in slices 10–13, which is designated component III. This component has an apparent molecular weight of 50,000 and is of unknown origin. These results show that, when the proteins in CSF are separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, it is possible to identify three separate components that have inhibiting activity in the radioimmunoassay and therefore are antigenically related to MBP. It was not possible to calculate the absolute amounts of the three components because the inhibition values were quite low (5–15%) and, except for component I, the extent of their antigenic relatedness to MBP is unknown.

Samples of CSF from 48 different patients were analyzed by the technique described above and the total inhibiting activity corresponding to components I, II, and III was determined for each sample (Fig. 2). Components I and II were detected in all 23 of the samples from patients with definite MS, in 3 of the 4 samples from patients with suspected MS, and in 19 of the 21 samples from patients with other neurological diseases. The range of inhibition values was quite large. However, the mean for both component I and component II was approximately the same in the definite MS group and in the other neurological diseases group. Component III was detected in all 23 of the samples from patients with definite MS and in 3 of the 4 samples from patients with suspected MS but it was not detected in any of the samples from patients with other neurological diseases. The range of values for component III was approximately the same as for components I and II. However, samples that contained large amounts of one component did not necessarily contain large amounts of the other two components. These results indicate that most samples of CSF, from both MS and non-MS patients, contain small amounts of MBP (component I) and fragments of MBP (component II), and that CSF from patients with MS contains, in addition, a larger MBP-related protein with an apparent molecular weight of 50,000 (component III) that is not present in non-MS CSF.

DISCUSSION

Previous studies (1–3), using conventional radioimmunoassay techniques to measure MBP-related protein in CSF, have shown that samples of CSF from MS patients contain more inhibiting activity than do samples from non-MS patients. It has been suggested that the inhibiting activity in CSF from MS patients represents MBP that is released into the CSF as a consequence of demyelination occurring in the central nervous system in these patients. The work reported here describes the application of a new technique, combining radioimmunoassay with sodium dodecyl sulfate/polyacrylamide gel electrophoresis, to the detection and measurement of MBP-related proteins in CSF. This technique ensures that all the MBP-related protein in the CSF is detected by the assay and also provides a means of identifying which components have inhibiting activity in the assay. The results indicate that non-MS CSF contains small amounts of MBP and fragments of MBP and that MS CSF contains comparable amounts of these two components as well as a larger MBP-related protein with an apparent molecular weight of 50,000 (component III) that is not present in non-MS CSF. This suggests that the MBP and fragments of MBP that

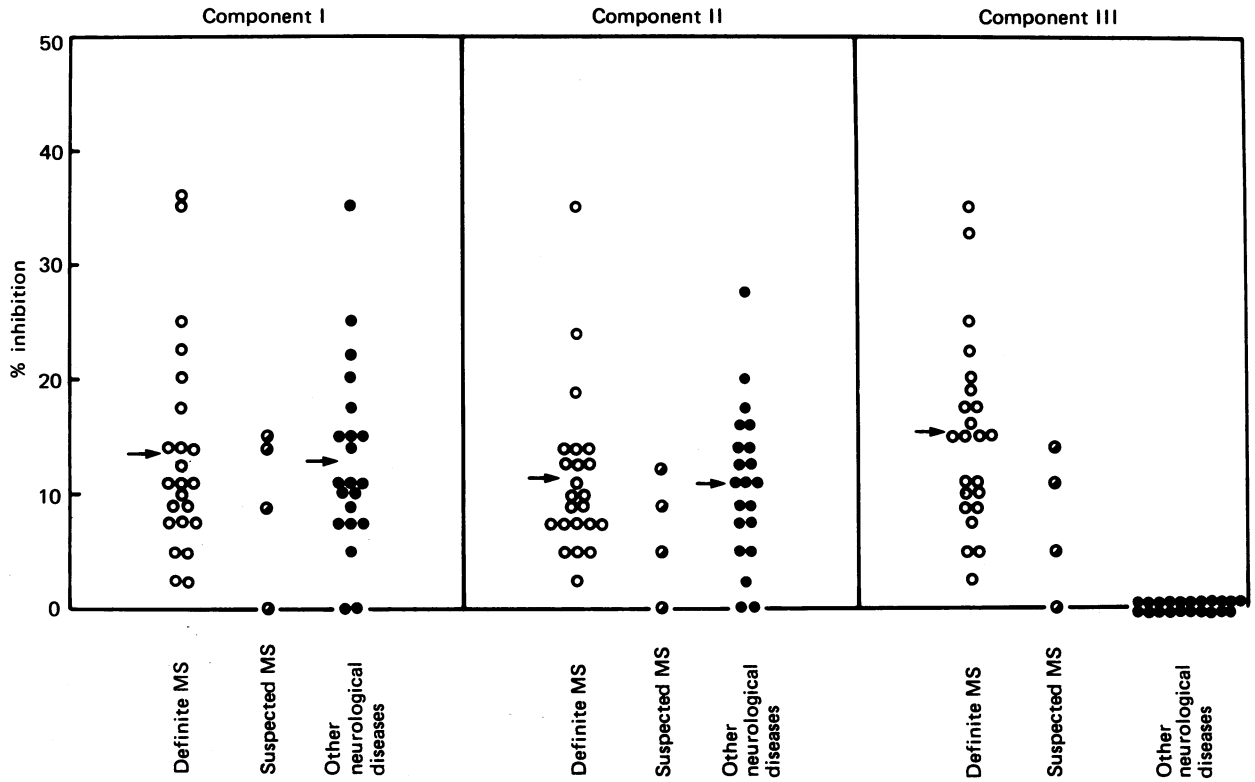

FIG. 2. Distribution of components I, II, and III in MS and non-MS CSF. The CSF samples were analyzed as described in Fig. 1. The total inhibiting activity corresponding to each component was determined by adding the percentage inhibition values in slices 18–23 (component I), in slices 24–28 (component II), and in slices 10–13 (component III). The arrows indicates the mean for each group.

are present in most CSF samples may be due to normal myelin turnover rather than to active demyelination and that the differences in inhibiting activity between MS and non-MS CSF that have been reported by other workers (1–3) may be due largely to the presence of component III in MS CSF and its absence from non-MS CSF.

The origin of component III is unknown. Because it has inhibiting activity in the radioimmunoassay it must be antigenically related to MBP, but it is not a normal constituent of human myelin (unpublished data). Component III may be one of the following: (*i*) an abnormal form of MBP present in the myelin of MS patients, which causes it to break down; (*ii*) a modified form of MBP produced during the demyelination process; or (*iii*) a nonmyelin protein which, because of its antigenic similarity to MBP, induces an autoimmune response directed against the patient's myelin. The isolation and characterization of component III should help to distinguish among these possibilities. Whatever its origin, it seems likely that component III is specific to patients with MS, and it can be hypothesized that it plays some role in the process of demyelination in MS.

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