An Improved Polyacrylamide Gel Electrophoretic Method for Demonstration of Oligoclonal Bands of Immunoglobulin G in Cerebrospinal Fluid

RITA LEE, B.Sc., W. CHEW LIM, Ph.D., BERNARD R. CASEY, M.B., CH.B. AND HENRY C. FORD, M.D., Ph.D.

A two-step procedure is described in which analytical disc gel electrophoresis in polyacrylamide is used to demonstrate oligoclonal bands of immunoglobulin G in cerebrospinal fluid. The principal advantage of the procedure is that unconcentrated samples of cerebrospinal fluid as small as 200 μL can be assayed. The results obtained in samples from 100 patients with various neurologic diseases show that the procedure is sensitive and specific for multiple sclerosis. (Key words: Multiple sclerosis; Oligoclonal bands; Polyacrylamide gel electrophoresis; Cerebrospinal fluid; Immunoglobulin G) Am J Clin Pathol 1983; 79: 112-114

THE DEMONSTRATION of oligoclonal bands of immunoglobulin G (IgG) in cerebrospinal fluid is now an accepted laboratory aid in the diagnosis of multiple sclerosis. Of the various assay methods available, agarose gel electrophoresis and isoelectric focusing have received the most attention. Electrophoresis of cerebrospinal fluid in cylindrical gels of polyacrylamide is a technic with sensitivity and specificity comparable to the more widely used assay methods, and it offers the advantage that prior concentration of the cerebrospinal fluid is not required. The method suffers from the disadvantage that haptoglobin polymers and oligoclonal bands of IgG migrate to similar regions in the gel, which may lead to difficulty in the interpretation of results.

We have developed a two-step procedure in which haptoglobin polymers are removed from the cerebrospinal fluid sample prior to electrophoresis in polyacrylamide gels. In the present paper, we report the results obtained using this procedure to assay 100 samples of cerebrospinal fluid.

Materials and Methods

The final conditions of ion exchange chromatography used to remove haptoglobin polymers from a cerebrospinal fluid sample were as follows: a column of DEAE-Sephadex A-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared within a disposable polypropylene pipette tip (Lancer, St. Louis, MO; product No. HRI 8889-220003). Sephadex G-25, coarse, (Pharmacia) was swollen in 50 mmol/L Tris-HCl buffer, pH 8, containing 100 mmol/L NaCl and was added to the pipette tip to a height of 15 mm. DEAE-Sephadex A-50, swollen in the same buffer, was added to the column to give a final bed volume of 160 μL, exclusive of the Sephadex G-25. The column was washed with several milliliters of the buffer and then 200 μL of cerebrospinal fluid was applied. The eluate was collected and was combined with the eluate from a subsequent 100-μL wash with 50 mmol/L Tris-HCl buffer, pH 8, that contained 150 mmol/L NaCl. The combined eluate was then subjected to analytical disc gel electrophoresis on polyacrylamide gels.

The electrophoretic conditions for the separation and identification of the oligoclonal bands are described in detail elsewhere. In brief, gels 12.5 cm long were cast in glass tubes (140 X 6 mm internal diameter). Twenty microliters of 10% (w/v) sucrose solution was added to the combined eluate (total volume, 350-450 μL), which was then layered onto the top of the gel. Electrophoresis was conducted at 4 mA/gel until the marker dye (bromphenol blue) was 0.5 cm from the bottom of the tube. The gels were removed, fixed with a sulfosalicylic acid-trichloroacetic acid solution, stained with Coomassie brilliant blue, and destained with a weak solution of acetic acid.

To establish the final conditions of ion exchange column chromatography, under which a separation of IgG and haptoglobin polymers could be achieved, a sample of cerebrospinal fluid was used that was known to contain both polyclonal IgG and haptoglobin polymers. Column chromatography was performed as described above, except that after application of the sample, the column was washed successively with 100-μL aliquots...
of the eluent buffer (50 mmol/L Tris-HCl, pH 8) that contained increasing concentrations of NaCl, and the eluate from each wash was collected separately. Proteins in each eluate were assessed after electrophoresis and staining as described.

All specimens of cerebrospinal fluid were obtained from patients with neurologic complaints. In each instance, the attending physician was questioned concerning the likelihood of multiple sclerosis in his patient. Usually, this information was obtained before the clinician was aware of the assay result.

**Results and Discussion**

Under the final conditions chosen for the ion exchange chromatography (100–150 mmol/L NaCl eluent), the cerebrospinal fluid IgG passed unretarded through the column, whereas all detectable haptoglobin polymers were retained (Table 1). In other experiments using samples of cerebrospinal fluid known to contain oligoclonal bands, it was shown that the bands also passed unretarded through the column under the final conditions of chromatography. Typical results are depicted in Figure 1.

The results obtained when the procedure was applied to cerebrospinal fluid samples from 100 patients are given in Table 2. Oligoclonal bands were demonstrated in 12 of 13 patients with definite multiple sclerosis. It is well-recognized that there is a substantial, although small, group of patients with clinically definite multiple sclerosis in whom oligoclonal bands cannot be demonstrated in the cerebrospinal fluid. It has been suggested that such patients may follow a more benign course than those in whom oligoclonal bands are observed. Cerebrospinal fluid samples were received from a number of sources. Therefore, it was not possible to apply a rigorous classification scheme for the diagnosis of multiple sclerosis to the patients in this study. The application of such a scheme to our patients most likely would change the diagnosis in those individuals in whom multiple sclerosis was considered to be unlikely, possible, or probable.

Most samples submitted for assay were obtained from patients in whom multiple sclerosis was thought not to be present. In only one of these cases, a patient with hysterical paraplegia, was an oligoclonal band observed. A possible relationship between hysteria and multiple sclerosis has been noted.

It is with those patients in whom the clinical diagnosis of multiple sclerosis is uncertain that the assay may be of greatest potential value. Long-term follow-up of patients with possible and probable multiple sclerosis may

<table>
<thead>
<tr>
<th>NaCl Conc in Eluent Buffer (mmol/L)</th>
<th>Protein Eluted</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>Haptoglobin polymers</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>Present</td>
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<td></td>
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<tr>
<td>175</td>
<td>Absent</td>
<td>Present</td>
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<tr>
<td>200</td>
<td>Absent</td>
<td>Present</td>
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<tr>
<td>250</td>
<td>Absent</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>Absent</td>
<td>Absent</td>
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**Fig. 1.** Results of polyacrylamide disc gel electrophoresis of cerebrospinal fluid from a patient with definite multiple sclerosis (right) and from a patient in whom multiple sclerosis was not present (left). Before electrophoresis, the cerebrospinal fluid samples were subjected to ion exchange chromatography as described in the Methods section of the text. The arrows point to oligoclonal bands.
Evaluation of a Monoclonal Antibody-based Immunoradiometric Assay for Prostatic Acid Phosphatase

SALLY N. DAVIES, M.B., B.S. AND NATHAN GOCHMAN, PH.D.

This report evaluates a new immunoradiometric assay for prostatic acid phosphatase in serum, based on a dual monoclonal antibody reaction system (Hybritech-TANDEM). A solid-phase antibody binds the acid phosphatase molecule and a second monoclonal antibody to a different antigenic site serves as the 125I-radiolabel. The method was tested on 67 patients with various stages of prostatic carcinoma and 134 patients without the disease. It also was compared with a conventional polyclonal radioimmunoassay (NEN) and an enzymatic activity method (duPont ACA). The upper limit for the TANDEM assay on normal male serum was found to be 2.0 μg/L. Based on this upper limit of normal, the diagnostic sensitivity of the method for all cases of prostatic carcinoma was 60%. We could not distinguish the enzyme released in abnormal amounts due to prostatic carcinoma from that released in conditions such as benign prostatic hypertrophy. The principal advantage of the TANDEM assay is that it is as sensitive as the polyclonal method but faster and less expensive.

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Table 2. Oligoclonal Bands in Cerebrospinal Fluid and the Clinical Likelihood of Multiple Sclerosis

<table>
<thead>
<tr>
<th>Clinical Impression (Multiple Sclerosis)</th>
<th>Number of Cases</th>
<th>Number of Cases with Oligoclonal Bands</th>
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<tbody>
<tr>
<td>Not present</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>Unlikely</td>
<td>9</td>
<td>1</td>
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<tr>
<td>Possible</td>
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<td>4</td>
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<td>Probable</td>
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<tr>
<td>Definite</td>
<td>13</td>
<td>12</td>
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References