Detection of Oligoclonal IgG Bands in Cerebrospinal Fluid by Isoelectric Focusing on Thin Layer Agarose Gels

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ABSTRACT

The detection of oligoclonal bands of IgG in cerebrospinal fluid by isoelectric focusing is a useful aid in the diagnosis of multiple sclerosis. At present, this test is performed on polyacrylamide gels which are technically very difficult to prepare and are not suitable for the routine laboratory. A technique is described for isoelectric focusing on thin layer agarose gels which permits oligoclonal banding detection in the routine laboratory in four hours. The advantages of this procedure over isoelectric focusing on polyacrylamide gels include: (a) it takes less time (four hours compared with at least 24 hours); (b) it requires less specimen (2.5 ml compared with reference laboratory requirements of at least 4 ml); (c) it is technically easier to perform; and (d) it can be done routinely in the clinical laboratory.

Introduction

Patients with multiple sclerosis (MS) present many and varied symptoms which often make diagnosis difficult for the physician. There are, however, consistent abnormalities in the cerebrospinal fluid of MS patients which have permitted the development of laboratory tests that can be of use for confirming a diagnosis of MS. These abnormalities include: (1) the increased concentration of cerebral spinal fluid (CSF) IgG when expressed as a percentage of the total CSF protein, and (2) the characteristic of this IgG to separate into a number of fractions or bands of restricted heterogeneity called oligoclonal bands (OCB) when subjected to electrophoresis.

The increase in CSF IgG is due to the synthesis of IgG within the central nervous system which is characteristic of MS patients and those patients with demyelinating diseases. To ensure that an increase in the level of CSF protein is not due to a damage in the blood-CSF barrier, a ratio of four measured protein values is used, as shown in table I. An R value greater than 0.66 is an indication of increased central nervous system (CNS) production of IgG and, in conjunction with oligoclonal banding, is suggestive of multiple sclerosis.

The methods available to demonstrate
TABLE I

Ratio of Four Measured Protein Values*

<table>
<thead>
<tr>
<th></th>
<th>CSF IgG/serum IgG</th>
<th>CSF albumin/serum albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ranges (adults):</td>
<td></td>
<td></td>
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<tr>
<td>R-value</td>
<td>0.34 - 0.66</td>
<td>0.5 - 0.66 mg/dl</td>
</tr>
<tr>
<td>CSF IgG</td>
<td>0.5 - 6.1 mg/dl</td>
<td></td>
</tr>
<tr>
<td>CSF albumin</td>
<td>13.4 - 23.7 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Serum IgG</td>
<td>639 - 1349 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Serum albumin</td>
<td>3000 - 5200 mg/dl</td>
<td></td>
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</tbody>
</table>

*Used to determine if CSF IgG is synthesized within the central nervous system.

the presence of OCB include cellulose acetate, agarose, and polyacrylamide gel electrophoresis, and procedures using isoelectric focusing. Several studies have been performed that have compared the abilities of these techniques to detect OCB, and the most sensitive appears to be isoelectric focusing. Although more positives can be detected using isoelectric focusing, the use of polyacrylamide gels as a support matrix for this technique has not enabled the procedure to be used as a routine diagnostic test. An isoelectric focusing procedure with thin-layer agarose for the detection of OCB is reported here. This procedure uses agarose, instead of polyacrylamide, as the support medium for isoelectric focusing. The agarose has a property of low electorendosmosis which is necessary for a stable pH gradient to be formed by the ampholytes. There are presently several suppliers of such agarose.* The linearity of a typical pH gradient formed by ampholytes in IsoGel is shown in figure 1.

The procedure produces results in the routine laboratory in less than four hours, retains the sensitivity of polyacrylamide isoelectric focusing, requires only 2.5 ml of CSF, and is suitable for most clinical laboratories since the staining and destaining procedures are rapid and simple.

Materials and Methods

Reagents

IsoGel agarose and GelBond film, a polyester support for the agarose were purchased from FMC Corporation.* Ampholyte, pH range 3 to 10, was purchased from Bio-Rad.† All chemicals were of reagent grade.

Procedures

Cerebrospinal fluid: Upon receipt, CSF was concentrated 80-fold with a MINICON CS-15 concentrator.‡ Samples were stored at 4°C for analysis within two days or at −20°C for longer periods of time.

Gel Preparation: The IsoGel agarose was used at a concentration of 0.5 percent (w/v) in deionized water. The gel was prepared by heating the IsoGel-water mixture to 100°C for 10 minutes.

* P. O. Box 308, Rockland, ME.
† P. O. Box 519, Rockville Center, NY.
‡ Amicon Corp., Lexington, MA.
followed by cooling to 65°C. Ampholyte was then added at 2.5 percent v/v. Gels were prepared of 100 × 150 × 1 mm in size by an open casting method. The GelBond film, hydrophobic side down, was placed onto a glass plate (warmed to prevent premature gelation) and the IsoGel/ampholyte mixture (15 ml) quickly poured and left to gel. Once the agarose had gelled, the gel was placed at 4°C in a humidified chamber for one hour.

**Focusing:** An LKB 2117 Multiphor System§ was used for electrofocusing. During the focusing process, the gel was placed onto the cooling plate and maintained at a temperature below 10°C. The surface of the gel was blotted once with Whatman #1 filter paper, and 5 μl of the concentrated spinal fluid samples applied to the gel by a sample applicator mask* with sample openings 2 × 10 mm. The gel was focused for 60 minutes at 25 watts constant power using an LKB 2103 power supply. Paper strips were used as the anode and cathode that were wetted with 1 M phosphoric acid and 1 M sodium hydroxide, respectively.

**Fixation and Staining:** After focusing, the gel was immediately removed from the cooling plate and fixed for 10 minutes in deionized water containing 30 percent methanol, 5 percent trichloroacetic acid, and 3.5 percent sulphosalicylic acid. The gel was then washed for 20 minutes with two changes of 95 percent ethanol and dried. It was then stained for five minutes with 0.2 percent w/v Coomassie Blue R-250 in deionized water containing 35 percent ethanol and 5 percent glacial acetic acid and destained using the same solution without the Coomassie Blue.

**Results**

Examples of oligoclonal bands detected by isoelectric focusing in IsoGel agarose are shown in figure 2. For controls, each run includes diluted normal human serum as a negative control, serum from a patient with a monoclonal IgG component as a positive control, and pI markers to define the alkaline region of the gel. The normal human serum and negative CSF samples produce a diffuse pattern in the alkaline region. A patient positive for OCB produces bands similar to that of the serum with the monoclonal IgG component.

The patient's CSF that was positive for OCB had a CSF IgG concentration of 8 mg per dL. The use of this technique enables OCB detection in CSF samples with IgG levels as low as 4 mg per dL, the normal range for CSF IgG being 1 to 6 mg per dL. Examples are shown in figure 3.

**Discussion**

The present authors believe isoelectric focusing using an electroendomosis-free agarose offers a satisfactory alternative to agarose electrophoresis or polyacrylamide gel isoelectric focusing for the detection of OCB. Agarose electrophoresis is attractive owing to its simplicity, but it provides low resolution. Although polyacrylamide gel isoelectric focusing provides high resolution, it is difficult to perform. For the routine clinical lab, problems associated with polyacrylamide include (1) the toxicity of acrylamide, (2) the difficulty of the polymerization procedure and subject to failure, (3) long time periods are required for electrofocusing, and (4) staining and destaining require overnight procedures.

In many labs, the simplicity in using agarose gel electrophoresis outweighs its disadvantage in sensitivity. An alternative is to rely on reference laboratories for this test, but they require large quantities of CSF (generally a minimum of 4 ml) with, usually, a delay of up to two weeks before results are obtained. This

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* LKB Instruments Inc., Rockville, MD.
§ Whatman Inc., Clifton, NJ.
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**Figure 2.** Two cerebrospinal fluid (CSF) samples were tested for oligoclonal bands (OCB). From top to bottom: a serum with a monoclonal IgG component as a positive control, a CSF sample positive for OCB, PI markers for defining the pH range of the gel, a CSF sample negative for OCB, and normal human serum as a negative control. Oligoclonal bands can be detected in the alkaline region of the gel (pH > 7) for the positive control and the first CSF patient.

Can be a considerable disadvantage when several tests have to be obtained from one spinal tap and when results are needed fairly quickly. Clearly, a simple technique that uses isoelectric focusing would be of tremendous benefit to such labs. Agarose isoelectric focusing offers comparable resolution to polyacrylamide gel isoelectric focusing and is easy to perform.

**Figure 3.** Cerebrospinal fluid samples positive for OCB with differing levels of CSF IgG. Oligoclonal bands can be detected with CSF IgG levels as low as 4 mg per dl.
References


