Application of Fluoroimmunoassay to Cerebrospinal Fluid Immunoglobulin G and Albumin

ROY W. STEVENS,* DANIEL ELMENDORF, MICHAELYN GOURLAY, ERNST STROEBEL, AND HASSAN A. GAFAAR

Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201

Received for publication 22 June 1979

Special solid-phase fluoroimmunoassay protocols were used to measure the amount of immunoglobulin G (IgG) and albumin in 1,511 samples of cerebrospinal fluid. The fluoroimmunoassay is an inhibition test conducted on the surface of a plastic probe, whose fluorescence is inversely related to the concentration of spinal fluid IgG or albumin. A microcomputer interfaced with the fluorometer calculates the sample IgG or albumin from a calibration curve based on standard cerebrospinal fluid values. The test and interpretation take less than 90 min. Correlation coefficients for over 100 cerebrospinal fluid samples tested by both fluoroimmunoassay and radial immunodiffusion were: IgG, 0.90 (slope, 1.04); albumin, 0.95 (slope, 0.99). The within-run precision (coefficient of variation) was: IgG, 4.4%; albumin, 6.3%. Run-to-run precision on a midrange sample was: IgG, 7.7%; albumin, 11.7%. These findings establish the simplicity, speed, and precision of the modified fluoroimmunoassay system for specific cerebrospinal fluid proteins.

Abnormal concentrations of specific proteins, especially immunoglobulin G (IgG), in cerebrospinal fluid (CSF) are important indicators in the differential diagnosis and treatment of a number of neurological disorders (11). Chief among these are encephalitis, neurosyphilis, subacute sclerosing panencephalitis, multiple sclerosis, and systemic lupus erythematosus affecting the central nervous system. These abnormal concentrations of specific CSF proteins can occur in fluids with normal total protein.

Three immunological procedures, diffusion (8, 12), electrophoresis (9, 18), and nephelometry (5, 14–16; R. W. Stevens, H. Lamonda, N. Trombley, and H. A. Gaafar, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, M34, p. 79), are commonly used in clinical laboratory determinations of CSF IgG and albumin. Tables of normal values have been compiled, and abnormal IgG and albumin concentrations and IgG/albumin ratios in neurological disorders have been described (3, 6, 10, 13).

At our suggestion special protocols were developed by the manufacturer to adapt the existing (4) solid-phase serum-protein fluoroimmunoassay (FIA) (FIAx system, International Diagnostic Technology, Santa Clara, Calif.) for analysis of IgG and albumin in CSF. To accommodate the ultra-low-level IgG and albumin in CSF, the fluorescent-antibody and specimen volumes and ratios of the FIAx serum protein system were adjusted, and unique microcomputer programs were used.

We have investigated the precision of this modified system and its agreement with the radial immunodiffusion (RID) techniques, using reagents supplied by the manufacturer for investigational use.

(This report was presented in part at the American Society for Microbiology Second Conference on Diagnostic Immunology, June 1978, Atlanta, Ga.).

MATERIALS AND METHODS

FIA. The FIAx eystem includes a horizontal shaker (200 oscillations per min, 2.5-cm stroke) with a 45° angle tube holder, a filter fluorometer, and a microcomputer (optional).

The special protocols for determinations of IgG and albumin in CSF are identical, so only the procedure for IgG is described here.

For the test, 5 μl of CSF in 250 μl of phosphate-buffered saline, pH 7.4, is mixed in a 12- by 75-mm tube with 5 μl of fluorescein isothiocyanate-labeled, monospecific, goat anti-human-IgG serum. The mixture is incubated on the horizontal shaker for two 10-min periods. During the first period a plastic probe with a shallow well surface, which has a high capacity to adsorb protein, is shaken in another tube containing 500 μl of human IgG. During the second period this probe is shaken in a rinse tube containing 500 μl of phosphate-buffered saline. The probe is then inserted in the CSF-antiseraum mixture, which is shaken for another 20 min. A final 10-min phosphate-buffered
saline rinse removes all CSF and antiserum proteins not coupled to the probe.

To measure the amount of antiserum complexed to IgG on the probe, the probe is inserted onto the fluorometer stage. Its fluorescence is converted to digital signal units, which are displayed on the instrument panel and simultaneously transmitted to the microcomputer. Probe fluorescence is inversely related to the IgG concentration in the CSF.

Four fluids of known protein concentration are used to establish the calibration curves. The calibrator fluids used in this study contained 0.6, 1.5, 3.0, and 9.2 mg of IgG per dl (or 3.6, 10.2, 17.5, and 52.5 mg of albumin per dl). A calibration curve plotted from their fluorescence signals (Fig. 1) is used to determine IgG concentrations from the specimen signals.

The microcomputer is used to construct the best-fit curve, compare the curve with an ideal curve for the CSF program, and report any deviation. If the calibration curve is acceptable, the specimen concentrations are computed and printed out in milligrams per deciliter. Specimens with fluorescence signals above the upper (or below the lower) reference value are reported as too high (or too low) for computation.

RID. Agar gel plates (Immunoplate III, Hyland Laboratories, Costa Mesa, Calif.) containing antiserum specific for human IgG (or albumin) and precut sample wells were used exactly as prescribed by the manufacturer for limit diffusion ("precision") measurements. The immunodiffusion standards contained (per deciliter) 1.9, 4.1, and 7.9 mg of IgG or 12.3, 25.2, and 50.0 mg of albumin. After 48 h of incubation at 37°C the diameters of the RID precipitation zones formed by the standards and the CSF specimens were measured to the nearest 0.1 mm. The standard measurements were squared and plotted on linear graph paper against known concentrations. Specimen concentrations were then determined from the calibration curve.

Total protein assay. Total protein was determined by applying Folin reagent to a trichloroacetic acid precipitate of the CSF specimen (17). This test is relatively insensitive to the CSF albumin/globulin ratio and eliminates interference by nonprotein substances.

Specimens. The 1,511 samples studied were from lumbar CSF specimens submitted to our reference service for diagnostic tests over 6 months. A total of 1,840 specimens were accessioned, but 329 showed evidence of plasma or microbial contamination or were of insufficient volume for all laboratory tests. These were excluded from the study. To determine the correlation of FIA results with those for the established RID assay, 108 fluids were tested undiluted by each procedure in lots of 54 samples. After all 108 had been examined, those with IgG (or albumin) concentrations greater than the maximum reference value were diluted 1:4 in normal saline and reexamined.

To determine normal IgG (and albumin) FIA values, we selected 50 specimens with normal results in all other laboratory tests (fluids, clear and colorless; qualitative tests for hemoglobin, negative; cardiolipin complement fixation tests for syphilis, nonreactive; colloidal gold curves, normal type A; total protein values, 15 to 45 mg/dl). These specimens were from an equal number of male and female individuals 18 to 60 years of age; no diagnosis or other information on disease was given.

Accuracy and precision. For FIA within-run precision measurements, 19 samples from one pool were tested in a single test run. For run-to-run precision, one pool of fluids was examined on each of 25 test days. In addition, World Health Organization IgG International Reference Preparation 67/95 was rehydrated with 1 ml of distilled water and diluted 1:200 in saline to contain 3.83 mg of IgG per dl (7). This dilution was tested in quadruplicate by FIA and RID.

RESULTS

Comparison of FIA and RID. Agreement between the FIA and RID results was excellent after samples outside the calibration range were excluded (Table 1).

The specific protein concentrations of nearly all 108 specimens compared fell within the FIA calibration range of 0.6 to 9.2 mg/dl for IgG (3.6 to 52.5 mg/dl for albumin). No sample had less IgG (or albumin) than the minimum FIA calibrator; two contained extraordinarily high albumin concentrations.

With RID, however, the relatively high minimum calibrator values (1.9 mg/dl for IgG, 12.3 mg/dl for albumin) precluded measurement of one or both of these proteins in 12 specimens. One contained too little of either IgG or albumin to be measured, six contained too little IgG only, and five others contained too little albumin. The maximum RID calibrator value of 7.9 mg/dl for IgG was exceeded in five specimens; the maximum of 50.0 mg/dl for albumin was exceeded in four of the same specimens.

Accuracy and precision of FIA. Within-run precision for the FIA system for IgG was
better than for RID, and run-to-run precision was nearly as good (Table 2). For albumin, however, the RID system was more precise.

For the diluted World Health Organization International Reference Preparation containing 3.83 mg of IgG per dl, the FIA mean was 3.73 mg/dl; the coefficient of variation was 4.1% (for values of 3.56, 3.60, 3.87, and 3.90 mg/dl). The RID mean for the same dilution was 4.45 mg/dl, and the coefficient of variation was 7.9% (for values of 4.1, 4.1, 4.8, and 4.8 mg/dl).

Normal values. Normal CSF values (in milligrams per deciliter) for IgG and albumin, found by FIA in 50 normal samples, were (mean ± standard deviation): IgG, 2.33 ± 0.53 (range, 1.14–3.90); albumin, 19.46 ± 4.30 (range, 10.6–31.3). IgG as a percentage of total protein was 7.42 ± 1.88 (range, 3.9–12.8).

Analysis of specimens. Of the 1,511 fluids tested, 947 had normal total protein concentrations (15 to 45 mg/dl). Most of these (81.6%) contained IgG, as a percentage of total protein, within 2 standard deviations of the mean normal value (Table 3). However, 167 (17.6%) were ≥2 standard deviations above normal, and 67 (7.1%) were ≥4 standard deviations above normal. One sample was >2 standard deviations below normal. Six others contained too little IgG to measure; three of these six also had too little albumin. None of the 947 samples had IgG or albumin in excess of the maximum reference values.

In the group of 564 specimens with total protein values outside the normal range, only 4 had <15 mg of protein per dl. The IgG concentrations in all four and the albumin concentration in three of these were less than the minimum calibrator values. Of the remaining 560 samples which had total protein values >45 mg/dl, the IgG levels of 52 and the albumin levels of 73 were greater than the maximum calibrator values. Virtually all of these off-scale high samples were fluids with total protein concentrations >65 mg/dl (IgG, 41 of 52 or 90%; albumin, 72 of 73 or 99%).

DISCUSSION

Our results with the special FIAx protocols for CSF determinations showed excellent agreement with findings obtained after 48 h with the limit-diffusion RID procedure. A recent study of the standard FIAx test for serum immunoglobulins showed results for IgG in close agreement with those found by timed-diffusion (16-h incubation) RID (4).

The special FIA system for CSF analysis has greater sensitivity and relatively longer calibration curves than RID. The FIA calibration curve range from 0.6 to 9.2 mg of IgG per dl (3.6 to 52.5 mg of albumin per dl) accommodated all 108 specimens in the comparison study. In the tests of all 1,511 diagnostic specimens only 10 contained too little IgG to be measured, and 6 of

**TABLE 1. Comparison of FIA and RID for determination of IgG and albumin in CSF**

<table>
<thead>
<tr>
<th>Determination</th>
<th>No. of specimens tested</th>
<th>Values outside calibration range</th>
<th>Results compared (no.)</th>
<th>Mean concn (mg/dl)</th>
<th>Intercept</th>
<th>Slope of regression line</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>FIA</td>
<td>108</td>
<td>Too low</td>
<td>0</td>
<td>1</td>
<td>101</td>
<td>3.75</td>
<td>0.274</td>
</tr>
<tr>
<td>RID</td>
<td>108</td>
<td>Too high*</td>
<td>7</td>
<td>5</td>
<td>101</td>
<td>4.18</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIA</td>
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<td></td>
<td>0</td>
<td>2</td>
<td>102</td>
<td>24.4</td>
<td>-0.179</td>
</tr>
<tr>
<td>RID</td>
<td>108</td>
<td></td>
<td>6</td>
<td>4</td>
<td>102</td>
<td>24.2</td>
<td></td>
</tr>
</tbody>
</table>

* Diluted 1:4 and retested.

**TABLE 2. Precision of FIA and RID for determination of IgG and albumin in CSF**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Within run*</th>
<th>Run to Run*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mg/dl)</td>
<td>Range</td>
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<tr>
<td>IgG</td>
<td></td>
<td></td>
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<tr>
<td>FIA</td>
<td>2.99</td>
<td>2.83–3.23</td>
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<tr>
<td>RID</td>
<td>4.02</td>
<td>3.85–4.50</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIA</td>
<td>24.1</td>
<td>22.1–27.0</td>
</tr>
<tr>
<td>RID</td>
<td>23.4</td>
<td>22.5–24.0</td>
</tr>
</tbody>
</table>

* One sample, n = 19. SD, Standard deviation; CV, coefficient of variation.

* One pool for FIA, n = 25; one pool for RID, n = 8.
the 10 contained too little albumin. Thus, specific protein levels of only about 0.7% of the specimens could not be determined by FIA. An estimate of the protein values might have been obtained by testing a greater sample volume in the same reaction mixture.

At the other extreme, 52 specimens contained too much IgG to be measured by FIA of an undiluted specimen; 73 contained too much albumin. All of these had total protein >65 mg/dl. Specific protein concentrations of such specimens might be determined if the sample is diluted 1:4 in saline.

The IgG values in normal adult CSF established with the special FIA protocol are comparable to those reported with other immunological assays. Published means range from 0.9 to 5.0 mg/dl (5, 6, 8) or from 6.4 to 11.4% of total protein (14). The accuracy of our FIA values of 2.3 mg and 7.4% is supported by the number of samples studied, by the method used to select samples, and by the accuracy of the total protein assay. In infants and young children normal total protein, and, thus, IgG concentrations are presumed to be lower (1).

Total protein concentration alone is an inadequate measure of CSF composition, since a significant number (7.1%) of fluids with normal total protein contained a markedly high concentration of IgG. A smaller percentage (0.7%) of fluids had a markedly low concentration of IgG, which may occur in autoimmune disorders with central nervous involvement (10). Thus, IgG measurement is an important adjunct to total protein findings in differentiation of neurological disorders.

The CSF FIA tests were completed very quickly: from materials setup to report printout for 40 specimens took about 90 min. The fluorescence of each sample probe was read, and the protein concentration printed in about 5 s.

This efficiency of solid-phase immunoassay was largely due to the separation of the specific antigen-antibody reactions (2). The dipstick probe was removed from the specimen reaction mixture and washed; neither reagent nor specimen blanks were needed. Specimen filtration or clarification was also unnecessary, since neither materials in suspension or solution (protein aggregates, drugs, contrast media, etc.) nor particulates (cells, tissue debris, etc.) would be carried to the fluorometer. Furthermore, the solid-phase assay was unaffected by an excess of antigen within the limits of the reference curve, since the immunofluorescence, unlike nephelometry, is independent of the character of the antigen-antibody complexes formed.

The test precision meets the special requirements of clinical laboratory determinations, and the results obtained with the World Health Organization reference standard indicate the accuracy of FIA for CSF specimens.

In conclusion, the advantages of simplicity, speed, and precision of the FIA system recommend its use for determinations of CSF IgG and albumin.

ACKNOWLEDGMENT
Gerald I. Kaufman kindly provided statistical analysis for correlation of FIA and RID results.

LITERATURE CITED


