Sensitivity of Immunofixation Electrophoresis for Detecting IgM Paraproteins in Serum

Sidney N. Kahn and Mahin Bina

Detection and characterization of small IgM paraproteins by immunoelectrophoresis are often difficult because of (a) slow diffusion of the macromolecular immunoglobulin, and (b) the obscuring of the light chain, especially kappa, by the light chains of background polyclonal immunoglobulin (the "umbrella effect"). Various methods of sample pretreatment have been tried in an attempt to overcome this problem. Using immunofixation electrophoresis (IFE), we were able to detect and characterize IgM paraproteins in serum at concentrations as low as 0.25 g/L, even when the polyclonal immunoglobulin background was increased. With routine IFE, our laboratory identified 58 patients with IgM paraproteinemias in a four-year period. In 36, the paraproteins were present at < 5 g/L; 10 of these were detectable only by IFE.

Immunoelectrophoretic detection of IgM paraproteins present in serum at low concentrations and determination of their light-chain isotypes are hampered by the slow diffusion of the IgM pentamer and by the obscuring "umbrella effect" (1) of the light chains of polyclonal immunoglobulins that make up normal background (2-5). Several methods have been proposed to overcome this problem, including reduction with mercaptoethanol (7), immunosubtraction (8), immunoelectrofocusing (9, 10), and removal of interfering polyclonal IgG, either by anion-exchange chromatography or by absorption with staphylococcal Protein A (11).

Although previous studies indicate that immunofixation electrophoresis (IFE) alone is capable of detecting and characterizing IgM paraproteins without additional procedures (3, 5, 12, 13), there are few quantitative data on the sensitivity of the procedure. We examined the limits of detection of our IFE procedure for both heavy and light chains of IgM in the presence of both normal and increased concentrations of polyclonal IgG. We also reviewed the laboratory records of 58 patients with IgM paraproteinemias, to determine the nature of the immunoglobulin abnormality.

Materials and Methods

Agarose gel electrophoresis. We used Titan high-resolution agarose gel (Helena Laboratories, Beaumont, TX) as described previously (14), applying 2 μL of undiluted serum to each lane for protein staining. For immunofixation, we diluted the samples in electrophoresis buffer before application to the gel. Amido Black was used for all staining procedures.

Immunofixation. The dilutions of serum used in our routine immunofixation procedure are 1:10 for IgG; 1:5 for IgA, IgM, and kappa light chain; and 1:2 for lambda light chain. Monospecific goat IgG antibodies against human IgM (mu heavy chain) and kappa and lambda light chains were obtained from Atlantic Antibodies, Scarborough, ME.

Cellulose acetate strips soaked in undiluted antibody and drained of excess liquid were applied to the surface of the gel after electrophoresis (15). After incubation at room temperature for 10 min, the antibody-impregnated cellulose acetate strips were removed and the gel plate was soaked for 10 min in 100 mL of 100 g/L NaCl, then pressed dry on filter paper for 5 min under a 1.5-kg weight. The gel was stained after two repetitions of these washing and pressing steps.

Sera. To determine the effect of background immunoglobulin concentration on the sensitivity of IFE for detecting and characterizing IgM paraproteins and their light chains, we selected three sera containing IgM paraproteins in concentrations of 17, 30, and 37 g/L and migrating in the mid-gamma region. All three sera were from patients with Waldenström's macroglobulinemia and had markedly decreased background polyclonal immunoglobulin. We quantified the paraprotein in each serum by densitometry as described previously (14). We set up a range of paraprotein concentrations by making dilutions of the paraproteinemias sera with each of two sera. One diluent serum was normal by agarose gel electrophoresis and had normal concentrations of polyclonal immunoglobulin as measured by immunoturbidimetry (IgG = 8.1, IgA = 4.1, IgM = 1.3 g/L); the other had increased concentrations of immunoglobulin by immunoturbidimetry (IgG = 19.4, IgA = 4.8, IgM = 3.6 g/L), with a polyclonal distribution seen on agarose gel electrophoresis. The concentration of paraprotein in each test serum was first adjusted to approximately 10 g/L by addition of the appropriate diluent serum, followed by serial twofold dilution with the same serum. The serial dilutions of paraprotein were analyzed by both agarose gel electrophoresis and IFE, using the routine dilutions in buffer of 1:5 for IgG and kappa light chain and 1:2 for lambda light chain.

Retrospective studies of patients. In our laboratory, IFE is performed for any of the following reasons: (a) an abnormal band visible on agarose gel electrophoresis, (b) unexplained decreased polyclonal immunoglobulin, (c) apparently increased transferrin or C'3 bands, (d) requests from particular clinical services (e.g., oncology, allergy, and immunology), or (e) a clinical diagnosis consistent with paraproteinemia (e.g., anemia, back pain, possible myeloma, polynephropathy, amyloidosis, lymphoma, leukemia).

During the study period, approximately 5500 serum protein electrophoreses and 1350 immunofixation electrophoreses were performed. Repeated agarose gel electrophoresis on serum from the same patient accounted for an estimated 10% to 15% of the total. We did not routinely ascertain clinical diagnoses.

Results

Sensitivity of IFE. Figures 1 and 2 illustrate the detection limits of agarose gel electrophoresis and IFE for mu heavy and kappa light chains. Figure 1 shows an IgM kappa paraprotein on a normal, and Figure 2 on an increased, polyclonal immunoglobulin background. Only the lower concentrations of paraprotein are shown, but equally clear results were obtained for all the concentrations tested (up to 10 g/L). The heavy and light chains reacted unequivocally
on IFE at paraprotein concentrations ≥0.25 g/L, regardless of the concentration of the background polyclonal immunoglobulin. The other two paraproteins studied (IgM kappa and IgM lambda) gave identical results (not shown).

Figure 3 illustrates the great sensitivity of IFE for detecting abnormalities in patients' samples. A prominent IgG kappa band and three very small bands reacting as IgM lambda were readily detected on an IgG background of normal intensity.

Characterization of IgG light chains by routine IFE. We reviewed the initial agarose gel electrophoresis and IFE results on sera from 58 patients with IgM paraproteinemia detected over a four-year period. We included only cases that were presumptively monoclonal; i.e., only one light-chain isotype was detected in association with the mu heavy chain.

Forty-eight sera contained IgM paraproteins that were visible on agarose gel electrophoresis. Twenty-six of the 48 paraproteins could not be quantified by densitometry, owing to their low concentration in relation to the polyclonal immunoglobulin background (14). Thirteen paraproteins were present at concentrations between 5 and 20 g/L, and nine were >20 g/L. In one case, the paraprotein migrated between transferrin and C'3 and could not be resolved from them by the densitometer.

Ten sera that were not visibly abnormal on agarose gel electrophoresis contained IgM paraproteins that were detectable only by IFE.

A single IFE done under routine conditions sufficed to determine the light-chain isotype in 55 of the 58 cases (39 kappa, 19 lambda). In two cases with no visible abnormality on agarose gel electrophoresis, routine IFE was not definitive but IFE, done using a different serum dilution, was all that was necessary for light-chain characterization. In only one case (Figure 4), a band that was barely visible on agarose gel electrophoresis reacted with anti-mu heavy chain, but not with antisera to light chain, despite analysis at several serum dilutions. No additional studies (e.g., reduction with mercaptoethanol) were performed because of...
the extremely low concentration of the IgM band and because the immunochemical findings (two faint IgG kappa bands visible on agarose gel electrophoresis and a further two IgG bands detected only by IFE) did not appear to be related to the clinical diagnosis.

Discussion

IFE has become the method of choice for routine detection of immunoglobulin abnormalities because of its superiority to immunoelectrophoresis in (a) technical ease, (b) ease of interpretation, (c) greater sensitivity, and (d) turnaround time (2–5, 12, 13, 16–20). Immunelectrophoresis remains a useful supplement for analyzing occasional cases, e.g., an intact paraprotein co-migrating with a free monoclonal light chain. The present study confirms that IFE is the only method required to identify IgM paraproteins, with a detection limit of ≤0.25 g/L even when the concentration of polyclonal immunoglobulin is increased.

Small, apparently homogeneous bands are frequently visible in the gamma globulin region on high-resolution agarose gel, but not on cellulose acetate electrophoresis (21, 22). IFE is the only convenient method for determining whether the abnormality represents C-reactive protein in the acute-phase reaction, an "oligoclonal" response, an immune complex, or a monoclonal immunoglobulin produced by a clone of benign or malignant cells. The very high prevalence of such bands in both hospital and healthy populations (21, 22) implies that most probably do not represent an immunocytic dyscrasia. In some cases, they may represent an "oligoclonal" immune response of restricted heterogeneity. The case shown in Figure 3 may represent such an abnormality.

The concentration of a monoclonal immunoglobulin in serum is approximately proportional to the number of neoplastic cells that produce it. Therefore, one of the earliest indications of an immunocytic dyscrasia may be the detection of a small monoclonal immunoglobulin band on electrophoresis. Determination of the significance of such small bands detected by current highly sensitive methods will require extensive, long-term prospective studies with use of these techniques.

References