Macroglobulinemia: The Usefulness of DL-Penicillamine and Paper Electrophoresis in Diagnosis

Donald J. Campbell and Thomas Boenisch

The change in mobility of an abnormal globulin peak during paper electrophoresis in the presence of DL-penicillamine is the most useful of simple procedures suggested for differentiation between macroglobulin and myeloma protein. However, not all cases indicating macroglobulin by this procedure will correlate with criteria based on ultracentrifuge analysis or antigenic reaction.

The laboratory diagnosis of macroglobulinemia is difficult, and it is generally agreed that the only method that gives certainty is to determine the sedimentation constant of the abnormal serum protein with the ultracentrifuge. In many cases, due to its size, the protein will not migrate in starch or agar during electrophoresis. In paper electrophoresis, macroglobulins show up as a discrete band in either the beta- or gamma-globulin region, but this same performance is exhibited by myeloma proteins of lower molecular weight as well. The term "M-components" has been used to describe these discrete bands that appear in abnormal concentration, usually in the beta- or gamma-globulin region.

Depolymerization of the macroglobulin by various sulphydryl compounds appears to offer simpler means of identifying these proteins. Deutsch and Morton (1), in 1957, were the first to demonstrate this; other investigators (2-7) confirmed their findings. In general, starch gel electrophoresis was performed with and without sulphydryl compounds and macroglobulin was identified by a change in mobility. In our hands, this procedure lacks uniformity.

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Recently, Leder (8) reported that DL-penicillamine altered the paper electrophoretic behavior of a macroglobulin. We found this approach to be far simpler and more readily reproduced than any other proposed. As paper-electrophoresis equipment is available, we felt our experience with Leder’s procedure for investigating macroglobulinemia would be of interest.

We have correlated our findings with immunoelectrophoretic studies using specific antisera,* which has been most useful. It should be mentioned that the antisera were used directly as supplied by the manufacturer. The antigen-antibody reaction obtained with these sera was of sufficient intensity to rule out the necessity of cross absorption to further increase the specificity.

Methods and Materials

The treatment of the serum under investigation was carried out according to Leder’s directions (8). The sample was aliquoted into two 1.0-ml portions, and both were incubated at 25° for 24 hr. after 0.25 ml. of 7.2% (w/v) DL-penicillamine in physiologic saline was added to one portion.

Paper electrophoresis was carried out subsequently. A Durrum-type electrophoresis apparatus was used, in which Schleicher and Schuell 2043a filter-paper strips (2.9 \times 30.5 cm.) were suspended in form of an inverted V. The veronal buffer of pH 8.6 and ionic strength 0.05, contained 0.005% Ca ions (9). After 1 hr. equilibration of the system, separation was carried out for 6 hr. at a constant current of 6.6 ma per eight strips. Six lambda of the nonsulfhydryl treated and 7.5 \lambda of the sulfhydryl-treated portion were applied to the strip. Staining was carried out according to the manufacturer’s directions (10).

By the method described above, we studied ten serum protein samples, all containing abnormal discrete M-globulins, as defined by paper electrophoresis. All were submitted to DL-penicillamine treatment as well as immunoelectrophoresis in agar gel. Five of the ten specimens showed no change in mobility on paper with DL-penicillamine. Antigenically, these five were all of the gamma-type myeloma protein, and clinically all were diagnosed as having multiple myelosis. We included Case 1 (DA) of this group in Fig. 1; it showed no change in mobility of the M-component and was typical of the other

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four cases not shown. As indicated by Fig. 1 and 2, the remaining 5 cases showed some change in mobility of the M-component by paper electrophoresis after DL-penicillamine treatment. Details of our results, including clinical diagnosis, are shown in Tables 1 and 2.

Results and Discussion

Cases 3 and 4 can be considered true Waldenström macroglobulinemia. The M protein was 16 S by ultracentrifuge; antigenically, a strong response was obtained to beta 2 M antisera prepared from physiologic 16 S macroglobulin.

Both Cases 2 and 5 exhibited a change of M-component mobility and antigenically reacted well with beta-2M antisera. However, the sedimentation constants were lower than those usually encountered in macroglobulinemia. Imhof (14) found that 95% of 92 cases of macro-
globulinemia had sedimentation values over 13S. Values between 8S and 13S were referred to as "atypical macroglobulinemia," possibly reflecting a gradual change from myeloma protein through to macroglobulin. Other authors (12, 13) regard a sedimentation constant of 16S to be borderline.

It is also interesting to compare Cases 2 and 6. Both exhibit a beta 2 globulin with some change in mobility on paper after DL-penicillamine. Antigenically they differ, Case 6 being beta-2A (myeloma) with no beta 2M (macroglobulin) antigenicity. Unfortunately, ultracentrifuge data was not available on Case 6.

As shown by Table 1, the Sia water test was of essentially no value, as a bewildering sequence of false positives and negative results were obtained serially on these patients. Serum viscosity was determined on some of the cases. This was performed with an Ostwald viscometer.

Fig. 2. Further specimens showing mobility of M-component. Solid line indicates absence of penicillamine; dotted line, presence of penicillamine.
Table 1. Test Results in Cases of Macroglobulinemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Albumin (g/m.100 ml.)</th>
<th>Globulin (g/m.)</th>
<th>SIA water test</th>
<th>Sed. rate (mm./hr.)</th>
<th>Serum viscosity</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4</td>
<td>7.5</td>
<td>Neg.</td>
<td>57</td>
<td>2.7</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>8.0</td>
<td>Neg.</td>
<td>30</td>
<td>4.7</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>3.1</td>
<td>Neg.</td>
<td>—</td>
<td>2.1</td>
<td>Primary macroglobulinemia</td>
</tr>
<tr>
<td>4</td>
<td>3.1</td>
<td>6.1</td>
<td>Neg./pos.</td>
<td>58</td>
<td>—</td>
<td>Lymphoblastoma</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>4.1</td>
<td>Pos.</td>
<td>55</td>
<td>2.0</td>
<td>*</td>
</tr>
<tr>
<td>6</td>
<td>2.9</td>
<td>5.5</td>
<td>Neg.</td>
<td>58</td>
<td>—</td>
<td>Multiple myeloma</td>
</tr>
</tbody>
</table>

*Lymphocytic hyperplasia, malignant lymphoma?*
†Carcinoma of stomach; malignant lymphoma†; macroglobulinemia (secondary).

Table 2. Protein Fractions Obtained in Testing

<table>
<thead>
<tr>
<th>M-component in ultracentrifuge study (% total protein)*</th>
<th>Paper electrophoretic mobility of M-component</th>
<th>Antigenic property (immunoelectrophoresis)</th>
</tr>
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<tbody>
<tr>
<td>Patient</td>
<td>M</td>
<td>units</td>
</tr>
<tr>
<td>---------</td>
<td>---</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>6.9</td>
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<tr>
<td>2</td>
<td>55</td>
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<td>20</td>
<td>13.9</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Ultra centrifuge data kindly supplied by Dr. Cyril Kay, Department of Biochemistry, University of Alberta.

at 37°, and the values obtained are the ratio of serum/water flow times. By this method, normal serum had a relative viscosity ranging from 1.4 to 1.8.

The sedimentation rates (10–15 mm./hr. normal) are given for interest. They are not corrected for anemia, and the high rates reflect the pronounced effect of rouleaux formation. The elevated viscosity of the serum had virtually no effect in counterbalancing this.

Conclusion

We have confirmed that paper electrophoresis of serum containing abnormal globulins in the presence of L-penicillamine is a useful and simple technic for differentiation between macroglobulin and myeloma protein. However, some cases which indicate macroglobulins by this technic may not meet other criteria of macroglobulinemia based on
ultracentrifuge analysis or antigenic reaction. In such cases, the clinical and hematologic pictures are usually uncertain as well. These cases may include the category of “atypical macroglobulinemia” as described by Imhof (14). Five of 6 cases diagnosed as multiple myeloma showed no change in mobility of the abnormal globulin during paper electrophoresis in the presence of DL-penicillamine.

References