Problems of C-Reactive Protein Determination in Patients with Monoclonal Immunoglobulins

To the Editor:

Urdal et al. (1) remind us that high concentrations of bilirubin, lipids, or rheumatoid factors are known to interfere with many techniques of C-reactive protein (CRP) titration. We observed that certain monoclonal immunoglobulins known to induce false results in certain biochemical analyses (2–4) are also likely to alter CRP measurements in certain techniques.

CRP was measured in 190 patients whose sera contained a monoclonal immunoglobulin. Ninety-four patients had IgG monoclonal immunoglobulin, 22 had IgA, and 74 had IgM. For CRP titration we used two classic immunochemical techniques: nephelometry with the BNA® analyzer (Berhingwerke, Marburg, Germany), using specific antibodies coating latex particles (detection threshold: 3 mg/L), and turbidimetry with a Turbitimer® analyzer (Berhingwerke), using a specific antisera (detection threshold 5 mg/L).

In patients with monoclonal IgG or IgA, there were no discrepancies between results obtained with the two methods, regardless of the existence of an inflammatory syndrome (CRP ≥ 10 mg/L). In contrast, of the 74 patients with IgM monoclonal component, 9 (12.2% of cases) showed marked discrepancies between the two methods: CRP concentration was measured as high by the nephelometric technique (>100 mg/L in 6 cases, between 10 and 100 mg/L in 3 cases), whereas readings were normal (<5 mg/L) with the turbidimetric technique. In these 9 patients, neither the clinical pattern nor acute-phase proteins titration revealed any inflammatory syndrome. This shows that the nephelometry results were inaccurate and artificially increased. The nine cases with the discrepant IgM monoclonal component were patients with Waldenström disease, who had a kappa-type IgM at high plasma concentration (mean 38 g/L, range 11–81 g/L). However, if, as we showed, monoclonal IgM interferes in nephelometric titration of CRP, this interference does not appear to be caused by high IgM concentration. Indeed, of the 52 sera with monoclonal IgM concentration >11 g/L, only 9 were falsely titrated (17.3%). It therefore appears that the abnormality observed is due to a particular structure of the monoclonal component, which induces a nonspecific reaction between the IgM and the latex particles.

References

Autoantibody Specific for Lactate Dehydrogenase Isoenzyme 4

To the Editor:

There have been several reports of complexes between isoenzymes of lactate dehydrogenase (LD; EC 1.1.1.27) and autoantibodies, or macro-LD (1–3). Podlasek et al. (3) described a series of these complexes and classified them into three categories, based on the antibody specificity, as (a) reactive against only M subunits, (b) reactive against both H and M subunits, and (c) reactive against isoenzymes containing both M and H subunits. We report an autoantibody that apparently reacts only with the LD4 isoenzyme: we believe this is the first case of such antibody specificity.

The patient was a 61-year-old man who presented with testicular atrophy and the following biochemical and hematological abnormalities.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patient range</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD, U/L</td>
<td>287</td>
<td>100–225</td>
</tr>
<tr>
<td>Testosterone, mmol/L</td>
<td>6.4</td>
<td>11–40</td>
</tr>
<tr>
<td>Leukocyte count, x 10^9/L</td>
<td>11.8</td>
<td>3.5–10.0</td>
</tr>
<tr>
<td>Neutrophil count, x 10^9/L</td>
<td>9.2</td>
<td>1.5–6.5</td>
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Electrophoresis of the LD isoenzymes on a Beckman Instruments (Brea, CA) Paragon electrophoresis system (LD Agarose Gels, Beckman cat. no. 655940; LD barbital buffer and developing substrate, cat. no. 655795) is shown in Figure 1.

The investigation of the abnormal band migrating cathodally to LD4 involved immunofixation with human antiserum and electrophoresis of an equilibrium mixture of the patient's serum and a normal subject's serum. Immunofixation revealed both LD activity and an IgG kappa immunoglobulin at the position of the abnormal band. Electrophoresis of an equilibrium mixture

Fig. 1. Electrophoretogram of LD isoenzymes: (1) normal subject, (2) patient's sample, and results of immunofixation against (6) polyclonal antiserum, (7) anti-IgG, (8) anti-IgM, (9) anti-kappa, and (10) anti-lambda. A point of application.
mixture of the patient's serum with normal serum showed a pattern identical to that seen with the patient's serum alone, indicating the presence of an excess of an antibody with specificity for LD4 only. As can be seen from the results of the immunofixation, no antibody is associated with the non-LD4 bands.

We believe this incidental finding of a specific anti-LD4 has no clinical significance in this patient apart from perhaps reducing the clearance of LD4 and hence producing an artificially increased total LD activity. It does, however, represent an autoantibody with unusual specificity and leads to a rare case of abnormal LD isoenzyme quantitation.

References

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High Concentrations of Eosinophil-Derived Neurotoxin in Patients' Urine Mimic Lysozyme Far-Cathodic Bands in Agarose Gel Electrophoresis

To the Editor:

We want to draw the attention of clinical chemists concerned with urinary protein analysis to possible misinterpretation of a band migrating far to the cathode in agarose gel electrophoresis. Under standard conditions (Tris/barbital buffer, pH 9.3), a very basic protein band is classically interpreted as being lysozyme. Lysozymuria suggests either an increase in lysozyme concentration in serum or an alteration of renal tubular function. In the latter case, other low-molecular-mass proteins such as β₂-microglobulin and retinol-binding protein are increased in urine. Therefore, in this context, the presence of a cathodic band is easily interpreted.

The former case may correspond to the so-called "isolated lysozymuria," which could be associated with monocytic and myelomonocytic leukemia (1). However, in these situations, the identity of lysozyme has to be carefully determined because other basic proteins with the same apparent electrophoretic mobility as lysozyme but from a different origin may be responsible for such a cathodic band.

Recently (2), we detected from a patient's urine a quantitatively important protein band migrating as lysozyme on agarose gel (Figure 1a). However, no band with molecular mass corresponding to lysozyme could be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The diagnosis of chronic eosinophilic pneumonia (Carrington disease) was made in this patient, based on clinical criteria associated with blood and alveolar hypereosinophilia. This led us to suspect the eosinophil granulocytes as the source of this unusual protein. The protein was easily purified from the agarose gel and its 19-kDa molecular mass was established by SDS-PAGE. In addition, the protein had ribonuclease activity and gave a strong immunoreaction by Western blot analysis with rabbit antibodies against eosinophil-derived neurotoxin (EDN), whereas anti-eosinophil cationic protein (ECP) antibodies gave only a faint reaction. Finally, the 19-kDa protein was used to generate a specific rabbit antibody that was demonstrated to react with EDN.

By systematic and careful examination of urine agarose gel electrophoretograms, we found that the presence of far-cathodic bands was not uncommon. In most cases these bands represented <1% of total urinary protein. The bands were identified by immunofixation on agarose gel with either anti-lysozyme or anti-EDN rabbit antibodies (Figure 1b). Urine from a patient with altered tubular function contained mainly lysozyme and only a trace of EDN, as shown with the corresponding antibodies, whereas urine from a hypereosinophilic patient reacted with anti-EDN but not with anti-lysozyme. Urines from nonhypereosinophilic patients with physiological proteinuria did not exhibit the typical basic band after staining with Coomassie Blue. Nevertheless, they frequently gave a positive reaction with anti-EDN antibodies. This is in agreement with the finding of Reimert et al. (3), who described the purification of EDN from normal urine.

We conclude from these observations that excretion of high amounts of low-molecular-mass basic proteins from eosinophil-EDN and possibly ECP must be taken into account in the pathophysiological interpretation of proteinuria. In addition, our preliminary results indicate that the evolution of hypereosinophilic-associated diseases under corticosteroid treatment can be monitored by estimation of the cathodic band by agarose gel electrophoresis.

References
3. Reimert CM, Minuva U, Kharasmi A,

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