Macro-Prostatic Acid Phosphatase in a Patient’s Serum

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A patient without prostatic carcinoma had a high concentration of prostatic acid phosphatase (PAP; EC 3.1.3.2) in his serum. This PAP was bound to IgG ("macro-PAP"), and IgG autoantibodies against PAP were demonstrated in serum. The patient’s IgG prolonged the biological half-life of radiolabeled PAP in rats, suggesting that the formation of IgG–PAP complexes was responsible for decreased PAP catabolism. Furthermore, macro-PAP was inactivated in serum more slowly than PAP. These factors accounted for the increases in the enzymatic activity and antigenic concentration of PAP measured in the patient’s serum. Inappropriate therapy was prescribed on the basis of this laboratory result. The diagnosis of prostatic carcinoma requires clinical or histological evidence of malignant disease, and should not rely solely on PAP measurements.


Measuring prostatic acid phosphatase (PAP, EC 3.1.3.2) is widely used for diagnosing prostatic carcinoma and for measuring its progression and response to therapy (1). Whereas there has been ample discussion about the cutoff value that most reliably distinguishes normal from abnormal PAP concentrations, very high concentrations almost always indicate a diagnosis of prostatic cancer (2). Few patients with reportedly high PAP activity in serum did not have prostatic carcinoma: a patient with pancreatic carcinoma in whom the tumor produced an acid phosphatase indistinguishable from PAP (3); a patient with myelomonocytic leukemia (4); and an elderly woman with osteoarthritis (5). Here we describe another such exception. Analysis of this patient's serum revealed a novel type of circulating PAP, macro-PAP, which resulted from the combination of an autoantibody with its antigen, i.e., PAP.

Case Report

In 1983, this 63-year-old man was found to have homogeneous splenomegaly by computerized axial tomography scanning. A bone-marrow aspirate showed several lymphoid follicles but no evidence of tumor cells. Two years later, his PAP was measured for the first time and was grossly above normal by all three tests used (normal values listed parenthetically): tartrate-sensitive acid phosphatase activity, 55 U/L (3.6 U/L); radioimmunoassay ("Rianen"), New England Nuclear (NEN), Dreisch, F.R.G.), 102 µg/L (c.2.2 µg/L); and enzyme-linked immunoassay (Merck, Darmstadt, F.R.G.), 31.2 µg/L (<0.47 µg/L).

The prostate was normal on rectal examination and there was no clinical, biologic, or histologic (three fine-needle aspirations) evidence of prostatic carcinoma. Results of bone scan and a radiographic bone survey were also normal except for a severe left coxarthrosis.

An IgM kappa paraprotein was seen on immunoelectrophoresis and values of IgG and IgM were above normal (28.1 and 10.8 g/L, respectively). Cyproterone acetate, 300 mg/day, was started but was interrupted six months later because of poor tolerance.

In October 1986 the patient had a total hip replacement. He has been well since, and values for PAP, IgG, and IgM have decreased (in September 1987, the PAP tartrate-sensitive acid phosphatase test value was 9.5 U/L, by RIA 16.1 µg/L; values for IgG and IgM were 12.6 and 5.0 g/L, respectively). The splenomegaly and the M band have persisted.

Materials and Methods

For gel filtration we used Sephacryl S-300 (Pharmacia, Uppsala, Sweden), equilibrated with phosphate-buffered saline (pH 7.4, conductivity 14 mΩ −1, Oxoid Ltd., Basingstoke, U.K.) in a 2.5 × 90-cm column (serum sample applied, 5 mL). Affinity chromatography on Protein A–Sepharose CL 4B (Pharmacia) was performed in fivefold excess of Protein A, as compared with the IgG loaded on it, so as to obtain maximum binding of IgG (serum sample applied, 1 mL; column volume of swollen gel, 15 mL). Sepharose CL 4B was used as a control, and the buffer was phosphate-buffered saline. Fractions of the effluents were tested for enzymatic activity of PAP.

Total acid phosphatase activity was measured according to Hillmann’s method (6) in a Coba Fara centrifugal analyzer (Hoffmann-La Roche, Basle, Switzerland) at 37 °C. Reagent kits were provided by Bio Mérieux, Charbonnières-les-Bains, France (cat. no. 63501) and were used according to the manufacturer’s instructions. Tartrate-resistant acid phosphatase activities were determined in the same way but with sodium tartrate, 75 mmol/L, present in the reaction mixtures. Activities of the tartrate-inhibited enzyme (so-called prostatic acid phosphatase) were obtained by calculating the difference between total and tartrate-resistant activities.

IgG from normal and pathological sera were purified by chromatography on Protein A–Sepharose. The IgG was eluted with glycine HCL (0.2 mol/L, pH 2.8), and F(ab)2 fragments were obtained by digestion with pepsin (EC 3.4.23.1; Sigma Chemical Co., St. Louis, MO) (7).

PAP from human seminal fluid, purity 99% by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (sodium dodecyl sulfate–polyacrylamide gel electrophoresis; CIE PAGE), 600 U per milligram of protein; Calbiochem, Lucerne, Switzerland) and 125I-labeled PAP (125I-PAP tracer; Rianen, NEN) were used. 125I-PAP was shown by SDS-PAGE to consist of two noncovalently linked chains of ~46 and 50 kDa (8, 9), and no contaminant was detected. After adding 1 µg of PAP or 0.01 µCi of 125I-PAP to 1 mL of the various sera, IgG (10 mg/mL), or F(ab)2 fragments (5 mg/mL), we incubated the mixtures overnight before analyzing them.

Two anesthetized rats were intravenously injected with 125I-labeled preparations of the patient’s PAP–IgG and 500-µL blood samples were obtained from the rats at different
times afterward. The protein-bound (precipitable by trichloroacetic acid, final concentration 100 g/L) radioactivity in these sera was determined.

In vitro inactivation of PAP was measured in serum samples that had been kept at −80 °C. The tartrate-inhibitable acid phosphatase activities were determined after leaving the samples at room temperature (22 °C) for different time intervals.

Results

PAP in the patient's serum was increased as measured by functional assays and immunosassays. By chromatography it became apparent that the size of the PAP was larger, ≥250 kDa, than its expected size of 100 kDa (Figure 1). In normal serum supplemented with purified PAP and in serum of a patient with prostatic carcinoma, PAP enzymatic activity was recovered in 100-kDa material.

To see whether PAP was combined with IgG, we depleted the patient's serum of IgG by Protein A–Sephrose chromatography. This procedure also removed a large fraction of the patient's PAP activity (Table 1), which was recovered in part after elution of the IgG with glycine HCl, pH 2.8. This finding suggested the presence of PAP–IgG complexes, with the IgG having anti-PAP activity. When 125I-PAP was added to the patient's serum or to his purified IgG, a large fraction was retained by the Protein A–Sephrose (Table 1). 125I-PAP added to various control sera or to IgG preparations (sera and IgG from two patients with systemic lupus erythematosus, one with prostatic carcinoma, and one normal subject) did not bind to Protein A.

By gel filtration we determined that the patient's IgG and its F(ab)2 fragment were responsible for a shift in the size of 125I-PAP from 100 kDa to 250 kDa and 200 kDa, respectively. This was not the case when the control IgG and F(ab)2 were used (Figure 2).

Experiments were performed to determine whether macro-PAP differed from PAP in its elimination and inactivation rates. First, we studied the elimination of 125I-PAP in vivo in rats (Figure 3): when PAP was complexed with the patient's IgG, its elimination rate was significantly decreased. Second, we followed in vivo the time-dependent loss of tartrate-sensitive acid phosphatase activity in serum left at room temperature (Figure 4): the inactivation of macro-PAP in the patient's serum was much slower than that of PAP in the serum of a patient with prostatic carcinoma (the estimated half-life being 3.5 times longer).

To confirm this, we measured the inactivation rate of PAP in the sera of 19 patients with prostatic carcinoma: after incubation for 14 h they had lost 83% (SD 15%) of the initial PAP activity, the decrease exceeding 90% in all five sera containing high initial PAP activities (>15 U/L). In contrast, the serum containing the macro-PAP decreased by less than 46%.

Discussion

We concluded that the very high concentration of PAP was attributable to the presence of circulating immune complexes formed by one molecule of PAP and one autoantibody directed at PAP. This was strongly suggested by several findings: (a) the acid phosphatase was most likely PAP, as ascertained by its expected immunoreactivity in two assays with specific polyclonal antibodies against PAP; (b) the difference in the apparent molecular mass between PAP and the patient's "macro-PAP" was ~150 kDa, the size of an IgG molecule; (c) Protein A removed most of the macro-PAP. Moreover, there was clear evidence for antibodies against PAP when radiolabeled PAP was used: this PAP combined with IgG, and also with F(ab)2 fragments, establishing that PAP bound to the antigen-recognition site of the IgG.

Several other enzymes present in blood were described in the form of "macro-proteins" consisting of enzyme–IgG complexes, and in many instances the autoantibody nature of the IgG was clearly established (10–12). The macro-PAP found in the patient described here can be added to this list of falsely increased enzyme concentrations that can mislead clinicians. Here, the patient had three unnecessary fine-needle aspirations of the prostate, and six months of treatment with anti-androgen drugs. Similarly, the measure-

Table 1. Binding of PAP to Protein A–Sephrose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Substrate</th>
<th>PAP%</th>
<th>125I%</th>
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</thead>
<tbody>
<tr>
<td>Patient's serum</td>
<td>Prot A–Seph</td>
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<td>41</td>
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<td>Patient's serum</td>
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<td>3</td>
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<td>Prostatic cancer patient's serum</td>
<td>Prot A–Seph</td>
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<td></td>
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<tr>
<td>Normal serum</td>
<td>Prot A–Seph</td>
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</tr>
<tr>
<td>Patient's IgG</td>
<td>Prot A–Seph</td>
<td>41</td>
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</tr>
<tr>
<td>Normal IgG</td>
<td>Prot A–Seph</td>
<td>5</td>
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* Determined by enzyme immunoassay.
* After addition of 125I-PAP to sample.

Fig. 1. Gel filtration of the patient's serum on Sephacryl S-300
The PAP activity is detected in fractions larger than IgG (~250 kDa), whereas its expected molecular mass is 100 kDa

Fig. 2. Gel filtration on Sephacryl S-300 of radiolabeled PAP (PAP*) mixed with (a) the patient's IgG, (b) normal IgG, (c) the F(ab)2 fragment of the patient's IgG, and (d) the F(ab)2 fragment of normal IgG
The size of PAP increased on exposure to the patient's IgG and F(ab)2 fragment
ment of the creatine kinase (CK, EC 2.7.3.2) BB fraction in plasma is falsely increased when a macro-CK type 1 is present (CK BB–Ig complex); many patients with a macro-CK type 1 were wrongly diagnosed as having myocardial infarction and were managed accordingly, i.e., with stays in coronary care units and coronary arteriographies (13). An abnormally high concentration of a circulating enzyme that is not in accordance with other findings merits further analysis. The chromatographies used here were simple and sufficient to demonstrate enzyme–IgG complexes.

In general the formation of an antigen–antibody complex leads to the rapid elimination of the antigen. Because there was no evidence for overproduction of PAP, we speculated that the functional half-life of the enzyme was prolonged by its combination with antibody. In rats, the PAP–IgG complexes were eliminated at a slower rate than was PAP alone. In addition, macro-PAP was inactivated in serum at a slower rate than PAP; the immunoglobulin may have interfered with the enzymatic inactivation of PAP. Similar phenomena may explain the high concentration in plasma of other enzymes that have been complexed with antibodies (macro-amylase, macro-CK, etc.).

The concomitant decrease of macro-PAP and IgG concentrations indicates some relationship between autoantibodies and production of polyclonal IgG. However, more work is necessary to define such a correlation, because we do not know yet the precise nature of the antibody (polyclonal/monoclonal) and its concentration in blood.

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References