Detection of Alkaline Phosphatase/Immunoglobulin Complexes
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We report another patient with a circulating alkaline phosphatase/immunoglobulin complex in his blood, and describe a simple method of demonstrating such complexes. On electrophoresis on cellulose acetate, the complex was relatively slow moving and there was no activity in the normal bone/liver isoenzyme region. When the serum was treated with trypsin, the slow band disappeared and the normal pattern was restored.

Since the first description (1) of alkaline phosphatase (ALP, EC 3.1.3.1) bound to an immunoglobulin (Ig) in the blood, several papers have addressed this subject (2-4). In most cases the complex was manifested as a slow-moving isoenzyme band on gel electrophoresis and was confirmed by demonstrating ALP to be present in the IgG precipitation arc after immunoelectrophoresis. Heat and other inhibition studies are useful in identifying the ALP type but not the complex. This ALP is usually of liver or bone origin, or both.

While studying a patient with this abnormality during 14 months, we established a simple and rapid technique for its detection.

Case Report

The patient, a 72-year-old man with clinical gout, was admitted with acute pulmonary edema. This was successfully treated, but his chest roentgenogram showed a peculiar "moth-eaten" appearance of the ribs. A full skeletal survey showed extensive sclerotic and lytic areas suggestive of secondary deposits from carcinoma of the prostate. Biopsy of the enlarged prostate confirmed this diagnosis. The patient's serum acid phosphatase (EC 3.1.3.2) activity was 18 U/L (normal <12) and his serum alkaline phosphatase activity was 292 U/L (normal 35-100). Other tests showed mild uremia and moderate hyperuricemia (urate 0.69 mmol/L).

ALP isoenzyme electrophoresis was done, to assess possible hepatic involvement. This showed that all the ALP activity lay in a band that did not correspond to known ALP isoenzymes. The patient was treated with stilbestrol and, over the next 14 months, the serum ALP activity decreased gradually to 111 U/L, but the constituent with unusual electrophoretic mobility persisted as the only band.

We further studied the various samples collected during this period, to learn more about the abnormal isoenzyme.

Methods

Electrophoresis of ALP was performed with the Helena System (Helena Laboratories, Beaumont, TX 77704), using Titan III ISO-VIS cellulose acetate plates (cat. no. 3000) and Electra HR buffer (cat. no. 5805) according to the manufacturer's specifications, except that, for more sensitive visualization, we used β-naphthyl acid phosphate (8 mmol/L) as substrate and Fast Violet B (ALP isoenzyme technical bulletin 27; Gelman Instrument Co., Ann Arbor, MI 48106) as color developer.

After the electrophoretic separation the strip was placed on another cellulose acetate membrane spread with 0.5 mL of substrate, and incubated at 37 °C for 20 to 30 min in a moist box. The strip was then removed and the color developer was poured onto the reaction surface. With this technique, bands appear within a minute, and the strip is then immersed in an acetic acid solution (50 mL/L) to remove the yellow background.

Complex dissociation. We attempted to dissociate the complex by repeated freezing and thawing (5), or use of Triton surfactant (2), n-butanol (3), or trypsin (3). This last was done by adding 250 μL of serum to 1.0 mg of trypsin (bovine pancreas, Type I; Sigma Chemical Co., St. Louis, MO 63178) and incubating for 2 h at 37 °C.

ALP inhibition studies were done by kinetic analysis and electrophoretic separation after treatment by heat (6, 7) or with L-phenylalanine (8), L-homoarginine (9), or urea (10). We measured total ALP activity kinetically at 37 °C, using p-nitrophenyl phosphate as substrate.

We identified the immunoglobulin nature of the complex and applied the enzyme staining procedure, as outlined, to the electrophoretic gel after immunofixation (11).

The relative molecular mass of the complex was estimated by gel filtration and gradient polyacrylamide gel electrophoresis.

Results

Figure 1 shows the position of the ALP band after electrophoresis. Note the absence of activity in the normal bone/liver isoenzyme positions. After treatment with trypsin, however, the abnormal band disappeared and the pattern became normal. This did not happen after repeated freezing or thawing or after treatment with Triton surfactant (which dissociates lipoprotein complexes) or n-butanol. Nor did trypsin treatment of other sera alter the position of the bands.

Table 1 gives the results of the kinetic inhibition studies. The values obtained for the patient's serum lie between those for bone ALP and liver ALP. Electrophoresis and visualization after treatment with inhibitors confirmed this resemblance to normal bone and liver isoenzymes. Incubation of the patient's serum with serum containing liver or bone ALP (1-3) showed a low residual capability for binding liver ALP or bone ALP.

We confirmed the immunoglobulin nature of the complex after immunofixation by demonstrating ALP activity in the precipitate produced by anti-IgG and anti-lambda chain sera, but not in those produced by anti-M or anti-A sera. Some faint activity was, however, noted with kappa antiserum; this phenomenon has been reported previously (cited by Hattori et al., ref. 4).

The relative molecular mass of the complex and liver ALP was estimated to be about 330 000 and 150 000, respectively. This is compatible with a 1:1 molar ratio of ALP to IgG, as previously suggested (4).
Fig. 1. Cellulose acetate electrophoresis of ALP from liver (patient with biliary obstruction), bone (Paget's disease), placenta (pregnant patient), intestine (extracted from small bowel mucosa) and from our patient's serum, before and after treatment with trypsin.

Discussion

The diagnostic significance of ALP/IgG complexes is not known. No clinical feature has been common to the reported cases. Many have had chronic disease of the liver, intestine, or chest, but this may merely reflect the hospital population most likely to be treated. The main pathological condition in our case was prostatic carcinoma. One other reported case has had prostatic pathology (3), an adenoma. Again, this is probably coincidental. The binding apparently is an acquired, possibly temporary, phenomenon, because it has been reported in a patient with a previously normal ALP isoenzyme pattern (3) and has disappeared in others (4). In our patient it has been present for at least 14 months although the total ALP activity has declined to near normal values.

Now that the existence and nature of such complexes has been established, they can be identified without extensive inhibition and immunological studies. The position of the band on cellulose acetate, the absence or reduction of "activity" in the liver/bone isoenzyme region, and the return to a "normal" pattern after the sample is treated with trypsin indicates an ALP/protein complex.

Not all types of cellulose acetate are suitable for separation of ALP isoenzymes. Crofton and Smith (2) found that their slow ALP complex ran closely behind liver ALP, while De Broe et al. (3) found that their complex could be confused with intestinal ALP. However, we have found the electrophoretic system described to be completely satisfactory for the separation of ALP isoenzymes. Since preparing this paper, we note that Koett et al. (12) have also stressed the value of the Helena system in ALP isoenzyme electrophoresis.

Bauer et al. (13) described a simple technique using the binding of enzyme/IgG complexes in human serum to Protein-A Sepharose CL-4B. This will not detect complexes to other immunoglobulins, or to IgG3, and so has theoretical limitations.

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References