be accompanied by the determination of phenol concentrations in the patient's plasma. The growing acceptance of high concentrations of phenol in clinical practice for various dermatological and nerve block applications, as well as the known risk to the patient, mandates cardiac monitoring in association with rapid and accurate measurements of phenol in blood.

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References

Quantification of Serum Amyloid P by Enzyme-Linked Immunosorbent Assay

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This enzyme-linked immunosorbent assay procedure for quantifying serum amyloid P (SAP) in human plasma makes use of affinity-purified polyclonal antibodies to SAP in a "sandwich"-type format. The procedure is sensitive, reproducible, simple, and easily automatable. Results correlate well with those by a rocket immunoelectrophoresis method performed with the same antibodies. Sera from apparently normal individuals had a mean SAP content of 44.17 mg/L and increased with age.

Additional Keyphrases: amyloidosis • sex- and age-related effects

The generic name amyloidosis is applied to a family of diseases characterized by organ function disturbances attributed to amyloid deposits that replace and destroy normal tissues (1, 2). P-component has been found associated with amyloid fibrils in all forms of amyloid disease, with the possible exception of the intracerebral amyloid plaques associated with Alzheimer disease (3, 4). P-component isolated from both plasma (SAP) and amyloid deposits has the property of calcium-dependent binding to particular specific ligands (5–7). Human serum contains two known pentraxins, C-reactive protein and SAP (8). Unlike C-reactive protein, however, SAP is not an acute-phase protein (9), is glycosylated (10), and is present in serum as a complex of two pentameric discs instead of one (5, 11). SAP is synthesized in the liver and can be reproduced by hepatocytes in tissue culture (12). The short half-life of SAP, 7.8–8.5 h, suggests rapid, continuous production to maintain a stable and substantial concentration in serum, 40–50 mg/L (13–15). The function of SAP, especially its pathological role in amyloid formation or deposition, is unknown. For a better understanding of SAP, a simple assay for its determination is needed. We report here an enzyme-linked immunosorbent assay (ELISA) for the quantification of human SAP.

Materials and Methods

Serum samples. We studied sera from 150 apparently healthy subjects (100 males, 50 females) selected at the Center for Preventive Medicine (Institut Pasteur, Lille). Isolation of SAP. SAP was purified as described by De Beer and Pepys (16). The SAP preparations were analyzed without pretreatment in 4–30% gradient polyacrylamide gel electrophoresis (Pharmacia, Bromma, Sweden) precisely according to the manufacturer's instructions.
Antisera. Monospecific rabbit anti-human SAP protein was raised by repeated immunization of New Zealand White rabbits with isolated (purified) SAP, as described previously (17).

Purification of antibodies to SAP. To purify the anti-SAP antibodies, we precipitated the antiserum with Na2SO4 and subjected the precipitate to Protein A affinity chromatography. The specific antibodies were isolated by passing immunoglobulin fractions through CNBr-activated Sepharose CL-4B coupled to the purified SAP.

Enzyme immunoassay. Antibodies to SAP were coated onto the ELISA plate; the same antibodies were labeled with horseradish peroxidase (EC 1.11.1.7) (18). The coating and washing buffer consisted of phosphate-buffered saline, pH 7.4. The same buffer containing 10 g of bovine serum albumin per liter was used to dilute standard, samples, and labeled antibodies. We coated 96-well microtiter plates by incubating them for 16–18 h at 25 °C with 100 μL of phosphate-buffered isotonic saline containing antibody to SAP, 20 mg/L. The solution was then aspirated and the wells were washed four times. We then incubated the wells with 100 μL of diluted antigen for 2 h at 37 °C, washed the wells, and added 100 μL of labeled antibody solution at a dilution determined by previous titration experiments. After a further incubation for 2 h at 37 °C, the plate was washed again. Finally, we added 100 μL of freshly prepared o-phenylenediamine substrate (75 mg of o-phenylenediamine in 25 mL of 0.1 mol/L citrate/phosphate buffer, pH 5.5, and 16 μL of H2O2). We allowed the enzymatic color reaction to develop for 30 min in the dark, then stopped it by adding 100 μL of 1.0 mol/L HCl to each well. The absorbance was read at 492 nm.

Calibration curve. The protein content of purified SAP, determined by the method of Lowry et al. (19), was used to construct a primary standard curve. A pool of sera from healthy patients served as a secondary standard.

Analytical recovery. To determine recovery, we added purified SAP to plasma samples of known SAP concentration, and assayed.

Results

Isolation of SAP. SAP, >99% pure, was isolated from pooled human sera. The molecular mass obtained for our preparation was the same (250 kDa) as that of commercially available SAP (Calbiochem, La Jolla, CA). As determined in 4–30% gradient non-denaturing polyacrylamide gel electrophoresis.

Specificity of the antisera. The antiserum was shown to be monospecific for human SAP by immunodiffusion against normal human serum and purified SAP. The appearance of a single band and the absence of non-specific precipitin lines verified antisera specificity. Specificity was confirmed by immunoblotting, as outlined before (20), by using anti-rabbit-IgG–peroxidase conjugate. A single band corresponding to SAP was obtained.

Standard curves. When we used pure SAP, the binding curve was sigmoidal, characteristic of this type of immunoassay. The most linear region of the curve (from 10 to 100 μg/L) was selected as the primary standard curve (Figure 1). Curve slopes for sera were identical to that for the primary standard. This led us to use the secondary standards for the assay.

Recovery and reproducibility. When various amounts of purified human SAP were added to plasma of known SAP content, analytical recoveries ranged from 94.5% to 104%. The intra- and interassay coefficients of variation were respectively 3.8% and 7.9%.

Correlation. We compared the results obtained by the proposed method (x) with those obtained by rocket immunoelectrophoresis (y) (21), performed with the same antibodies, for 50 different samples covering a wide range of SAP concentration (about 10–100 mg/L). Good agreement was found: \( y = 0.998x + 0.202 \) mg/L (\( r = 0.96, P < 0.0001 \)).

Concentrations of SAP in normal sera. To characterize the method, we quantified SAP in sera of 100 apparently normal subjects, 50 males and 50 females, ages four to 83 years. Their mean SAP concentration was 44.17 mg/L. However, the concentrations in the females were ~7–8 mg/L lower than in the age-matched males \( n = 50 \). Similar results for the SAP concentration in normal subjects have been reported by others (13–15). To eliminate the influence of sex, we also studied 100 apparently normal male subjects, arranged in four age groups. We found significant differences in SAP content with age (Figure 2), in contrast to the results reported by Skinner et al. (22). SAP concentrations in females are also influenced by age (data not shown).

Discussion

Three methods are widely used for the quantification of SAP: rocket immunoelectrophoresis, radioimmunoassay, and rate nephelometry (15, 22–24). We report here an ELISA for determining this protein. We consider this technique the method of choice because it is rapid, sensitive, specific, reproducible, and technically simple. In comparison, radioimmunoassay procedures are more time consuming, require greater technical expertise,
and require the isolation and labeling of SAP. Currently available rocket immunoelectrophoresis techniques for the measurement of SAP have a detection limit for SAP of only 10 mg/L.

Two other enzyme immunoassays of SAP have been reported (25, 26), but these techniques are not simple. In one, the amount of SAP is determined by an indirect ELISA involving calcium-dependent binding of SAP to trinitrophenyl-conjugated proteins, and immunological specificity is lacking (25). In the other, purified SAP is required for each assay, and the assay is less sensitive (26).

The physiological function of SAP remains unknown but the protein is a substantial component of both normal serum and amyloid deposits in humans and animals. Some preliminary results indicate an increase in the SAP concentration in malignancy, in Waldenström macroglobulinemia, and in rheumatoid arthritis (22, 27, 28). Concentrations of SAP are extremely low in neonates and a little higher in children (29). Amyloidosis occurs frequently in elderly subjects, but the SAP concentration in plasma is normal in patients with amyloidosis (22, 23). The significant increase of SAP with age in this study is a finding that deserves further investigation.

Interest in SAP has recently increased as evidence accumulates that it may play a role in amyloidosis. Labelled normal human SAP introduced into the circulation of patients with different forms of amyloidosis is rapidly and specifically deposited at sites of amyloid accumulation and can be imaged (30). The technique described here makes it possible to participate in investigations of various problems associated with this particular protein. The assay should be a useful tool for further clinical and basic research investigations.

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References
Monoclonal Antibody-Based Discrepancies between Two-Site Immunometric Tests for Lutropin

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We compared five two-site immunometric assays (including three commercially available kits) for measuring lutropin (LH) in serum. Four of the assays involved monoclonal antibodies directed against the alpha–beta dimer, intact LH; these assays measured significantly lower concentrations of LH in 19 (out of 83) samples than did a commercial method not involving such antibodies. In five serum samples, two of the intact LH assays failed to detect any significant immunoreactivity above the detection limit. Findings of normal in vitro LH bioactivity in these samples did not confirm the low immunoreactivity of the intact LH assays. The inability of these assays to detect bioactive LH creates confusion in daily, routine testing as well as in research monitoring of bio/immuno ratios. The data presented here confirm our previous findings (Clin Chem 1991;37:333–40) and emphasize the need to avoid the use of monoclonal antibodies specific for the intact LH dimer.

The use of monoclonal antibodies in diagnostic applications increased dramatically during the 1980s. Monoclonal antibodies are particularly well suited for implementing the potentials inherent in a two-site immunometric assay design (e.g., sensitivity, specificity, range, and kinetics). Doubts have been raised, however, about whether a monoclonal antibody may be "too specific" and thus miss some subtype(s) of an antigen present in multiple forms (1, 2). In a recent paper (3), we showed that different two-site immunometric assays involving one or both of two anti-lutropin (lutenizing hormone, LH) monoclonal antibodies underestimated the LH content in about 25% of samples from healthy individuals, compared with results from assays not involving these two antibodies. These antibodies have low cross-reactivity with human chorionic gonadotropin (hCG), and recognize only the intact LH dimer, not the free subunits. Furthermore, the assays based on one or the other "specific" antibodies could detect no LH in one individual, a 31-year-old healthy mother of two children.

Today, several immunometric LH assays, claimed to be highly specific for LH and unaffected by hCG, have become commercially available. Some of these reportedly make use of monoclonal antibodies that recognize only the intact LH dimer. In this study, we report the results of comparing two such LH tests with a commercially available immunofluorometric assay (IFMA) of LH (4) and two inhouse IFMAs involving two intact-specific monoclonal antibodies as described earlier (3). We also measured the in vitro biological activity of LH in selected samples.

Materials and Methods

All assays are calibrated against the 1st International Reference Preparation (IRP) 88/40, human pituitary LH for immunoassay. All determinations were performed in duplicate, according to the manufacturers' instructions.

Commercially Available LH Immunoassays

Amerite LH-30 (Amersham International plc, Amersham, Bucks., U.K.): This assay makes use of an anti-whole-LH monoclonal antibody on a solid phase and a peroxidase (EC 1.11.1.7) labeled anti-beta-LH monoclonal antibody as tracer antibody. A 50-μL sample is incubated in one step for 30 min at 37 °C. The detection limit (2 SD above the mean for the zero standard) is <0.12 int. unit/L and the standard range extends from 2

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