Letters to the Editor should be typed double-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Two Cases of Cytosol Aminopeptidase–Immunoglobulin Complex

To the Editor:

There have been many reports of soluble complexes between immunoglobulins and enzymes in human sera. Maekawa et al. (1) reported a case with various enzyme–immunoglobulin (lg) complexes, including a cytosol aminopeptidase (c-AP; EC 3.4.11.1), also known as leucine aminopeptidase (LAP), immunoglobulin complex. Here we report the second and third cases with c-AP–lgG complex in patients with rheumatoid arthritis (RA) or acute myeloblastic leukemia (AML).

Leucinamide-splitting aminopeptidases in human serum consist of c-AP, microsomal aminopeptidase (EC 3.4.11.2), and cystyl-aminopeptidase (EC 3.4.11.3). We separated each aminopeptidase by electrophoresis on Cellogel membrane with barbital buffer (0.06 M, pH 8.6). Activity staining was performed with L-leucinamide as the substrate, as described by Kanda et al. (2). We detected the existence of complex between c-AP and immunoglobulins by using counterimmunoelectrophoresis in a modification of Tozawa’s technique involving Titan III separating medium (3). The IgG fraction was prepared from the patient’s serum as described by Sudo et al. (4) for studying reconstitution of enzyme–lgG fractions.

Case 1: A 37-year-old woman with RA was admitted to our hospital for fever and swelling of her feet. Her laboratory data included an LAP concentration of 179 U/L (reference interval: 26–44 U/L) and a lactate dehydrogenase (LD; EC 1.1.1.27) concentration of 546 U/L (162–323 U/L), both increases that were attributed to Staphylococcus aureus infection. The remainder of her biochemistry profile was almost within normal range. With the use of antibiotics, her symptoms lessened and the LD and LAP activities decreased. However, the electrophoretic pattern for LAP remained abnormal, with an extra band that migrated more slowly than the normal c-AP, when her LAP activity was greatest (Figure 1). It was suggested that this abnormal pattern correlated with RA rather than the acute symptom of myelitis. Counterimmunoelectrophoresis suggested that the abnormal band was c-AP–lgG(kappa) complex.

Case 2: This patient, a 14-year-old girl with AML, has been followed in our hospital. Her laboratory data included an LAP value of 114 U/L and an LD concentration of 808 U/L. An extra band, located between the origin and the “fast γ” region, was observed in association with the increases of LAP activity (Figure 1). Counterimmunoelectrophoresis suggested that the abnormal band was c-AP–lgG(λ) complex.

We mixed the serum IgG fraction from case 2 with serum containing high c-AP activity and incubated the mixture overnight at room temperature. A new band, corresponding to the extra band reported above, appeared in electrophoresis. This finding suggested that the extra band originated from the complex between IgG and c-AP.

Because the concentration of serum LAP and the fractional determination of each aminopeptidase are useful for the assessment of liver disease (5), viral infection (6, 7), and other diseases, we routinely evaluate serum LAP activity as a part of a patient’s laboratory examination. If measurements of serum LAP activity become more widely undertaken, more cases of “macro” LAP will probably be reported.

References

Yutaro Azuma
Masato Maekawa
Masami Kubo
Takashi Kanno

Dept. of Lab. Med.
Hamamatsu Univ. School of Med.
Handa-cho 3600
Hamamatsu City, 431-31
Japan

Use of Cholesterol/Triglyceride Ratio and the Friedewald Formula

To the Editor:

Gonzalez G-Estrada et al. (1) criticized the Friedewald and DeLong formulas for use in estimating low-density-lipoprotein (LDL) cholesterol. Unfortunately, their evaluation is replete with errors, exaggerates the problems associated with derived or calculated LDL values, and proposes a remedy that is impractical for routine use.

The authors imply that a large portion of patients will have a serum cholesterol/triglyceride (C/TG) ratio too low for either formula. In their control group, 24 of 62 (38.7%) had a
C/TG ratio too low for reliable estimation of LDL cholesterol by the current formulas.

Their patient population with "vascular disease" was not adequately defined. Vascular disease could include hemorrhoids and varicose veins, which have no association with lipid values, as well as angina pectoris, arteriosclerosis, and hypertension, which do. The mix of these diseases was not specified, nor did the authors indicate whether any attempt was made to exclude from the control group any patients with diseases such as diabetes mellitus, which are frequently associated with lipid abnormalities.

pipetting serum or plasma after dilution and ultracentrifugation without marker dye involves the assumption that the top 1.5 mL is solely very-low-density lipoprotein (VLDL). Marker dye is not used in many chemistry laboratories doing ultracentrifugal analysis, but clearly some method of evaluation is required before accepting such a high percentage of outliers among the controls as was shown in their Figure 1. It is very unlikely that the outliers are all explained by an abnormal type of lipoprotein such as seen in Type III hyperlipidemia because that is a very uncommon disorder. The more likely source is contamination of the 1.5-mL aliquot by inclusion of a portion of the LDL fraction. The authors do not give any validation for their ultracentrifugation method nor do they indicate whether other tests were performed to exclude abnormal forms of lipoprotein.

Conversely, the possibility of a low serum C/TG ratio due to the presence of chylomicrons should have been addressed. Confidence in their conclusions is further shaken by numerous errors in the published article:

1. Friedewald's formula is incorrectly stated. On page 1673, paragraph 1, "TG" is substituted for "C" or "TG".

2. Reference to "LDL-C" on line 26 of the last paragraph of page 1674 is erroneous and should be replaced by "C(TG)/VLDL".

3. The legend for Figure 1 should read "mg/L" instead of "mmol/L". This is obvious from the text of the last paragraph of page 1673. (The fact that this Figure is a presentation of ratios is irrelevant because the gram molecular weights of triglyceride and cholesterol are quite different and mmol/L data would be plotted much further to the right without revision of the scale.)

4. "TG" should read "C" or "TC" on line 17 of last paragraph of 1674.

Reference

William G. Gillespie
108 Park Lane
New Castle, PA 16105

Editor's note: The author of the article in question has been ill and is unable to reply to the above Letter. The original article was accepted (pending reotyping) by our previous editor, who is now retired. The above Letter is being printed after careful review by the reviewers of the article cited, one of whom notes that the cited authors clearly stated that the controls were "apparently healthy subjects."

Variants of Prostate-Specific Antigen Separated by Concanaevalin A

To the Editor:

Above-normal concentrations of prostate-specific antigen (PSA; a 33-kDa glycoprotein, ~7% carbohydrate by weight) (1) are an indication of the presence of prostatic diseases, most commonly benign prostatic hyperplasia (BPH) or prostate cancer. We have shown previously significant overlap of PSA concentrations in BPH and prostate cancer (2, 3). A single determination of PSA is not specific for the diagnosis of prostate cancer. Various lectins (proteins that bind to terminal sugar residues of glycoproteins), e.g., concanaevalin A (Con A), have been used to stain normal, hyperplastic prostate, and prostatic adenocarcinoma (4). We studied the molecular variants of PSA for their abilities to bind Con A-Sepharose, hoping to be able to use such information to differentiate BPH from prostatic cancer.

We centrifuged 0.5 mL of Con A-Sepharose 4B (Pharmacia, Inc., Piscataway, NJ 08854) at 12,000 × g (room temperature, 20 min) to remove the supernatant. We then added 1 mL of Tris buffer (50 mmol/L, pH 7.5, containing 150 mmol of NaCl, 1 mmol of MgCl2, 0.5 g of NaN3, 0.1 mmol of ZnCl2, and 10 g of bovine serum albumin per liter), vortex-mixed, and centrifuged as above. The supernatant liquid was aspirated with a disposable pipette and discarded. We then added a serum sample, 0.5 mL; incubated this with the gel sediment at room temperature for 2 h with gentle shaking; and centrifuged the mixture as before. The serum supernate, the Con A-nonreactive (CNR) fraction, was aspirated for PSA analysis by the Tandem E2 solid-phase two-site immunoenzymometric assay (Hybritech, Inc., San Diego, CA 92121) (5).

We analyzed two serum samples from each patient, one sample treated with Con A-Sepharose and the other untreated. All patients' samples, calibrators, and controls were analyzed in duplicate, and the results were averaged according to the manufacturer's recommendation. Any specimen with PSA concentration >100 μg/L was diluted with the zero diluent.

The percentage of Con A-nonreactive PSA was calculated as follows: CNR% = (PSA in treated serum/PSA in the original serum) × 100%

We analyzed 48 serum specimens, 30 from patients with various clinical stages of prostate cancer and 18 from patients with BPH. The percentages of CNR PSA in serum from these two groups of patients is shown in Figure 1. The percentages of CNR PSA of cancer patients varied from 12% to 34% (mean 24.6%, SD 6.0%). For BPH patients, the percentage varied from 3% to 29% (mean 17.8%, SD 6.8%). Although the ranges overlapped, the differences were statistically significant (P <0.001).

Recently, Barak et al. (6) reported complete differentiation of BPH and prostate cancer patients by using Con A-Sepharose. Their study showed no overlap of CNR fractions of PSA between two groups of such patients from Israel. Their procedure was similar to ours except for the centrifugation and calculation, minor differences that should not be sufficient to cause the difference in outcome. The only major difference was the patient populations.

Fig. 1. Distribution of the Con A-nonreactive percentage of PSA for BPH and prostate cancer

The horizontal lines indicate the mean, the vertical lines the standard deviation

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