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Regan Isoenzyme in Normal Human Sera

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SUMMARY

The Regan isoenzyme of alkaline phosphatase was demonstrated in the sera of 50 normal healthy individuals by a very sensitive immunochemical test system. Several properties of the isoenzyme isolated from a pool of the normal sera were studied and were found to be identical to those of placental alkaline phosphatase.

INTRODUCTION

The discovery of both placental and fetal antigens in human cancer sera and tissues has great importance in the area of cancer diagnosis and prognosis (6–8). The most thoroughly studied "carcinoembryonic" antigens are CEA,[1] α-fetoprotein, and Regan isoenzyme. It has not yet been clearly elucidated whether the so-called CEA's are truly tumor specific (1–3, 6–11). CEA has been demonstrated in the plasma of normal healthy individuals by radioimmunoassay and column chromatography (3). However, a glycoprotein of smaller size than CEA has been extracted from normal adult tissues and shown to have common antigenic determinants to CEA (6). As soon as a sensitive radioimmunoassay for α-fetoprotein was developed, detectable levels of the protein in normal individuals were found (9). Studies of the sera of 140 hospital personnel undergoing pre-employment or annual physical examinations showed 4 Regan-positive patients. Also, a number of patients with nonmalignant disease have been shown to have the isoenzyme present in their sera (1, 7). Results from our laboratory showed that sera from 89 of 91 normal healthy adults contained an alkaline phosphatase isoenzyme that reacted with monospecific antiserum to placental alkaline phosphatase (11).

The present communication reports the results of our studies on the confirmation of the existence of the Regan isoenzyme in normal sera.

MATERIALS AND METHODS

A very sensitive immunochemical test system developed in our laboratory was used to assay for the Regan isoenzyme (11). The basis of the method is isolation and 10- to 20-fold concentration of the Regan isoenzyme by reaction with polymerized monospecific rabbit anti-Regan serum. Heat-inactivated serum (65°, 10 min) is incubated with the polymerized antibody for 1 hr and subsequently centrifuged. The pellet containing the enzymatically active enzyme-antibody complex is immediately assayed for enzyme activity with 72 mM phenylphosphate as substrate. Control tubes containing 0.15 M NaCl instead of inactivated serum were carried out through the procedure to check on the spontaneous hydrolysis of phenylphosphate at alkaline pH values.

RESULTS AND DISCUSSION

The sera of 50 healthy normal individuals were assayed for the presence of the Regan isoenzyme. The results obtained are presented in Chart 1. All samples contained detectable levels of a heat-stable isoenzyme that reacted with monospecific antibody to placental alkaline phosphatase.

Several properties of the Regan isoenzyme of alkaline phosphatase, including heat stability, pH optimum, L-phenylalanine sensitivity, and immunological specificity, show its identity with placental alkaline phosphatase and distinguish this alkaline phosphatase isoenzyme from other isoenzymes in serum (4). Reports in the literature that indicate the presence of CEA in normal human sera use immunological reactions for identification (1–3, 6–11). A small cross-reactivity of the antibodies with normal serum proteins could account for the results obtained.

To demonstrate unequivocally that the heat-stable isoenzyme detectable in normal sera by reaction with monospecific antibody to placental alkaline phosphatase was indeed the

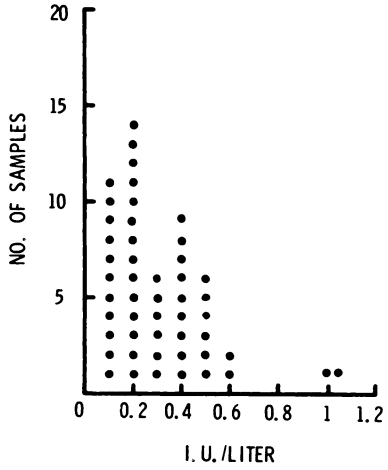

Chart 1. Distribution of the Regan isoenzyme among the group of 50 healthy adult individuals employed in this study.

[1] The abbreviation used is: CEA, carcinoembryonic antigen of the human colon.

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Table 1

Inhibition of alkaline phosphatase isoenzymes by monospecific antibody to placental alkaline phosphatase

Enzyme activity of the heated serum (65° , 10 min) and of the isolated enzyme-antibody complex was determined using 72 mM phenylphosphate in 0.1 M carbonate-bicarbonate buffer, pH 10.7.

Serum sample	% inhibition
Cancer	37
Pregnant	35
Normal pool (50 individuals)	33
Elevated normal	36

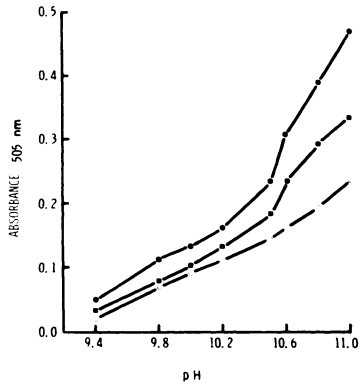

Chart 2. Effect of pH on alkaline phosphatase isoenzyme activities. ■, placental isoenzyme; ●, Regan isoenzyme; ○, normal pool isoenzyme. The enzyme-antibody complex activities are assayed using 72 mM phenylphosphate in 0.1 M carbonate-bicarbonate buffers adjusted to the desired pH. After a 2-hour incubation, the pH is readjusted to 9.0. Color development takes place after addition of 1.5% aminoantipyrine and 4% potassium ferricyanide. The absorbance of the wine-pink solution is read at 505 nm at 5 min.

Table 2

Inhibition of alkaline phosphatase isoenzymes by L-phenylalanine

Enzyme activity inhibition in the presence of 5 mM L-phenylalanine was measured using 20 mM phenylphosphate as substrate at pH 9.8.

Serum sample	% inhibition
Cancer	75
Pregnant	78
Normal pool (50 individuals)	75
Elevated normal	79

Regan isoenzyme, the activity profile as a function of pH and L-phenylalanine sensitivity of the normal serum isoenzyme was investigated. Regan isoenzyme from a cancer patient, placental alkaline phosphatase from a pregnant patient, and the heat-stable isoenzyme from a pool of the 50 normal individuals were isolated on the surface of the insoluble antibody. Data on the identical inhibition of all 3 isoenzymes by monospecific antibody to placental alkaline phosphatase are presented in Table 1. Their activities in the pH range 9.4 to 11 can be seen in Chart 2. All 3 isoenzymes showed the same behavior as a function of pH. It has been shown that pH activity curves for intestinal, liver, and bone isoenzymes are quite different from the placental isoenzyme curve (5). Further evidence for the identity of all 3 isoenzymes is presented in Table 2. As can be seen, L-phenylalanine exerts a strong inhibitory effect on enzymatic activity. This inhibition of enzymatic activity by L-phenylalanine is a well-known property of the isoenzyme of placental origin (5).

The results of the present investigation give strong support to the idea that the Regan isoenzyme of alkaline phosphatase is not a tumor-specific antigen. It also adds support to the validity of the finding of CEA and α-fetoprotein in normal human sera.

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