Brief Communication

Regan Isoenzyme: A Carcinoplacental Antigen

William H. Fishman, Norma R. Inglis, and Sidney Green

Department of Pathology (Oncology), Tufts University School of Medicine and the Cancer Research Department, New England Medical Center Hospitals, Boston, Massachusetts 02111

Summary

The Regan isoenzyme of alkaline phosphatase of human cancer tissue first described in this journal (9) is a carcinoplacental antigen. The experimental evidence shows lines of identity between alkaline phosphatase antigens of placental and tumor tissue origin and corresponding rabbit antisera to these antigens. Also, the loss in the enzyme activity of the antigen in both antigen-antibody complexes is identical within the limits of experimental error as a function of dilution of antisera. The reasons for considering Regan isoenzyme antigen carcinoplacental as opposed to fetal are presented.

Introduction

The discovery of so-called fetal antigens (1, 2, 10) in human cancer tissues and sera has assumed importance in cancer biology since their existence supports the hypothesis of gene derepression in cancer cells. This concept is attractive because it explains the many different "ectopic" manifestations of cancer as aberrations in the control of normal gene expression. A key requirement is that the antigen in both fetal and cancer tissues be identical, and it is not enough that their nonantigenic properties are closely similar.

Thus, with regard to isoenzymes, we have reported that the Regan isoenzyme of human cancer tissue is an alkaline phosphatase which is indistinguishable from the human placental isoenzyme with respect to inhibition by L-phenylalanine, heat stability (5 min at 65°), starch gel electrophoresis, neuraminidase cleavage, Michaelis constants, optimum pH, inhibition by p-hydroxymercuribenzoate (5). Also, on starch gel electrophoresis, the complex of either Regan isoenzyme or of placental isoenzyme with rabbit antisera to placental alkaline phosphatase was excluded from the gel and remained at the origin whereas liver, bone, and intestinal isoenzymes of alkaline phosphatase travelled to their expected positions on starch gel. Finally, the Regan and placental isoenzymes in the antigen-antibody complex experience the same substantial loss in enzyme activity in contrast to no loss in the activities of liver, bone, and intestine isoenzymes in the same serum systems (5). These findings have been interpreted to indicate that the antigenic sites of Regan and placental isoenzymes are identical to each other but different from those of liver, bone, and intestinal isoenzymes.

The evidence for this interpretation could be fortified considerably if (a) lines of identity were to be observed in the Ouchterlony double diffusion technique between antisera to Regan and placental isoenzymes and the corresponding antigens and if (b) the same loss of activity occurs as a function of antisera dilution in the antigen-antibody complexes obtained separately with both antisera and both antigens.

Materials and Methods

Accordingly, these antisera were tested against placental and Regan isoenzymes in sera and tissues in two ways. One was the use of the Ouchterlony double diffusion technique separately with both antisera (Figs. 1 and 2), and the other was the titration of the antigens against appropriate dilutions of the serum in which the loss of enzyme activity in the antigen-antibody mixtures was measured (Charts 1 and 2). The details of these experiments are described in the legends to the charts and figures. Many of the sera tested had been heated for 5 min at 65° to inactivate the non-Regan, nonplacental isoenzymes.

Results

Lines of identity of placental and Regan isoenzyme antigens in sera and tissue are seen in Figs. 1 and 2, respectively, and no "spurs" were observed. Here, the position of the antigen-antibody complex has been visualized by virtue of the phosphohydrolase activity in the complex since, in our experience, the antigen-antibody complexes that form with minute amounts of alkaline phosphatase protein fail to produce precipitin lines. Also, the differences in intensity in the lines of identity are due to unequal enzyme activities in the test sera.

An identical loss in activity (within experimental error) was observed in the antigen as a function of dilution of rabbit antisera. This was true for both Regan and placental isoenzymes titrated against antisera to either Regan isoenzyme or placental isoenzyme. In other experiments not
Regan Isoenzyme: A Carcinoplacental Antigen

Fig. 1. Ouchterlony double diffusion results on rabbit antisera to placental and Regan isoenzymes of alkaline phosphatase in human sera. Left, the center well contained rabbit antisera to placental isoenzyme. Dotted Wells 2, 4, and 6 contained 3 individual pregnancy sera, and Well 8 contained the placental isoenzyme antigen. Sera from 4 different Regan patients have been placed in Wells 1 (lung cancer), 3 (colon cancer), 5 (cancer of the pancreas), and 7 (lung cancer). Right, the center well contained rabbit antisera to Regan isoenzyme. Dotted Wells 3, 6, and 8 each contain a different pregnancy serum and sera from Regan patients were placed in Well 1 (ovarian cancer), 2 (lung cancer), 5 (ovarian cancer), and 7 (mammary cancer). Well 4 contained a specimen of ultrasonically disrupted C-SP cells (11).

Illustrated here, Regan antisera reacted in an identical fashion with both Regan and placental isoenzymes in the exclusion of the antigen-antibody complexes from starch gel.

Discussion

The term “carcinoembryonic antigen” was introduced by Gold (10) to refer to a cancer tissue antigen identical to one prepared from the gastrointestinal tract of a fetus. Others have earlier used the words “fetal antigen” without reference to cancer to describe material prepared from fetal serum and which was identical to antigens of hepatoma and teratoma (1, 2). Now, Elson and Cox (4) have termed HeLa cell alkaline phosphatase “fetal-like” because it is similar immunologically to placental alkaline phosphatase. Yet the fetus does not produce this isoenzyme (3).

None of these terms are completely satisfactory to describe the identical antigenicity of Regan isoenzyme in tumor and of the alkaline phosphatase in placenta and yet to recognize the absence of the placental isoenzyme from fetal tissues and serum. However, an operational terminology would regard placenta and fetus simply as developmental manifestations of the embryo and so both placental and fetal antigens are clearly embryonic from the biological point of view. Their significance in tumors can be indicated by the prefix “carcino,” and so all of these antigens thus far mentioned are carcinoembryonic antigens, some being carcinofetal antigens and others being carcinoplacental antigens. In this way, fetus and placenta become 2 separate unambiguous sources of carcinoembryonic antigens.
Chart 1. The titration of placental and Regan alkaline phosphatase isoenzyme antigens with increasing dilutions of antisera from a rabbit immunized with a purified preparation of Regan isoenzyme obtained from the lymph nodes (5). Suitable dilutions of antigen and antibody are made in 10% normal, nonpregnancy, pooled serum which had been heat inactivated for 2 hr at 55° to destroy all alkaline phosphatase activity. Thus, equal volumes of antigen (0.4 ml) are mixed with equal volumes of graded dilutions of antibody, they are allowed to stand for 5 min at room temperature, and this soluble antigen-antibody complex is assayed for alkaline phosphatase activity by the method of Fishman and Green (7), but at pH 10.7 in 0.05 M carbonate buffer and a substrate concentration of 72 mM disodium phenylphosphate, conditions that are optimum for the detection of placental and Regan alkaline phosphatase activity (6). Placental antigen was used at an initial activity of 11.6 placental isoenzyme units and Regan antigen at 10.5 units. (A unit of activity is defined as 1 mg of phenol/15 min/100 ml under the optimum conditions of pH and substrate concentration at 37°.) The activity of each antigen-antibody complex is subtracted from the control activity (antigen alone) to obtain the loss of activity which is plotted against the log of the dilution of antisera. The reciprocal of the dilution of antiserum that produces a 50% loss in activity is defined as the relative potency of the antiserum. The maximum loss in activity is obtained from the peak value of the curve and the ratio of this value to the activity in the absence of antisera is expressed as a percentage. The values obtained for relative potency and maximum loss in the two titrations appear in the chart. Pl, placental antigen; Rl, Regan antigen; Ab, antibody.

Chart 2. The titration of placental (Pl) and Regan (Rl) alkaline phosphatase isoenzyme antigens as a function of increasing dilutions of antisera from a rabbit immunized with a purified preparation of human placental alkaline phosphatase, SA-20 (5). The initial antigen activities were 11.0 and 10.9 placental isoenzyme units for placental and Regan antigens, respectively. See Chart 1 for full description.
On the basis of earlier and present experimental findings, the Regan isoenzyme qualifies as a carcinoplacental antigen.

Acknowledgments

Our thanks go to Dr. John P. Manning for preparing the antisera to Regan isoenzyme. C-SP cells were supplied by courtesy of Dr. R. Rustigian.

REFERENCES

Regan Isoenzyme: A Carcinoplacental Antigen

William H. Fishman, Norma R. Inglis and Sidney Green