Isolation of a Fetal Antigen from Human Colonic Tumors

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SUMMARY

An antigen has been isolated from perchloric acid-soluble extracts of human colonic tumors which has also been found to be present in human fetal intestine. It has been established that this particular antigen is not present in normal adult colon or gastric mucosa, but that it is found in gastric tumors. The physicochemical characteristics of this antigen are described.

INTRODUCTION

Since Hirszfeld et al. (6) first stated that an antigenic relationship exists between human tumor extracts and fetal tissue extracts, little progress has been made in this field. It took almost 30 years before general interest again centered on this very important observation, and numerous papers have been published on this subject since then (1, 4, 5, 8, 10, 16). Our article deals with the isolation of an antigen from human colonic tumors; this antigen is considered to be of fetal origin.

MATERIALS AND METHODS

Unpreserved gastric and colonic tumors were obtained after surgery. Normal colonic specimens were obtained either at autopsy or after surgery for benign tumors, such as polyps or for intestinal constriction or diverticulosis. In some instances the nontumorous part of the colon was utilized if it was located at least 10 cm away from the malignancy. Normal gastric tissues were obtained from stomachs which were removed because of duodenal ulcers. In all cases, histologic examinations were performed both for the normal and the tumor tissues. All tumors classified as glandular carcinomas were retained for extraction. Homologous fetal tissues were obtained from fetuses between the 2nd and 7th month of gestation.

Only the mucosa of normal tissues was utilized. It was dissected off the muscular layer and freed from connective tissue as carefully as possible. Nine normal colons, two of which originated from autopsies and three from tumor-bearing colons, 2 small intestines, 4 normal stomachs, and one esophagus were treated in this way. The tumors were cut clean of normal tissue remnants, and then the tissues were cut in small pieces and washed to eliminate the excess blood. Six to eight small tumors of the colon from different subjects were pooled before extraction. For the present study, at least five such pools were employed. Large-sized colonic tumors (five) and gastric tumors (four) were extracted individually.

Fetal intestine and stomachs were not cleaved but were extracted as a whole.

Extraction Procedures

Aqueous Extracts. These were prepared by grinding 2 volumes of tissue in 1 volume of buffered saline solution (Ultra-Turrax, 24,000 rpm, Janke & Kunkel K.G., Stauffen i. Br.). The homogenate was then centrifuged for 30 min at 9,000 rpm (MSE High Speed 18). The supernatant was kept for lyophilization.

Perchloric Acid Extracts. These were obtained as follows. The tissue was ground in a minimum of distilled water. Then an equal volume of 10% perchloric acid was added; the solution was stirred rapidly and centrifuged at once. The supernatant was neutralized and then precipitated with ammonium sulfate at saturation. After centrifugation the precipitate was collected in distilled water, dialyzed for 3 days against the same liquid, centrifuged again, and finally lyophilized. (This extract will henceforth be referred to as semipurified or SiP).

The protein concentration of both extracts was determined by the biuret method.

The SiP extract was further purified by column filtration on a Sephadex G-200 (100 x 4 cm column) with a 0.15 M Tris buffer (pH 8.2). Fractions were gathered after spectrophotometric readings at 2,800 Å. After dialysis against distilled water the peaks were lyophilized.

Antiserum Preparation

Antisera were prepared by injecting rabbits with aqueous and semipurified extracts of tumorous and normal fetal tissues, following the schedule adopted in our laboratory (7). We thus obtained antisera against colonic tumors (aTC) and gastric tumors (aTE), against the semipurified extracts of these malignancies, against a Sephadex column fraction of the TCSiP, and against fetal intestine and stomach (aEi, aEs). All antisera were absorbed by lyophilized normal human plasma (NHP 50 mg/ml) before use. Furthermore, all antitumor antisera were absorbed completely by normal tissue extracts. For this purpose, lyophilized extracts of normal colonic,
normal gastric, small intestine, and esophageal mucosa were added to the antisera in concentrations ranging from 20 to 200 mg dry weight/ml of antiserum; the protein concentration of the dry powder varied from 50–60%. The antisera were then incubated for 2 hours at 37°C and centrifuged in a Beckman Spinco Model L centrifuge at 15,000 rpm for 30 min. Absorption controls were performed by immunoelectrophoresis and double diffusion tests.

Analytic Methods

Analysis was carried out by Scheidegger’s (13) micromethod of immunoelectrophoresis and by Ouchterlony’s (11) double diffusion technic, in 1.5% agar and 0.05 M Veronal buffer (pH 8.2).

Electrophoretic migration in acrylamide agarose was performed following the indications of Uriel (15), utilizing a Tris-glycin buffer (pH 8.7). Staining was performed for proteins, lipoproteins, esterases, and peroxidas (14).

Analytic centrifugation was carried out in a Beckman Model E ultracentrifuge at 20°C utilizing the rotor ADN at 59,780 rpm and 0.1 M KCl as a solvent.

RESULTS

The immunoelectrophoretic pattern of colonic tumor semipurified extracts, developed by an absorbed aTC or aTCS:P antiserum, shows 2 precipitin lines with a β-electrophoretic mobility as compared to plasma proteins. A precipitin line of α mobility is clearly revealed only by certain antisera. The two β lines are of an almost identical mobility and migrated in acrylamide agarose, the same peak gave one band was revealed by an aTCS:iP antiserum (Fig. 2a). When migrated in acrylamide agarose, the same peak gave one band in the β-region and a faint spot in the α-region.

To further distinguish between the antigens, the semipurified extract was passed on a Sephadex G-200 column. In the fraction which composed the first peak, one β-precipitin line was revealed by an aTCS:P antiserum (Fig. 2a). When migrated in acrylamide agarose, the same peak gave one band in the β-region and a faint spot in the α-region.

Ultra centrifugation yielded 2 peaks, one of 4.1 S and another of 7.2 S. An initial centrifugation, which had been rendered very difficult by heavy polysaccharide contamination of the eluent by the Sephadex gel itself, gave one peak of 2.4 S and one of 2 S. This led us to believe in the presence of a substance tending to form dimers or polymers. This hypothesis is consistent with the formula applying to polymers which was used by Revet (12) in his determination. The formula, which is very valuable for spheric particules, is as follows:

\[ S_1 \over S_2 = \left( {M_1 \over M_2} \right)^{\frac{2}{3}} \]

where \( M_1 \) and \( M_2 \) represent two molecular masses and \( S_1 \) and \( S_2 \) the coefficient of the sedimentation constants under the same experimental conditions. If \( M_2 = 2 M_1 \), the \( S_2 = 1.58 S_1 \).

In our case \( S_2 = 1.54 S_1 \).

Since the two figures are very close, it is very likely that the second peak is a polymer of the first one.

All lines took on the classic colors for proteins (amido-black, Ponceau red). None were of lipidic nature nor was any enzymatic activity demonstrable. The tumor-specific antigen has been found to be heat resistant up to a boiling time of 40 minutes.

Immunoelectrophoretic studies with aqueous and semipurified gastric tumor extracts showed the presence of the colon tumor β-antigen in gastric tumors also. aTE and aTES:P antiseris yielded indetical results with aqueous or S:P colon tumor extracts (Fig. 2a-c). The chromatographically purified antigen has not been found in mucosa of normal stomach, colon, and small intestine. If it is present, its quantity is minute and escapes detection by the technics employed.

There is some evidence that the second antigen of β-mobility of the S:P extract is also present in normal adult tissue. Perhaps the isolated β-antigen and the one resulting in the α-line of the semipurified extract are of fetal origin. They can be demonstrated in aqueous or S:P extracts of colonic tumors by an anti-Ie antiserum; they can also be demonstrated in fetal intestine by an anti-TC antiserum.

Furthermore, the antibody giving the tumor-specific β-line can be completely absorbed by fetal intestine extract. There is, however, a remarkable difference in the quantity of this antigen present in fetal and tumor tissue extracts. The former is much poorer in antigen, which is translated by a considerably greater amount of S:P fetal-gut extract than tumor extract necessary in order to achieve the complete absorption of a specific antitumor antiserum. This holds true also for direct reactions; it always takes much higher concentrations of fetal extract than tumorous antigen in order to get identical results when an aTC antiserum is employed (Fig. 3a, b.)

DISCUSSION

An antigen of a β electrophoretic mobility has been purified from human colon tumors. This antigen can be considered tumor specific in the sense that this term is commonly employed, even though identity to an antigen normally present in the fetal intestinal tract has been proven. We think our antigen is similar, if not identical, to that already described by Gold and Freedman (5) and isolated and characterized by Krupey et al. (9), even though we did not find exactly the same sedimentation constant as the Canadian authors. We think, however, that we are dealing with a substance which, for a reason still unknown to us, easily undergoes polymerization while being processed for purification. The somewhat variable centrifugation results communicated by the above-mentioned authors also speak of this theory. Gold and Freedman (4, 5) reported the antigen as being specific for tumors arising from the entodermally derived digestive system epithelium. We are not yet able to confirm this observation since we have not completed our studies with tumors of a different origin. We expect, however, to come to the same conclusion because, until now, we have not detected the antigen in tumors other than those of the digestive tube.

We believe we have an antigen distinct from that described recently by Yachi et al. (17), who also located theirs in colonic and gastric tumors but did not find it perchloric acid soluble.
Whether this antigen is responsible for the formation of antibodies in the sera of patients afflicted with cancer of the digestive tract, as reported by Gold (3), is being investigated. When we studied this question with aqueous, nonpurified colonic tumor extracts (7), we did not find a specificity for the digestive cancer sera, but we did find many positive cases among malignancies of other organs, as well as evidence for a normal origin of the antigen(s) in question. These early observations join our present findings of the heterogeneity of the S$_1$P extract. It is composed of at least three different proteic components, two of which have almost the same electrophoretic mobility. If they give rise to the formation of antibodies, these might well be directed against one or the other protein, regardless of its origin. Further purification of other normal tissues also will be necessary to elucidate this last uncertainty.

Day (2), in pointing out the drawbacks of analytic immunology, has found the formula which most probably applies to our antigen. The antigen is cancer distinctive, but perhaps not uniquely specific. Whether this antigen is responsible for the formation of antibodies, these might well be directed against one or the other protein, regardless of its origin.

REFERENCES


Fig. 1. Immunoelectrophoresis of a semipurified colonic tumor extract (S$_1$P). At the left is an antiserum anti semipurified colonic tumor extract (aTCS$_1$P). At the right is an anti-fetal-gut antiserum (aIE). One sees the two β lines (left) and the α line (right). The negative pole is, as in all figures, at the bottom.

Fig. 2. a. Immunoelectrophoresis of a semipurified gastric (left) and colonic (right) tumor extract. Both extracts show identical lines with the two antisera employed [left antigastric tumor (aTE), right anticolic tumor (aTC)]. b. Ouchterlony plate with an anti gastric tumor antiserum to show identity between the lines revealed in gastric (TE) and colonic (TC) S$_1$P extracts. c. Ouchterlony plate to show the same reaction by use of an anti-S$_1$P colonic tumor antiserum (aTCS$_1$P). The external lines join each other; this is hardly visible on this photograph. Note the absence of reaction with semipurified extract of normal colonic mucosa (CNS$_1$P).

Fig. 3. a. Immunoelectrophoresis of a chromatographically purified colonic tumor antigen revealed by an anti-S$_1$P colonic tumor extract antiserum (left) and at right by the same antiserum absorbed further by fetal-gut extract (aTCS$_1$P; IE). b. Ouchterlony plate to demonstrate identity between colonic tumor and fetal-gut extracts. Note the weak line with gastric tumor extract. TC, colonic tumor; IE, fetal gut; CN, normal colon; TE, gastric tumor; NHP, normal human plasma; aTCab, absorbed anticolic tumor antiserum.
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