CARCINOEMBRYONIC ANTIGEN OF EXPERIMENTAL INTESTINAL TUMORS

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Intestinal tumors induced in rats by 1,2-dimethylhydrazine contain a water-soluble antigen that is absent in other organs and tissues, including in the mucous membranes of the intestines of control animals. This antigen can also be detected in embryos at the seventh and ninth days of development. In rats with intestinal tumors the antigen was found in 70% of cases in the blood serum. By analogy with human carcinoembryonic antigen, the rat antigen now discovered is soluble in 1 M perchloric acid.

KEY WORDS: carcinoembryonic antigen; intestinal tumors.

In 1965 Gold and Freedman [9] showed that tumors of the human large intestine produce an embryo-specific glycoprotein which they called carcinoembryonic antigen (CEA). Later the existence of CEA in tumors of the human gastrointestinal tract was confirmed by many investigations [4, 7, 8, 10, 11]. However, no CEA analog has hitherto been discovered in laboratory animals with tumors, and this adds considerably to the difficulty of making a detailed study of the pathogenetic nature of this phenomenon, which would be possible only under experimental conditions.

With the introduction in recent years of adequate experimental models of intestinal tumors [2, 6, 13, 14], the writers undertook an attempt to detect a CEA analog in rats with tumors in this situation.

EXPERIMENTAL METHOD

Tumors of the large intestine in noninbred albino rats, reared at the Rappolovo nursery, Academy of Medical Sciences of the USSR, were induced by subcutaneous injection of 1,2-dimethylhydrazine by the method described earlier [2, 5], as a result of which nearly all the animals developed intestinal tumors with varied histological structure [3]. The tumor tissue was homogenized with quartz sand, distilled water was added to the homogenate in the ratio of 1 : 3 and, after extraction for 16-18 h at 4°C, the extracted substances were separated by centrifugation. The resulting aqueous extracts of the tumors were used for prolonged immunization of rabbits, for which purpose they were injected intramuscularly, subcutaneously, or into the plantar pads of the forelimbs with the addition of an equal volume of Freund's complete adjuvant.

Some of the aqueous extracts were treated with an equal volume of 2 M $HClO_4$; the residue thus formed was separated by centrifugation, and the supernatant was dialyzed for 3 days, first against running tap water and then in distilled water. The method used was taken from papers describing the isolation and partial purification of CEA from tumors of the human large intestine [4, 12]. The resulting perchlorate extracts, after lyophilization, were dissolved in physiological saline (10 mg/ml).

Aqueous extracts of the various organs and tissues of intect male rats (liver, kidneys, spleen, lungs, skeletal muscles, myocardium, ovary) and also of the mucous membrane of the large intestine were prepared by the method described above, so that the protein concentration in the solutions was approximately 10 mg/ml.

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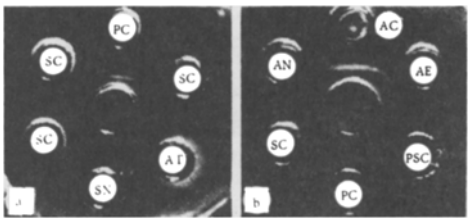

Fig. 1. Determination of carcinoembryonic antigen by immunodiffusion in agar: a) immune serum exhausted with extracts of organs and serum of normal rats; b) immune serum further exhausted with extract of mucous membrane of large intestine of normal rats. AC) Aqueous extract of intestinal tumors; PC) perchlorate extract of intestinal tumors; AN) aqueous extract of mucous membrane of intestine of normal rats; AT) aqueous extract of organs and tissue of normal rats; SC) blood serum of rats with intestinal tumors; PSC) perchlorate extract of blood serum of rats with intestinal tumors; AE) aqueous extract of 9-day rat embryo; SN) blood serum of normal rats.

A seromucoid fraction also was extracted with perchloric acid from some of the aqueous extracts of normal mucous membrane of the large intestine and from the serum of rats with tumors; the fraction was lyophilized and dissolved in physiological saline (10 mg/ml).

The immune sera were exhausted by incubation with the required antigen for 2 h at 37°C and then for 18-20 h at 4°C. After separation of the resulting precipitate by centrifugation, the sera were used in the double immunodiffusion test in agar [1]. The results of the test were read after 20-24 h.

EXPERIMENTAL RESULTS

After exhaustion of the serum and organ extracts (except extract of the large intestine) of normal rats the immune sera reacted with aqueous extracts of tumors of the large intestine with the formation of two precipitation lines (Fig. 1a). An identical reaction also was observed with perchlorate extracts of the tumors, indicating the glycoprotein nature of the antigens. One of these antigens, with greater diffusion mobility in agar and which participated in the formation of the precipitation line nearer to the well containing immune serum, also was present in aqueous and perchlorate extracts of normal mucous membrane of the rat large intestine, so that the tissue specificity of this antigen could be postulated. This hypothesis was confirmed by the fact that antibodies against this antigen were completely removed by exhaustion of the immune serum with extracts of normal mucous membrane of rat large intestine.

Meanwhile, after exhuastion as described above, the immune sera reacted with aqueous and perchlorate extracts of the tumors to form only one precipitation line (Fig. 1b), indicating that this antigen belonged specifically to the tumors investigated. Analysis of the blood serum of 30 rats with intestinal tumors showed that this "tumor-specific" antigen of tumors of the large intestine also was present in 22 of them (70%), and as extraction of the sera with perchloric acid showed, this antigen was contained in the seromucoid fraction (Fig. 1b).

Investigation of the blood serum of 37 normal rats (29 males and 8 females) showed the identical antigen in the blood serum of only one male. Autopsy showed multiple ulcerative lesions of the mucous membrane in the large intestine of this rat.

To determine whether the "tumor-specific" glycoprotein thus discovered is present during embryogenesis aqueous extracts of embryos in toto on the seventh, ninth, and 16th days of development and also the intestine of fetuses at the 20th day and young rats aged 8 days were investigated. The results showed that the "tumor-specific" glycoprotein was present only in 7- and 9-day embryos (Fig. 1b); this antigen could not be detected in the other extracts (by agar diffusion with a protein concentration of 10 mg/ml).

In some cases in rats with intestinal tumors in whose serum the tumor-specific antigen was detected, tumors of the male mammary gland, frequently found in rats treated with 1,2-dimethylhydrazine [2], also were present. Examination of extracts of these tumors failed to reveal the antigen in question. This confirms that the source of formation of the antigen was the malignant intestinal epithelium.

The investigation thus revealed the presence of carcinoembryonic antigen in induced tumors of the large intestine. Its characteristics – solubility in 1 M $HClO_4$, its presence in the serum of rats with tumors and in early embryos, its absence (within the limits of sensitivity of the method used) in unchanged intestinal mucous membrane of control animals – enable an analogy to be drawn with the phenomenon observed in patients with tumors of the gastrointestinal tract. The results of this investigation provide a basis for the development of methods for the comprehensive study of CEA during the development of intestinal tumors.

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