IMMUNOCHEMICAL IDENTIFICATION OF CARCINOEMBRYONIC ANTIGEN IN EXTRACTS OF OVARIAN ADENOCARCINOMA AND PSEUDOMUCINOUS CYSTOMA

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Carcinoembryonic antigen (CEA) was found in 3 of 17 individual extracts of adenocarcinoma and in 7 of 8 individual samples of a pseudomucinous cystoma of the human ovaries. Rabbits were immunized with the separate fractions (ammonium sulfate, sulfosalicylic acid, and phosphotungstic acid) of CEA-positive ovarian adenocarcinomas in order to obtain specific antisera against CEA.

KEY WORDS: human carcinoma – serologic diagnosis; tumor antigens.

Carcinoembryonic antigen (CEA), found in the intestinal mucosa of the human embryo and in tissues of an adenocarcinoma of the large intestine [2, 3, 7] has been recommended as a specific antigen for the serologic diagnosis of carcinoma of the colon and rectum [15]. It has since been discovered that CEA can be found in cancer not only of the gastro-intestinal tract [12], but also of other internal organs [8, 13, 14]. In particular, CEA has been found in the blood serum of patients with ovarian adenocarcinoma [13]. Furthermore, an unidentified antigen, immunologically similar to one of the antigens of tumor tissues from the large intestine, has been found in the mucinous contents of an ovarian cystadenoma [11].

Tissue extracts from individual human ovarian tumors and separate fractions obtained from some extracts of ovarian adenocarcinomas were studied for the purpose of immunochemical identification of the CEA, first described by Gold and Freedman [2], in them.

EXPERIMENTAL METHOD

Tumor tissue was obtained at operation and used within 2-3 h of being obtained. A weighed sample of tissue with the addition (1 : 1) of tris-glycine buffer, containing detergent (Triton X–100) was homogenized with powdered glass. The resulting homogenate was frozen and thawed twice and then centrifuged at 12,000 rpm. The supernatant was fractionated with ammonium sulfate and with sulfosalicylic and phosphotungstic acids.

Considering that CEA is a glycoprotein [2, 3] and is soluble in a semisaturated solution of ammonium sulfate, and since it is not precipitated by sulfosalicylic acid but is precipitated by phosphotungstic acid, the corresponding fractions were obtained from separate homogenates of an ovarian adenocarcinoma. The

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TABLE 1. Results of Immunochemical Determination of CEA in Various Human Ovarian Tumors and Normal Tissues

Name of Tissue	Number of individual samples tested	Result of determination of CEA		
		positive	negative	scatter of titers
Ovarian adenocarcinoma	17	3	14	1:2-1:64
Ovarian pseudomucinous cystoma	8	7	1	1:2-1:8
Metastatic ovarian carcinoma (carcinoma of the colon)	1	1	0	1:128
Ciliary epithelial cystoma, cubical epithelioma, dermoid and follicular cyst	10	0	10	
Normal tissues:				
ovary	10	0	10	
stomach	5	0	5	
small and large intestine	10	0	10	
lung	5	0	5	
spleen	5	0	5	
liver, kidney	10	0	10	
Donor's serum	10	0	10	
Total	91	11	80	

TABLE 2. Results of Semiquantitative Determination of CEA in Different Fractions Obtained from Extracts of Ovarian Adenocarcinoma

Fraction isolated from extracts of ovarian adenocarcinoma	No. of individual samples tested	Protein content (μg/ml)	Result of titration of CEA	
			scatter of titers	μg/ml*
1-AS	2	3800	1:2 —1:4	4—8
2-AS	2	4000	Not determined	
3-SSA	2	3500	1:2—1:4	4—16
4-PTA	2	3500	1:32—1:128	64—256

*Calculation based on conventionally accepted sensitivity of the test system [1]: 2 μg/ml corresponds to "blending" of the test system

order of obtaining the fractions and their nomenclature were as follows. Fractions soluble in semisaturated ammonium sulfate (1-AS) and insoluble fractions under these conditions (2-AS), were dialyzed and freeze-dried after dialysis, to give fraction 3-SSA. The supernatant after precipitation of the proteins with sulfosalicylic acid was further treated with a 5% solution of phosphotungstic acid in order to obtain fraction (residue) 4-PTA.

Fractions 1-AS and 4-PTA were tested as antigens for immunizing sick rabbits. The schemes of immunization did not differ in principle from those usually used in order to obtain anti-tissue antisera. The antisera were obtained 7-12 days after the complete cycle of immunization.

The antisera thus obtained were exhausted with dried human plasma (20 mg plasma was added to 2 ml) and with the corresponding fraction obtained from equal weights of normal human kidney and liver (15-20 mg of the freeze-dried tissue added to 1 ml of antiserum). After adsorption the antisera were tested by immunodiffusion analysis in the laboratory directed by Professor G. I. Abelev (A. I. Gusev), and also in the writers' laboratory using standard antisera against CEA* as the control (Fig. 1.).

Immunoelectrophoresis was carried out by the method of Grabar and Williams [4] and immunodiffusion analysis by Oucherlony's method in the modification of Khramkova and Abelev [1]. A standard test system enables different antibodies and antigens to be identified without the need for isolating them in the pure form.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that 3 of the 17 individual ovarian adenocarcinomas and 7 of the 8 pseudomucinous cystomas contained CEA in a titer of 1:2-1:64 when tested with standard antisera. Normal tissues of various internal organs, judging from the results of immunodiffusion analysis, contained no CEA.

*Monospecific antisera against CEA were kindly provided by Professor Gold (Canada) and Professor Martin (France), to whom the authors are grateful.

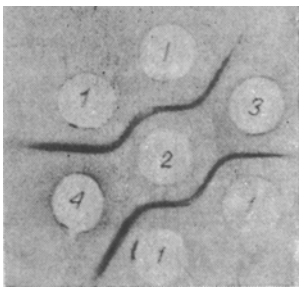

Fig. 1

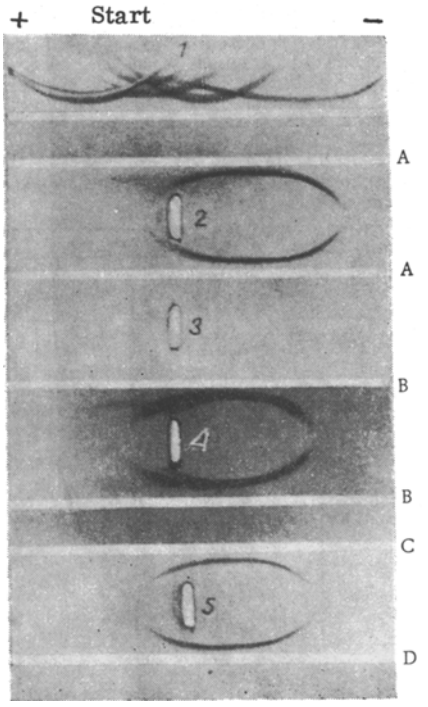

Fig. 2

Fig. 1. Comparative immunodiffusion analysis of various antisera against CEA: 1) fraction 1-AS of ovarian adenocarcinoma; 2) rabbit antiserum against fraction 1-AS after its adsorption with plasma and fraction 1-AS from normal human kidney and liver; 3) monospecific antiserum against CEA of intestinal tumor received from Gold; 4) monospecific antiserum against CEA received from Martin.

Fig. 2. Immunoelectrophoretic characteristics of antisera against individual fractions of ovarian adenocarcinoma: 1) human blood serum, developed with polyvalent antiserum; 2, 4, 5) extract of ovarian adenocarcinoma; 3) extract of normal ovary; antisera: A) against fraction 1-AS of ovarian adenocarcinoma after its exhaustion with human plasma, C) the same after additional exhaustion with the corresponding fraction of normal tissues, B) against fraction 4-PTA of ovarian adenocarcinoma after its exhaustion with human plasma, D) the same after additional exhaustion with the corresponding fraction of normal tissues. 1% Difco agar, veronal-medinal buffer, pH 8.6, ionic strength 0.05, 120 V, 40 mA, duration 40 min.

Semiquantitative analysis (titration) showed that the 4-PTA fraction obtained from a homogenate of CEA-positive ovarian adenocarcinoma had the highest content of an antigen immunologically similar to CEA (Table 2). Obviously, further physicochemical investigations of the CEA of the ovarian adenocarcinoma are required in order to determine conclusively that it is completely identical with the CEA of the intestinal adenocarcinoma.

It should be noted that the antisera obtained possessed a comparatively small range of "additional" antibodies against the various antigens contained in the preparations used for immunization. As Fig. 2 shows, after exhaustion of the antisera with dried plasma, one of the β-globulins identified as CEA was clearly demonstrable by immunoelectrophoresis. Subsequent exhaustion of the antisera with the corresponding fraction from the tissues of normal organs (liver, kidney) led to neutralization of "additional" antibodies (Fig. 2). Meanwhile, investigations have shown [5, 6, 9, 10] that after immunization of rabbits with saline, butanol, and perchlorate extracts of intestinal carcinoma tissue the antisera contain a wide range of antibodies against normal antigens of the intestinal mucosa. Such antisera require careful exhaustion with the addition of, besides plasma, fairly large doses of protein (up to 200 mg/ml antiserum) of various donor's tissues [9, 10] in order to yield a monospecific antiserum against CEA.

The results show that CEA can be found in individual primary tumors of the human ovaries and that its immunochemical and histochemical characteristics are interesting for the study of the particular features of CEA-positive and CEA-negative adenocarcinomas and pseudomucinous cystomas of the ovaries.

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