IMMUNOCHEMICAL STUDY OF ESTROGEN-BINDING ALPHA-GLOBULIN IN TISSUES AND SERUM OF CANCER PATIENTS

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Interaction between estrogens and proteins is known to play an important role in the realization of their biological action at all stages of ontogeny and, in particular, during embryogenesis [1, 3]. The beneficial effect of female sex hormones in the treatment of some types of cancer also suggests definite correlations between the level of these hormones and the onset of malignant neoplasms. Considering the important hormonal disturbances during tumor growth, it is interesting to study proteins which bind steroid hormones during carcinogenesis.

The aim of this investigation was to study one such protein, namely estrogen-binding alpha-globulin (EBAG), with affinity for 17 β-estradiol immobilized on sepharose 4B.

EXPERIMENTAL METHOD

EBAG was isolated from a butanol extract of human abortion material by affinity chromatography on a sorbent containing 17β-estradiol immobilized on sepharose 4B.

The butanol extract was prepared as follows. Butyl alcohol was added to the abortion material in the ratio of 1:5 to precipitate lipoproteins and allowed to stand for 24 h. The abortion material was then centrifuged at 6000 g for 1 h and the supernatant was transferred to a separating funnel to separate the butanol fraction. The aqueous-protein phase was then dialyzed in running water for 24 h, after which it was again centrifuged at 6000 g for 60 min. The extract of abortion material thus prepared was used to isloate estrogen-binding protein.

Sepharose 4B in a volume of 100 ml was transferred into 50% acetone, after which 1.5 g of trichlorotriazine, dissolved in 100 ml of acetone, was added and, by adding 1M NaOH drop by drop, the pH of the solution was kept at about 8.5. After the pH had become stabilized the reaction was stopped with 50% acetic acid. The Sepharose was then washed with a 50% aqueous solution of acetone, 500 mg of estradiol and 500 mg of sodium bicarbonate were added, and the mixture was left for 12 h with continuous stirring.

After washing to remove the unbound estradiol, 50 ml of adsorbent was transferred to a column, through which was passed the butanol extract of abortion material in a volume of 200 ml, after which the column was washed free from unbound proteins with the working buffer solution.

Elution was carried out with 1M NaCl and fractions each of 5 ml were collected. Optical density of the outflowing fractions was monitored by means of a continuous flow spectrophotometer.

Rabbits were immunized with the protein preparation thus isolated in accordance with the usual scheme. The resulting antiserum was subjected to specific absorption by healthy human plasma proteins, and a monospecific test system was set up with the preparation used for immunization.

For the immunochemical study of EBAG, titration and immunodiffusion with a standard test system were carried out by the method in [4].

EXPERIMENTAL RESULTS

The occurrence of EBAG in healthy human blood serum and in serum from cancer patients, and tumor tissues, was studied by immunodiffusion in agar.

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TABLE 1. Immunodiffusion Analysis of Human EBAG in Extracts of Tumor and Normal Tissues

Test material	Number of specimens		Titer of EBAG
	Total	Positive	
Tumor tissue			
Adenocarcinoma of the stomach	6	3	1:1—1:4
Wilms' tumor	17	9	1:1—1:8
Hypernephroma of the kidney	48	24	1:1—1:4
Lung	4	3	1:1—1:4
Mammary gland tumor	3	1	1:1—1:8
Teratoblastoma of testis	5	3	1:1—1:4
Brain tumor	22	6	1:1—1:4
Normal tissue			
Kidney	12	—	—
Liver	8	—	—
Spleen	12	12	—
Brain	9	—	—
Heart	9	—	—
Lacrimal gland	6	—	—
Lung	9	4	—
Adrenals	6	—	—
Ovaries	4	—	—
Uterus	4	—	—
Prostate	4	4	—
Mammary gland	4	4	—

TABLE 2. Immunodiffusion Analysis of EBAG in Serum of Cancer Patients

Location of tumor	Number of samples of serum		
	Total	Positive	%
Control (healthy human blood serum)	42	0	0
Kidney	170	74	41
Urinary bladder	11	6	54
Pharynx	9	4	45
Mammary gland	28	7	25
Lung	3 + 10	2	60
Rectum		4	45
Ovaries	4	2	50
Larynx	8	1	16
Stomach	26	13	50

The results of immunodiffusion analysis of EBAG in tissue extracts showed (Table 1) that EBAG is found in extracts of Wilms' tumors in 9 of 17 cases, in hypernephroma in 24 of 48 cases, in teratoblastoma of the testis in 3 of 5 specimens, in adenocarcinoma of the stomach in 3 of 6 specimens, and in mammary gland tumor in 1 case, i.e., this antigen is found in roughly half the specimens of malignant tissues. However, the fact that this protein is found in extracts of normal tissues of the spleen, prostate, and mammary gland does not permit it to be strictly specific for tumor tissue.

The discovery of EBAG in the blood serum of patients with malignant neoplasms in various situtations is interesting. As will be clear from Table 2, EBAG is found in a high percentage of cases of carcinoma of the stomach, pharynx, rectum, urinary bladder, and kidney; however, it was not found in healthy human blood serum (from blood donors).

The results of the immunochemical investigation thus show that EBAG takes part in the phenomenon of antigenic divergence, in which antigens not characteristic of that given tissue under normal conditions, but are present in other organs and tissues [2], appear in tumor tissue, i.e., this protein may belong to the group of tumor-associated antigens.

Further investigations will show whether the test for EBAG can be used for screening and monitoring the malignant neoplasms.

LITERATURE CITED

1. V. N. Dil'man, Endocrinologic Oncology [in Russian], Leningrad (1974).
2. E. D. Day, The Immunochemistry of Cancer, Thomas, Springfield, Il. (1976).
3. R. Kavetskii, Interaction Between Organism and Tumor [in Russian], Kiev (1977).
4. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).