ONCOLOGY

IMMUNOCHEMICAL IDENTIFICATION OF ONCO-OVARIAN ACID-SOLUBLE ALPHA-2-GLOBULIN

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The search for and identification of specific antigens of ovarian carcinoma remain urgent unsolved problems due to the mild nature of the clinical features of the early forms of this disease and the absence of any specific tumor markers of ovarian carcinoma [5]. The study of the particular features of the antigenic structure of primary ovarian tumor tissues and their metastases in the omentum by traditional immunochemical methods has led to the identification and isolation of a new carcinoplacental beta-2-globulin, known as ovarian-metastatic antigen 8 [6], its properties to be studied, and an immunoenzyme method of its determination in the blood serum to be developed [7] and its serum level established in healthy individuals and patients with gynecological forms of cancer [2]. However, it has not exhibited strict specificity for ovarian carcinoma (a raised serum level in 75% of cases), for in 45% of cases its serum level also is raised in patients with carcinoma of the body of the uterus [2]. This paper gives the results of immunochemical identification and the study of the properties of onco-ovarian acid-soluble alpha-2-globulin (ASAG-2).

EXPERIMENTAL METHOD

Tissue extracts from internal organs of adult humans and fetuses, from primary tumors of the ovaries, and from their metastases in the omentum were prepared in Tris-glycine buffer (pH 8.3) in a ratio of 1:2 (w/v). Antisera were obtained by immunizing rabbits with a fraction of pooled extract of ovarian carcinoma and metastatic tissue, insoluble in ammonium sulfate at 50% saturation. The antisera were adsorbed with dry normal human plasma, with normal human blood serum, blood serum from pregnant women, erythrocyte and leukocyte lysate, and also a mixture of extracts from tissues of adult human internal organs (liver, kidney, spleen, lung, stomach, large and small intestine, heart, adrenal medulla, thyroid gland, pancreas, prostate gland, skin, ovary, testis, omentum, and cartilage). The gamma-globulin fraction was salted out from the exhausted antisera (35% saturation with ammonium sulfate) and exhaustion was confirmed with the individual samples of extracts of organs and blood serum from healthy individuals and pregnant women listed above, by the agar precipitation test. In the absence of response of the antiserum to the biological objects listed above, it was tested with extracts from ovarian and metastatic carcinoma tissue. A specific test system of antiserum with active tumor extract was modeled, and this test system was used to study cross reactions with individual extracts of adult human and fetal organs, with individual samples of normal blood serum, serum from pregnant women, and other biological fluids (amniotic, ascites fluid, etc.), and also with known carcino-embryonic, placental, reactive, and other proteins.

Tissue samples from adult human internal organs (123), embryonic organs (48), primary ovarian carcinomas (24), metastases of primary ovarian carcinomas in the omentum (20), 80 blood sera from individual donors (40 women and 40 men), eight neonatal human blood sera, 16 blood sera from pregnant women (8-41 weeks of pregnancy), 24 blood sera from

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TABLE 1. Physicochemical Characteristics of ASAG-2

Property	Characteristics
Electrophoretic mobility in agar	Alpha-2-globule
Relative electrophoretic mobility in agar	0,76±0,05
Electrophoretic mobility in 7.5% polyacrylamide gel	Alpha-1-globule
Molecular mass, kDa	
By gel-filtration on a column: with Sephadex G-100	55±5
by electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate under reducing conditions (2-mercaptoethanol)	52±3
Isoelectric point	7,38
Solubility:	
at 50% satruration with ammonium sulfate in 0.6 sulfosalicylic acid	Insoluble
in 0.6M percholic acid	Soluble
in 5% TCA	
Thermostability	Resistant (95°C, 15 min)
Resistance to action of enzymes:	
protease	Inactives
nuclease	Does not affect activity
Interaction with:	
DEAE-Sephadex (pH 8.0)	Does not bind
Con A-Sephadex	Does not bind
Reaction for	
proteins	Positive
glycoproteins	Negative
ferroproteins	—»—
lipoporoteins	—»—

patients with ovarian carcinoma, 16 blood sera from patients with carcinoma of the uterus, and 32 blood sera from patients with benign tumors of the ovaries and uterus were studied.

Traditional methods of immunochemical analysis were used: the agar precipitation test [10], immunoelectrophoresis and isoelectric focusing [3], disk electrophoresis in 7.5% polyacrylamide gel, disk-immunoelectrophoresis, disk electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate under reducing conditions [8], gel-filtration [4], and ion-exchange and affinity chromatography [9].

EXPERIMENTAL RESULTS

The antigen found in extract of a metastasis of ovarian carcinoma in the omentum was identified with the aid of a standard test system in 12 of 24 extracts of primary ovarian carcinomas and in eight of 20 extracts from metastases of primary ovarian carcinoma in the omentum. It could not be found in tissues of adult and fetal human internal organs, except the fetal large intestine, in single samples of which it was identified in trace amounts. AFAG-2 likewise was not found by this method in healthy human blood serum, blood serum from pregnant women, newborn infants, and patients with genital tumors, in ascites and lymphatic fluid from patients with ovarian carcinoma, and in amniotic fluid at different times of pregnancy.

Immunochemical identification with the aid of standard test systems demonstrated nonidentity of ASAG-2 with alpha-fetoprotein, carcinoembryonic antigen (CEA), ferritin, alpha-2-globulin of pregnancy, alpha-2-C-reactive protein, carcinocerebral alpha-2-globulin [1], transferrin, chorionic gonadotropin, and ovarian-metastatic antigens 1-8 [6].

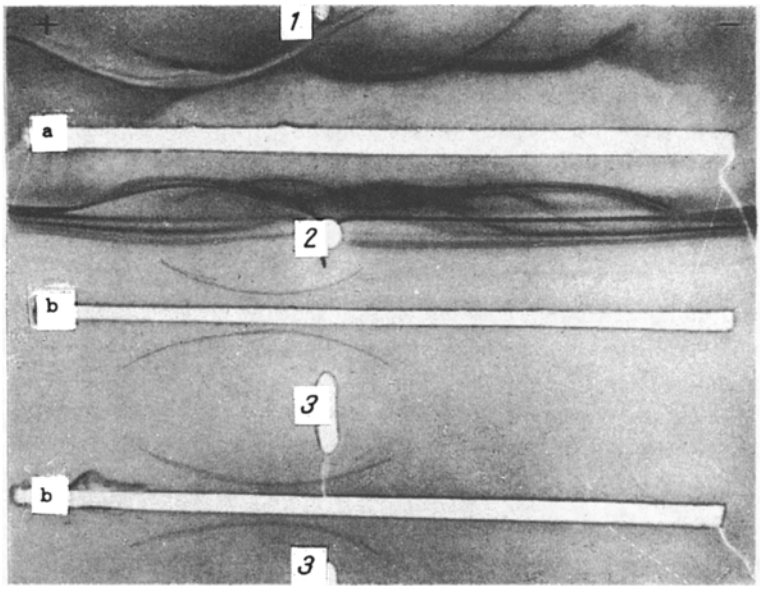

Fig. 1. Immunoelectrophoresis of ASAG-2 in agar. 1) Healthy human blood serum; 2) tissue extract of ovarian carcinoma; 3) tissue extract from a metastasis of ovarian carcinoma, in the omentum. a) Antiserum to healthy human plasma proteins; b) antiserum to ASAG-2, exhausted with healthy human plasma and mixture of extracts from tissues of human internal organs.

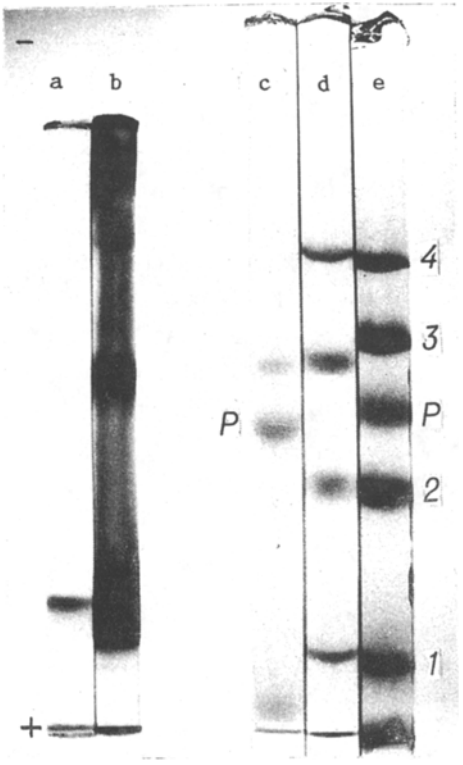

Fig. 2. Disk-electrophoresis of ASAG-2 in 7.5% (a, b) and in 10% polyacrylamide gel with sodium dodecylsulfate under reducing conditions (c, d, e). a) Preparation of ASAG-2 protein; b) extract of ovarian adenocarcinoma; c) preparation of ASAG-2 protein; d) markers for polyacrylamide gel electrophoresis: 1) soy trypsin inhibitor (20 kD), 2) ovalbumin (43 kD); 3) bovine serum albumin (67 kD); 4) phosphorylase B (94 kD); preparation of ASAG-2 protein mixed with markers; P) ASAG-2.

ASAG-2 is a thermostable acid-soluble protein (Table 1) with molecular mass of 55 ± 5 kD and electrophoretic mobility in agar of alpha-2-globulins (Fig. 1). During electrophoresis in 7.5% polyacrylamide gel, ASAG-2 migrates in the alpha-1-globulins zone. Electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate under reducing conditions (2-mercaptoethanol) showed that ASAG-2 has an oligomeric structure with molecular mass of 52 kD (Fig. 2), but under these circumstances some degree of heterogeneity of the molecule is found.

Certain physicochemical properties of ASAG-2 (Table 1), namely its solubility in 0.6 M perchloric acid, its insolubility in ammonium sulfate at 50% saturation, and its discovery in ovarian tumors and the embryonic large intestine, create a definite similarity with CEA of the large intestine [12]. However, the other properties of CEA (molecular mass of 200 kD, electrophoretic mobility of beta-2-globulin, insolubility in 5% TCA, glycoprotein nature) and the immunochemical nonidentity of CEA and ASAG-2 demonstrate their difference. The physicochemical and antigenic properties of ASAG-2 suggest that it is a new antigen, differing from other known carcinoembryonic, placental [11], reactive, and onco-ovarian proteins.

Thus, a new tumor-associated antigen of ovarian carcinoma (ASAG-2) has been identified, characterized, and isolated by traditional immunochemical methods. It has not been found by the immunodiffusion method in definitive tissues and biological fluids of healthy individuals, possible evidence of its conditional specificity for tumors. However, the range of its specificity for ovarian carcinoma will have to be studied on a more extensive material, including tumors of different organs. The problem of its origin from cells of the embryonic large intestine (preliminary results of immunodiffusion analysis) can perhaps be solved by the use of more sensitive methods (radioimmunoassay, enzyme immunoassay), just as its diagnostic capabilities as a marker of ovarian tumors must be evaluated.

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