ENZYME IMMUNOASSAY OF OVARIAN METASTATIC ANTIGEN 8

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The new tumor-associated antigen known as ovarian metastatic antigen 8 (OMA 8), which we discovered, has been studied by the immunodiffusion method [4]. The results as regards discovery of OMA 8 in tissues, blood sera, and other biological fluids showed that the new antigen is present in high concentrations in extracts of ovarian adenocarcinoma and its metastases in the momentum. It is also found in very small amounts in some specimens of chorion and mature placenta, but it is not found in extracts of tissues of definitive and embryonic human and animal organs or in blood serum from healthy human subjects and patients with genital tumors.

Regarding the nature of OMA 8, it is a thermolabile protein with mol. wt. 35 kD and electrophoretic mobility of the β_2 globulins. In its physicochemical and antigenic properties it differs from the known carcinoembryonic and placental proteins [4, 6].

The aim of this investigation was to develop a method of enzyme immunoassay of OMA 8 in human blood serum.

EXPERIMENTAL METHOD

Tissue extracts were obtained as described previously [4]. In view of the low content of OMA 8 in placental extracts, we isolated it from extracts of tumor tissue of an ovarian adenocarcinoma and its metastases in the momentum. The protein was purified by precipitation with ammonium sulfate, ion-exchange chromatography, and gel filtration [4]. The purity of the product was monitored by immunodiffusion in agar, immunoelectrophoresis, polyacrylamide gel disk electrophoresis, disk immunoelectrophoresis, and PAG-SDS electrophoresis [3, 5]. Antisera were obtained by immunizing rabbits with a purified preparation of OMA 8. IgG were isolated from the γ-globulin fraction obtained by ammonium sulfate precipitation from the exhausted antiserum [2]. A conjugate of IgG with horseradish peroxidase (from "Biolar," Olaine, Latvia), for which RZ = 3, was prepared by the periodate method [7]. The model ratio of antibodies and peroxidase in the conjugate was 1:1. A technique of EIA of OMA 8 was developed by the use of the principle of heterogeneous (solid phase) analysis [1]. Polystyrene plateaux (from "Dynatek," Switzerland) were used as the solid phase. Pure antibodies to OMA 8, isolated from antigen immobilized on CNBr-sepharose 4B, were used as layer 1. OMA 8 was immobilized on sepharose, freshly activated by CNBr, by the method of Kohn and Wilchek [8]. The standard curve had a concentration range from 1.95 to 250 μg/liter. The coefficient of variation in this zone did not exceed 10%. The mean level, determined in many series, was 100 $\pm$ 6 μg/liter. The coefficient of correlation in the "reciprocal" test was +0.91 and the "dilution" test +0.93.

Healthy human blood serum was obtained from a blood transfusion station, serum from patients with genital tumors on their admission to the Gynecologic Oncology Clinic. The analysis was carried out only after the results of histological tests on a genital tumor, removed at operation, were available. The patients ranged from 30 to 70 years of age, the healthy subjects from 25 to 45 years. Amniotic fluid was obtained on termination of pregnancy.

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TABLE 1. OMA 8 Concentration in Blood Serum and Amniotic Fluid

Subjects tested	Number of tests	Mean concentration, μg/liter	Median of concentrations, μg/liter	Number of positive	Percentage of positive
Healthy	80				
Women	40	10,0±0,1	9,1	0	0
Men	40	14,3±0,4	12,6	3	7,5
Neonates	16	25,0±1,1	28,0	16	1000
Pregnant women	33	15,2±1,0	14,2	13	39,3
8-12 weeks	14	13,9±1,6	14,0	6	42,8
21-40 weeks	19	15,3±1,7	15,0	7	36,8
Patients with	103				
Ovarian carcinoma	51	21,1±1,7	16,8	38	74,5
Benign epithelial tumors of the ovaries	24	13,1±1,0	14,6	9	37,5
Carcinoma of the body of the uterus	20	14,6±0,9	13,0	8	40,0
Myoma of the uterus	8	11,1±1,5	8,3	1	12,5
Amniotic fluid, 18-24 weeks	8	354±117,9	125	8	100.0

The number of blood samples taken for investigation was 80 from healthy donors, 16 from newborn infants, 33 from pregnant women, 51 from patients with ovarian cancer, 24 with benign epithelial tumors of the ovary, 20 with carcinoma of the endometrium, 8 with myoma of the uterus, and 8 samples of amniotic fluid from 18 to 24 weeks of pregnancy also were tested.

EXPERIMENTAL RESULTS

The results of EIA of OMA 8 are given in Table 1 and Fig. 1. The OMA 8 concentration in serum from healthy women varied from 2 to 15.4 μg/liter (mean 10.0 ± 0.1, median 9.1 μg/liter). The corresponding values for men were from 5 to 17.0 μg/liter (mean 14.3 ± 0.4, median 12.6 μg/liter). The normal serum OMA 8 concentration was taken to be the upper limit of normal for healthy women, namely 15.4 μg/liter.

The OMA 8 level in men was significantly higher ($p < 0.01$) than in women. A significant rise of the OMA 8 level ($p < 0.05$) likewise was found in pregnant women and newborn infants. A raised level was observed in 39.3% of pregnant women and in 100% of neonates.

In all groups of patients except those with myoma of the uterus the OMA 8 level was significantly higher ($p < 0.05$) than in healthy women. A higher than normal OMA 8 concentration was found in patients with ovarian cancer (74.5%), with benign epithelial tumors of the ovaries (37.5%), with carcinoma of the uterus (40%), and myoma of the uterus (12.5). Moreover, the OMA 8 level was significantly higher in ovarian carcinoma than in benign epithelial tumors of the ovaries ($p < 0.05$), carcinoma of the uterus ($p < 0.05$), and myoma of the uterus ($p < 0.05$).

The OMA 8 level in the amniotic fluid also was significantly higher than in the neonatal and adult human blood serum (Table 1).

It was thus possible to use EIA to study the serum level of the new tumor-associated antigen OMA 8 in normal healthy adults, neonates, pregnant women, and patients with genital tumors. The results showed that OMA 8 is present in healthy human blood, and its serum concentration is a little higher in men than in women. A raised level of this protein was found in all neonates tested, irrespective of their sex. The OMA 8 concentration in amniotic fluid was very high compared with that in the blood serum of newborn infants, pregnant women, and healthy and sick people. These findings suggest that OMA 8 is a protein of the embryonic period of life. Its blood level falls in the adult but rises again in patients with genital tumors, more especially with ovarian carcinoma. It is interesting to note that a raised OMA 8 level was found 2-3 times more often in malignant tumors of the ovaries and uterus than in benign tumors of these organs.

The function of the new protein is not yet clear. It may perhaps be a substance of hormonal or enzyme nature, but it is certainly related to the human reproductive system. By its distribution in the tissues and biological fluids OMA 8 can be classed with the tumor-associated antigens of ovarian adenocarcinoma, and with proteins of amniotic fluid and the placenta. Thus the way ahead is clear for further study of the new protein in connection with the diagnosis of tumors and of the pathology of pregnancy. Considering that a raised serum OMA 8 level was observed in 74.9% of cases, it will be interesting to continue the study of this protein in order to establish its true value in the diagnosis and prognosis of ovarian cancer.

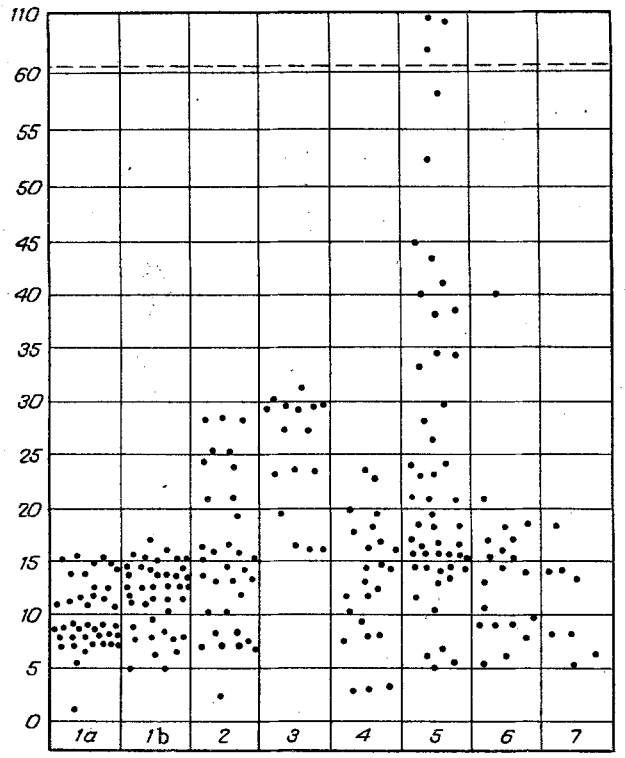

Fig. 1. Blood serum OMA 8 level in patients with genital tumors, pregnant women, and neonates. 1) Control — women (a, n = 40), men (b, n = 40); 2) pregnant women (n = 33); 3) neonates (n = 16); 4) benign epithelial tumors of the ovary (n = 24); 5) ovarian carcinoma (n = 51); 6) carcinoma of the body of the uterus (n = 20); 7) myoma of the uterus (n = 8).

The technique of enzyme immunoassay of OMA 8 in human blood serum, as described above, has thus revealed differences in its level in health and disease and has indicated the prospects for its further study.

LITERATURE CITED

1. B. B. Dzantiev and A. M. Egorov, Zh. Vses. Khim. Obshch. im. D. I. Mendeleeva, **27**, No. 4, 82 (1982).
2. J. C. Jaton, D. C. Brandt, and P. Vassalli, Immunological Methods, ed. by I. Lefkovits et al., Vol. 1, New York (1979), pp. 44-67.
3. H. Maurer, Disk Electrophoresis: Theory and Practice of Polyacrylamide Gel Electrophoresis [Russian translation], Moscow (1971).
4. P. G. Prokopenko, S. A. Borisenko, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 7, 81 (1988).
5. B. Takacs, Immunological Methods, ed. by I. Lefkovits et al., Vol. 1, New York (1979), pp. 81-105.
6. H. Bohn, Proteins of Placenta, Basel (1985), pp. 1-25.
7. A. G. Farr and P. K. Nakane, J. Immunol. Meth., **47**, 129 (1981).
8. J. Kohn and M. Wilchek, Biochem. Biophys. Res. Commun., **107**, 878 (1982).