IMMUNOCHEMICAL DETERMINATION OF PLACENTA-SPECIFIC AND INTERORGAN ANTIGENS IN PLACENTAL EXTRACT AND BLOOD SERUM OF PREGNANT RATS

M. F. Kan, S. K. Krivonosov, and Yu. S. Tatarinov

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KEY WORDS: placenta; antigen; immunoelectrophoresis.

By now about 17 antigens have been discovered in human placental extract [5, 7], but only seven of them are placental in origin, and the rest are interorgan proteins with broad and narrow specificity. One of the placenta-specific proteins, namely trophoblastic β_1-globulin (TBG), has been found to be specific not only for the period of pregnancy, but also for tumors of trophoblastic genesis [2].

The biological importance of these proteins for the mother–fetus system is largely unexplained [1, 7], and accordingly for the problem to be solved a suitable model must be created in a laboratory animal.

The aim of the present investigation was to identify organ-specific proteins of the rat placenta and study them immunochemically, taking into consideration the fact that the rodent placenta has a hemochorial type of structure similar to that in man.

EXPERIMENTAL METHOD

Samples of placental tissue (second half of pregnancy) were obtained from rats under ether anesthesia after decapitation. The tissue was carefully washed under running water, homogenized in an equal volume of Tris-glycine buffer, pH 8.3, containing the detergents X-100 and Tween-80, at the rate of 1 g of each to 1 liter of buffer. After freezing and thawing three times the homogenate was centrifuged at 6000 rpm for 30 min. The supernatant was separated after precipitation with 50% ammonium sulfate solution. The residue was dialyzed against tap water and 0.05% NaCl solution.

Rabbits were immunized with the resulting residue at the rate of 100–120 mg per injection (the dose was 3 ml, together with Freund's complete adjuvant), given alternately subcutaneously and intramuscularly in accordance with the following scheme: four injections at intervals of 7 days for 1 month. The animals were reimmunized after 45 days.

The antisera were absorbed with dry male rat plasma at the rate of 50 mg plasma to 1 ml of antiserum. Immunoelectrophoresis was carried out by the method in [6]. Crossed immunoelectrophoresis was performed by Laurell's method in the modification of Clark and Freeman [4]. Immunodiffusion analysis was carried out by the method in [8] in the modification in [3].

EXPERIMENTAL RESULTS

Up to 14 antigens with electrophoretic mobility of α_1- and β_2-globulins were discovered in rat placental extract (Fig. 1). Subsequent study of these proteins showed that 11 of them have interorgan specificity: They are found not only in the placenta, but also in extracts of liver, kidneys, uterus, stomach, ovary, adrenals, heart, and lungs of adult animals. After reexhaustion with freeze-dried extracts of tissues of the above-mentioned organs (at the rate of 10–15 mg of extract to 1 ml of antiserum) it was found that two proteins (α_1- and α_2- globulins) could still be detected in extracts of certain organs of pregnant animals (liver, kidney, uterus, etc.), whereas the protein with electrophoretic mobility of β_1-globulin was found only in extract of rat placenta (Figs. 2 and 3; Table 1).

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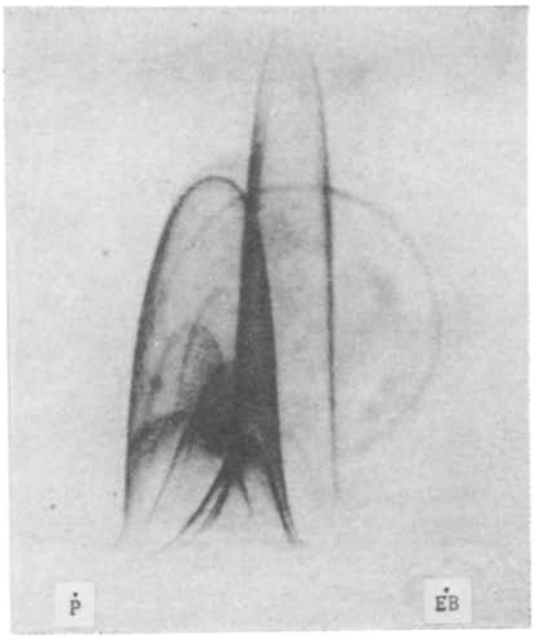

Fig. 1

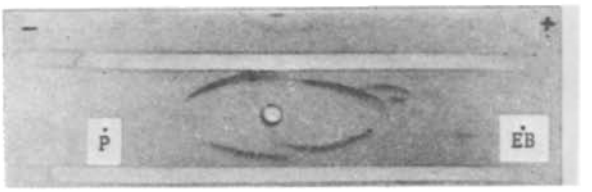

Fig. 2

Fig. 1. Crossed-immunoelectrophoresis of rat placental antigens. Conditions of electrophoresis: 1% solution of agarose (from Serva, West Germany), veronal-medinal buffer, pH 8.6, ionic strength 0.05. Electrophoresis in first direction for 40 min with potential gradient of 10 V/cm. 10 µl of placental extract placed in well. Top part contains 10% (v/v) of rabbit antiserum against rat placental proteins. Electrophoresis in second direction for 20 h with potential gradient of 2 V/cm. Anode above. EB) Evan's blue, P) pyronine.

Fig. 2. Immunoelectrophoretic characteristics of α_1-, α_2-, and β_1-globulins of pregnant rats. Conditions of electrophoresis: 1.5% agar (from Difco, USA), veronal-medinal buffer, pH 8.6, ionic strength 0.05, duration 40 min, potential gradient 10 V/cm. Immunodevelopment with antiserum for 20 h. EB) Evans blue, P) pyronine.

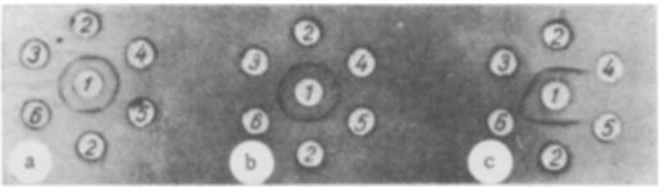

Fig. 3. Immunodiffusion analysis of rat placental proteins with standard test system. 1) Monospecific antiserum; 2) solution of standard antigen. Standard test system for identification of: α_1-globulin (a), α_2-globulin (b), specific β_1-globulin (b). Tissue extracts of pregnant rats: 3) placenta, 4) liver, 5) kidney, 6) uterus.

TABLE 1. Results of Immunochemical Determination of α_1-, α_2-, and β_1-Globulins (number of positive tests) in Organ Extracts from Pregnant and Intact Rats

Material	No. of specimens	α_1-Globulin		α_2-Globulin		β_1-Globulin	
		abs.	%	abs.	%	abs.	%
Placenta (I trimester)	30	11	36,7	12	40	10	33,3
Placenta (II and III trimesters)	50	50	100	50	100	50	100
Liver	51	51	100	51	100	0	0
Kidneys	51	29	56,9	30	58,8	0	0
Lungs	24	9	37,5	14	58,3	0	0
Heart	20	5	25	10	50	0	0
Spleen	51	51	100	51	100	0	0
Adrenals	20	10	50	8	40	0	0
Stomach	20	0	0	0	0	0	1
Small intestine	16	0	0	0	0	0	0
Large intestine	16	0	0	0	0	0	0
Uterus	25	25	100	25	100	0	0
Testes	16	2	12,5	0	0	0	0
Brain	21	0	0	0	0	0	0
Skeletal muscle	12	0	0	0	0	0	0

Legend. These antigens were not found in organ extracts from intact animals.

TABLE 2. Results of Immunochemical Study of α_1-, α_2-, and β_1-Globulins (number of positive reactions) in Blood Serum of Pregnant, Parturient, and Nonpregnant Female Rats and Also of Males

Group of animals	Number of specimens	α_1-Globulin	Titer	α_2-Globulin	Titer	β_1-Globulin	Titer
Females:							
I trimester of pregnancy	30	30	1:4—1:16	30	1:4—1:32	25	1:4—1:8
III trimester of pregnancy	30	30	1:32—1:128	30	1:32—1:128	30	1:32—1:.28
3rd-4th day after parturition	25	25	1:1—1:4	25	1:2—1:4	0	—
Males:	32	2	1:1	1	1:1	0	—
Nonpregnant females	50	44	1:2—1:4	46	1:2—1:4	0	—

α_1- and α_2-Globulins were found in blood sera not only of pregnant rats, but also of intact animals. Their relative content increased as pregnancy advanced and reached peak values toward its end, but 3-4 days after parturition it had fallen to a definite background level (Table 2).

β_1-Globulin was found only in the blood serum of pregnant animals. Its content reached peak values toward the end of pregnancy, and on the 3rd-4th day after it had completely disappeared from the maternal blood stream (Table 2).

Two antigens possessing electrophoretic mobility of α_1- and α_2-globulins were evidently acute-phase nonspecific proteins, "accompanying" pregnancy in rats, for they are found in very small quantities in adult blood serum. The fact that these antigens were found in several organ extracts from pregnant animals (liver, placenta, adrenals, uterus) can be taken as evidence of protein synthesis in these organs during pregnancy. The organ-specific protein of the placenta (β_1-globulin) discovered by comparative immunodiffusion analysis proved to be identical with the rat β_1-globulin described previously (SBG).

It can be concluded from these results that pregnancy in rats is accompanied by the appearance of 14 antigens in the placenta, of which 11 are interorgan tissue proteins and are found not only in placental extracts, but also in extracts of several other organs of adult rats. Two antigens with electrophoretic mobility of α_1- and α_2-globulins are acute-phase proteins associated with pregnancy. The specific β_1-globulin is distinguished by organ-specificity for rat placenta.

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DETECTION OF AUTOANTIBODIES AGAINST BLOOD CLOTTING FACTORS BY PASSIVE HEMAGGLUTINATION TEST AND ELISA

N. N. Tsybikov, M. P. Rudnik, and B. I. Kuznik

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In the writers' view [4] a physiological immune mechanism of regulation of enzyme homeostasis in the blood clotting system exists in healthy humans and intact animals. Under these circumstances the procoagulant enzyme, unlike the proenzyme (inactive form of the procoagulant) is able to give an autoantigenic stimulus and to induce the formation of autoantibodies. These latter inhibit and eliminate the active procoagulant, and so take part in the creation of the general anticoagulant potential of the body. Experimental proof has been obtained of this hypothesis. For instance, immunoglobulins belonging to the IgG class and possessing anticoagulant activity have been isolated from the gamma-fraction of human and bovine plasma [9], and immunocompetent cells realizing an acceptor function relative to thrombin and factor Xa [8] have also been discovered in the blood stream. It has also been found that coagulopathies arising in immunodeficiencies both experimentally and clinically can be partly explained by a fall in the level of anticoagulant antibodies [3, 5].

The aim of this investigation was to discover antibodies against blood clotting factors by the passive hemagglutination test (PHT) and enzyme-like immunoadsorption assay (ELISA).

EXPERIMENTAL METHOD

Human, bovine, canine, porcine, and murine factor Xa (Stuart-Prower factor) was obtained from activated human and animal prothrombin complex by iron-exchange chromatography on DEAE-Sephadex A-50 (Pharmacia, Sweden) [1]. Bovine thrombin (Research Institute of Epidemiology, Kaunas) and human thrombin (Leningrad Institute of Hematology and Blood Transfusion) were additionally purified by gel-filtration on Sephadex G-100 (Pharmacia) [2]. Canine thrombin was obtained from prothrombin by the method described in [7]. All preparations were freeze-dried and stored at 4°C.

For the PHT, thrice washed sheep's erythrocytes were sensitized with a 0.25% solution of glutaraldehyde (Merck, West Germany) and with factors Xa and IIa of different origin. The erythrocytic diagnostic preparations were used in the form of a 2.5% suspension in buffered physiological saline (BPS) and kept at 4°C for not more than 1 week. The effectiveness of sensitization was monitored on the basis of reactions with specific antisera.

The following materials were used for ELISA. The IgG fraction was isolated from sheep antiserum against human IgG (N. F. Gamelya Institute of Epidemiology and Microbiology) by salting out twice with ammonium sulfate and by ion-exchange chromatography on DEAE-Sephadex. After concentration to 10 mg/ml with the aid of PEG-6000 (from Loba-Chemie, Austria) the im-

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