BINDING OF TROPHOBLAST-SPECIFIC β-GLOBULIN BY TROPHOBLAST MEMBRANES

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Trophoblast-specific β-globulin (TBG), identified by Tatarinov and Masyukevich [3], is a specific protein found in the blood serum of pregnant women and patients with trophoblastic disease [4]. TBG and the specific β_1-glycoprotein of pregnancy (SP_1), described by Bohn [11], are immunologically completely identical [6].

It has been shown that TBG is synthesized by trophoblast cells, from which it is secreted into the maternal blood [5, 12]; its concentration in maternal blood in the third trimester of pregnancy reaches 30 mg% [11]. Similar specific β-globulins of pregnancy have been found in the blood serum of primates [12, 13] and also of rats, mice, guinea pigs, and rabbits [6, 7, 9].

The biological role of TBG is not clear. TBG preparations have been shown to have some inhibitory action on mixed lymphocyte cultures and on mitogen-induced lymphocyte proliferation [2, 13]. It is generally considered that immunologically active substances participating in the creation of mutual tolerance of mother and fetus are adsorbed by trophoblast cell membranes and that the formation of a local concentration of such substances is responsible for biological protection of the fetoplacental unit against the maternal immune system [1].

The object of this investigation was an immunochemical study of TBG binding by trophoblast membranes.

EXPERIMENTAL METHOD

Pieces of tissue of the mature placenta were carefully washed free from blood and cut into small pieces. Physiological saline was added in the ratio of 1:1 and the material ground to a homogeneous mass. After freezing and thawing 3 times and further grinding the homogenate was centrifuged for 30 min at 8000g. The residue was washed off with physiological saline, washed 3 times with 4% acetic acid solution, and again washed off with physiological saline, the supernatant being removed each time by centrifugation. The washed homogenate was then neutralized with Tris-(hydroxymethyl)-aminomethane (from Reanal, Hungary) to pH 7.5. Washing was considered to be adequate if immunodiffusion analysis of the supernatant with polyspecific antisera failed to reveal serum antigens. Homogenates were prepared in the same way from tissues of control organs and of the rat placenta.

The homogenate thus obtained was mixed with blood serum of pregnant women in the proportion of 1 ml serum to 1 g homogenate and the mixture was incubated for 24 h at room temperature. After centrifugation the residue was carefully washed with physiological saline. Completeness of removal of unbound serum antigens was verified by immunodiffusion analysis with polyspecific antiserum against pregnant human blood serum. The adsorbed proteins were eluted with 4% acetic acid; the volume of eluate was equal to the volume of blood serum used for incubation. After neutralization, the eluate was tested for the presence of TBG and other serum proteins by immunodiffusion with specific test systems. Altogether seven homogenates of human placenta, six of rat placenta, and more than 20 homogenates of liver, kidney, lung, and muscle of persons dying from accidental trauma, were used in the experiments.

The technique of immunodiffusion analysis and the method of obtaining monospecific antisera with TBG were described previously [7, 8]. Monospecific antisera against immunoglobu-

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TABLE 1. Results of Immunochemical Determination of Adsorption of Blood Serum Proteins by Placental Membranes

Protein	Concentration in blood serum, μg/ml	Content in eluate after adsorption by placenta (M ± m), μg/ml	
		human	rat
TBG	250	7,0±0,8	6,6±0,7
α_2-globulin of pregnancy zone	300	0	0
Albumin	40 000	20,8±3,2	16,6±2,7
Ig:			
G	12 500	42,0±7,1	40,6±5,3
A	2 000	15,4±2,0	11,6±1,7
M	1 000	0	0
Transferrin	3 000	24,0±3,6	13,8±1,9
α_1-lipoprotein	3 500	0	0
β-lipoprotein	5 000	0	0
Gc-globulin	300	5,2±0,6	5,0±0,7
β_2-glycoprotein-1	300	4,6±0,5	4,2±0,6

Legend. 0) Not found; *) taken from Putnam, The Plasma Proteins, Vol. 3, New York (1977). [Asterisk missing in Russian original — Consultants Bureau.]

lins (Ig) G, A, and M, transferrin, and Gc-globulin were from Behringwerke (West Germany); antisera against albumin, α_2-globulin of the pregnancy zone, α- and β-lipoproteins, and β_2-glycoprotein-1 were obtained in the writers' laboratory.

EXPERIMENTAL RESULTS

The results of immunochemical study of adsorption of TBG and some other blood serum proteins are given in Table 1. Besides TBG, antigens tested also included α_2-globulin of the pregnancy zone, whose concentration in the blood serum of pregnant women is of the order of 10 times higher than the normal level of this protein in healthy donors, and also a number of proteins which are normal components of blood serum and whose levels do not change significantly in pregnancy. It was shown that only some serum antigens bind with placental membranes, including albumin, IgG and IgA, transferrin, Gc-globulin, and β_2-glycoprotein-1. For some of these proteins specific receptors have been found on the trophoblast membranes [1, 14]. Under these same conditions α- and β-lipoproteins and IgM were not adsorbed by human and rat placenta. As Table 1 shows, adsorption of antigens by placental homogenates took place selectively, and did not depend on the initial concentration of these proteins in the blood serum.

TBG binds with placental membranes and also is specifically adsorbed by homogenates of rat placenta and of certain adult human organs — liver, skeletal muscle, and kidney. The α_2-globulin of the pregnancy zone is adsorbed by liver membranes, whereas receptors capable of binding this protein during incubation of pregnant human blood serum with homogenates of the tissues washed in acid medium are not found in placenta, kidney, and muscle.

After treatment of pregnant human blood serum with neuraminidase, adsorption of TBG by placental membranes also took place. Neuraminidase removes part of the carbohydrate component containing sialic acids from the molecule of this glycoprotein, and this is reflected in a decrease in the electrophoretic mobility of TBG [9, 10]. The results are evidence that this part of the carbohydrate component of TBG does not participate in interaction of this protein with specific tissue receptors.

As Table 1 shows, only a small part of the TBG contained in blood serum is adsorbed by the placenta, and this is true also of other adsorbed serum proteins. However, to make a more precise quantitative study of the affinity of TBG for the membranes, membrane preparations isolated by ultracentrifugation will evidently have to be used.

The use of immunochemical methods thus established the fact of specific binding of TBG by membranes of the placenta, and also of the liver, muscle, and kidney. It seems likely

that interaction between this protein and tissue membranes is essential for it to perform its biological function.

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