COMPARATIVE IMMUNOCHEMICAL AND PHYSICOCHEMICAL STUDY OF THE SPECIFIC β-GLOBULINS OF PREGNANCY

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Specific β-globulins of pregnancy of rats, rabbits, and guinea pigs and human β_{1G}-globulin were shown to be similar in their physicochemical properties. Their dynamics and distribution in the pregnant organism also were similar. These proteins evidently perform similar functions in man and animals. KEY WORDS: β-globulins of pregnancy; β_{1G}-globulin.

In 1970 the writers [1] described a specific B_{1G}-globulin (BGG) found in the blood serum of pregnant women. Later this protein was found [3] in the blood serum of patients with trophoblastic tumors. Specific β-globulins have also been found [4, 5] in the blood serum of pregnant rats, rabbits, and guinea pigs, which like man have a hemochorial type of placenta. Human BGG and the analogous animal proteins possess an individual antigenic structure, and only in closely related species of mammals (man–monkey, rat–mouse) has the partial immunologic identity of these antigens been established [4, 8]. The discovery of functional analogs of BGG in laboratory animals has made it possible to create an experimental model for the study of the role of these proteins during pregnancy and in the development of trophoblastic diseases. In this connection it is interesting to compare some of the physicochemical properties of human BGG and the analogous animal proteins as an approach to the study of the biological function of this group of proteins.

This paper describes a comparative immunochemical and physicochemical analysis of the properties of human BGG and specific β-globulins of pregnancy of rabbits, rats, and guinea pigs.

EXPERIMENTAL METHOD

The method of obtaining monospecific antisera against human BGG and the specific β-globulins of pregnancy of rabbits, rats, and guinea pigs was described previously [4, 5]. Sera of pregnant women (HPS), rabbits (RbPS), rats (RPS), and guinea pigs (GPPS) were used as antigens. A standard test system was used for semiquantitative analysis [2, 3, 4].

The relative electrophoretic mobility of the test antigens was determined by immunoelectrophoresis by Uriel's method [6]. Serum albumin was used as the standard protein, and dextran, with zero electrophoretic mobility, was used to assess the endo-osmotic effect.

The molecular weight of the antigens was determined by gel-filtration in a thin layer of Sephadex G-200 [1]. Proteins with known molecular weight were used as the standard proteins: cytochrome c, ovalbumin, hemoglobin, serum albumin, immunoglobulin-g, placental lactogen, α-fetoprotein, α_2-globulin of the "pregnancy zone," and human BGG. Immunodevelopment was carried out by the method of double immunodiffusion in gel with a standard test system. After the end of gel filtration portions of Sephadex 1 cm wide were successively removed from the plate and placed in agar wells. The distances traveled by the antigen molecule were judged from the "bending" of the test systems in the corresponding well.

The presence of sialic acids was determined from the change in electrophoretic mobility in the test with neuraminidase. This enzyme removes neuraminic acid derivatives from the glycoprotein molecule, and thereby reduces the anodal electrophoretic mobility of the antigen. The test serum, in a volume of 1 ml, was incubated with 100 units of neuraminidase (from Calbiochem, USA) for 24 h at room temperature, after which the experimental and control samples were subjected to immunoelectrophoretic analysis.

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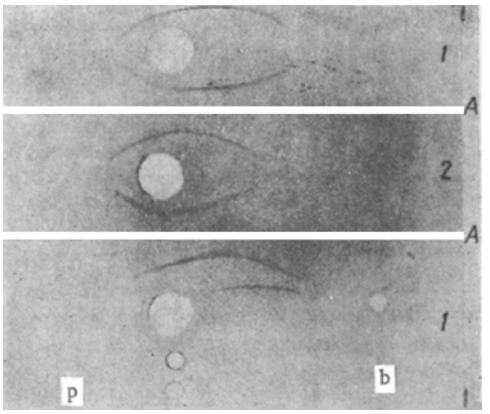

Fig. 1. Changes in electrophoretic mobility of specific rabbit pregnancy antigen after treatment with neuraminidase. 1) Control serum of pregnant rabbit; 2) serum of pregnant rabbit treated with neuramidase. A) Monospecific antiserum against rabbit pregnancy antigen; b) Evans' blue; p) pyronine.

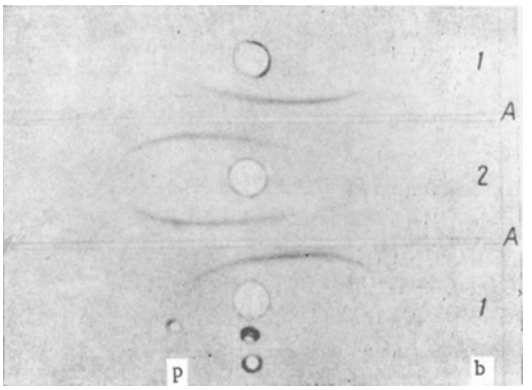

Fig. 2. Effect of neuraminidase on electrophoretic mobility of human BGG. 1) Control serum of pregnant woman; 2) serum of pregnant woman treated with neuraminidase. A) Monospecific antiserum against human BGG; b) Evans' blue; p) pyronine.

TABLE 1. Comparative Physicochemical Characteristics of Specific β-Globulins of Pregnancy of Man and Certain Laboratory Animals

Property and procedure	Human BGG	Rat β-globulin	Guinea pig β-globulin	Rabbit β-globulin
Molecular weight	120 ± 6 thou.	130 ± 4 thou.	120 ± 7 thou.	140 ± 4 thou.
Relative electrophoretic mobility	0.47 ± 0.02	0.45 ± 0.03	0.50 ± 0.03	0.56 ± 0.03
Thermolability	85°	85°	85°	80°
Treatment with trypsin	Destroyed			
Treatment with ribonuclease	Resistant			
Treatment with deoxyribonuclease	"			
Treatment with neuraminidase	Decrease in anodal electrophoretic mobility			
Precipitation with 0.25% rivanol solution	Not precipitated			
Precipitation with 1% copper sulfate solution	Not precipitated			
Precipitation with ammonium sulfate	20-50%	20-50%	20-50%	15-45%
Precipitation with perchloric acid	0.2 M	0.2 M	0.2 M	0.2 M

The thermolability of the individual antigens was determined by heating the serum of the pregnant animals and women to different temperatures for 1 h.

EXPERIMENTAL RESULTS

The physicochemical properties of the specific human, rabbit, rat, and guinea pig antigens of pregnancy are given in Table 1. The molecular weight of human BGG, determined by thin-layer gel-filtration, was 120,000 ± 6000. The molecular weights of the β-globulins of pregnancy of rats, guinea pigs, and rabbits were 130,000 ± 4000, 120,000 ± 7000, and 140,000 ± 4000 respectively.

On immunoelectrophoresis the test antigens formed precipitation arcs in the β-globulin zone. The relative electrophoretic mobility of the pregnancy antigens of rats and guinea pigs was similar to that of BGG (0.47 ± 0.02), whereas the mobility of the rabbit protein was a little higher (0.56 ± 0.03). The electrophoretic mobility of all the proteins studied was found to change in the same preparations during keeping and, for that reason, only fresh samples were analyzed.

Under the influence of neuraminidase the anodal electrophoretic mobility of the specific antigens of the rabbit (Fig. 1), rat, and guinea pig was reduced. Human BGG also showed a similar change in electrophoretic mobility (Fig. 2). All the antigens tested lost their antigenic properties after treatment with trypsin but retained them after treatment with ribonuclease and deoxyribonuclease.

The specific β-globulins of pregnancy were precipitated from serum by ammonium sulfate at between 20 and 50% saturation, they were precipitated by 0.2 M perchloric acid, but they were resistant to precipitation by 0.25% rivanol solution and 1% copper sulfate solution. All were thermolabile but retained their antigenic properties after heating to 60°C for 1 h.

As was stated previously [4], a characteristic feature of the specific β-globulins of pregnancy is that they are all present in a relatively high titer in the serum of pregnant animals (1:32 to 1:128) and in a lower concentration in the tissues of the placenta (1:2 to 1:8). Within the limits of sensitivity of immunodiffusion analysis they were hardly detectable in amniotic fluid or in fetal blood serum and they were not found in extracts of organs of pregnant rats, rabbits, or guinea pigs. The concentration of these proteins in the serum rises progressively in the course of pregnancy and falls sharply on the second to third day after parturition.

The distribution and dynamics of the specific β-globulins of pregnant rats, rabbits, and guinea pigs are thus similar to the distribution and dynamics of BGG in pregnant women [7, 8]. The antigens studied, like human BGG, are specific proteins with closely similar molecular weight and electrophoretic mobility. All are glycoproteins with similar properties relative to denaturing agents.

The results indicate that these proteins perform similar functions in man and animals. The further study of these proteins is indicated in order to ascertain their biological role.

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PHAGOCYTIC ACTIVITY OF STROMAL HEMATOPOIETIC TISSUE PRECURSOR CELLS

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Phagocytic cells were separated from a cell suspension with the aid of iron powder in a magnetic field. Clonogenic stromal precursor cells of hematopoietic tissue which do not belong to the histiocyte–macrophage group but to the mechanocyte group have high phagocytic activity. After treatment of a suspension of bone marrow cells with iron fewer than 1% of stromal precursor cells are left behind, and during monolayer culture they give rise to colonies of fibroblasts. In suspensions of spleen and peritoneal exudate cells about 10% of clonogenic precursors of fibroblasts remain. KEY WORDS: bone marrow; stroma; phagocytosis.

On explanation in monolayer cultures of hematopoietic tissue cells the stromal precursor cells give rise to colonies consisting of clones of fibroblasts [4]; the content of stromal precursors in the cell population can be judged from the number of colonies. Stromal cells maintain themselves independently of hematopoietic cells and under conditions of chimerism of the whole hematopoietic system or part of it they differ in origin from the hematopoietic cells [1-3]. To understand the functions of the hematopoietic and stromal components of hematopoietic tissue and the relations between them it is important to have detailed information on the stromal precursor cells. In particular, investigation of the phagocytic activity of these cells is an interesting problem.

In the investigation described below the ability of stromal precursor cells of hematopoietic tissue, exhibiting clonogenic properties in monolayer culture, to carry out phagocytosis in vitro was studied.

EXPERIMENTAL METHOD

Guinea pigs weighing 180-300 g were the donors of bone marrow, spleen, and peritoneal exudate cells. Cell suspensions were prepared by the method described earlier [2, 5] at 37°C in medium 199 plus 2% embryonic calf serum plus 15 mM Hepes. To obtain peritoneal exudate, 24 h before the experiment the animals were given an intraperitoneal injection of 20 ml nutrient broth. The method of Lundgren et al. [7] was used to remove phagocytes from the cell suspension. To 10 ml of the cell suspension in a concentration of $1 \cdot 10^7$ to $3 \cdot 10^7$ cells/ml, in a smooth-bottomed vessel treated with antifoam silane (to prevent adhesion of the cells), 0.4 g of a sterile powder of iron carbonyl was added. The mixture was incubated for 30 min at 37°C, with gentle stirring every 5 min. After careful and thorough resuspension the particles of iron and the cells phagocytosing them were removed with a powerful magnet, placed near the bottom of the vessel. The remaining cells were resuspended in fresh medium after centrifugation. The same volume of the original cell suspension for use as the control was treated in the same way except for addition of the iron powder. After counting, the cells of the suspension were explanted into 100-ml flasks with the addition of $2 \cdot 10^7$ bone marrow cells, irradiated in a dose of 4000 R, as the feeder. The cultures were grown on medium 199 with 10% embryonic calf serum and the gaseous phase consisted of air with 5% CO_2. After 10-12 days the cultures were fixed with ethanol and

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