COMPARATIVE IMMUNOCHEMICAL ANALYSIS OF SPECIFIC β-GLOBULINS OF THE "PREGNANCY ZONE" IN HUMANS AND OTHER MAMMALS

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UDC 612.124-06:612.63

It was shown by immunodiffusion analysis that the specific β_1-globulin of human pregnancy and the analogous β-globulins of other mammals (rats, guinea pigs) possess an individual antigenic structure. Animals of closely related species (rats and mice) were shown to have immunologically similar β-globulins, which are evidently synthesized in the placenta, in their blood serum during pregnancy.

KEY WORDS: β-globulins of pregnancy; immunodiffusion analysis.

Among human blood serum proteins in the so-called pregnancy zone four individual antigenic components are now generally distinguished, two with the electrophoretic mobility of α-globulins and the other two with mobility of β-globulins. One of the β-globulins of this zone, conventionally named by the writers [3] $\beta_{1\text{-g}}$-globulin (BGG), has been found in the blood serum of patients with chorionepithelioma of the uterus and teratoblastoma of the ovary [3, 4]. This globulin is immunologically identical with the specific β-globulin of pregnancy [2], β_1-glycoprotein [8], and the so-called pregnancy protein PAPP-C [9]. It has been shown [5] that BGG can be detected immunohistochemically in cyto- and syncytiotrophoblastic cells of the chorion. This protein is evidently synthesized in the trophoblastic cells of the placenta, from which it is secreted into the blood of pregnant women.

The object of this investigation was to make a comparative immunochemical analysis of human BGG and of the corresponding proteins of mammals which, like man, have a hemochorial type of placenta.

EXPERIMENTAL METHOD

Antiserum against human BGG was obtained by immunization of rabbits with a β-globulin fraction isolated from blood serum of pregnant women (40 weeks) by electrophoresis in agar gel. The scheme of immunization of the animals was described previously [2]. Antisera against serum β-globulins of pregnant rats and guinea pigs were prepared in a similar way. The resulting antisera were exhausted by dry plasma from blood donors and male animals respectively. Six batches of antisera which, after exhaustion with an excess of dry plasma, continued to precipitate human BGG or the β-globulins in the blood serum of pregnant rats and guinea pigs, respectively, were chosen for the work. These antisera were named as follows: 1) anti-BGG – antisera against human $\beta_{1\text{-g}}$-globulin; 2) anti-B_r – antisera against serum β-globulin of a pregnant rat; and 3) anti-B_{gp} – antiserum against serum β-globulin from a pregnant guinea pig.

Sera of pregnant women (PS_h) and also of pregnant rats (PS_r), mice (PS_m), and guinea pigs (PS_{gp}) were used as antigens.

Immunodiffusion analysis was carried out with the aid of a standard test system [6] or of two complete standard test systems [1]. Electrophoretic mobility of the specific serum β-globulins of the pregnant women was determined relative to the electrophoretic mobility of human serum albumin from the results of electro-

Department of Biochemistry and Laboratory for Problems in Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1223-1225, October, 1976. Original article submitted March 31, 1976.

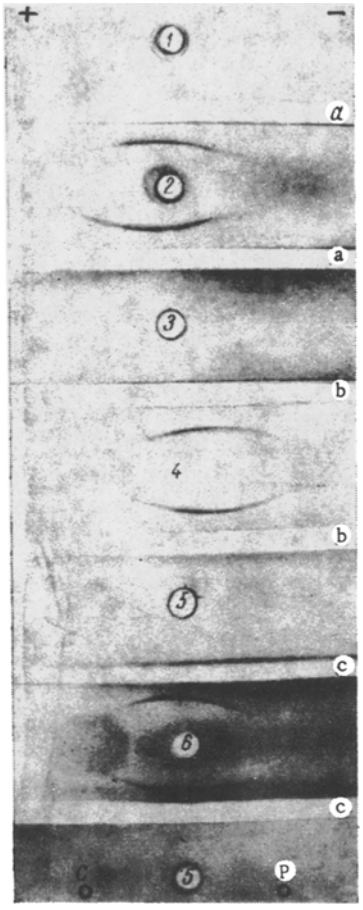

Fig. 1. Immunoelectrophoretic characteristics of specific β-globulins of pregnancy. Nonpregnant blood serum: 1) rat, 3) human, 5) guinea pig; pregnant blood serum: 2) rat, 4) human, 6) guinea pig. Antisera against specific β-globulins of pregnancy: a) rat, b) human, c) guinea pig. Experimental conditions: 1% Difco agar, veronal-medinal buffer, pH 8.6, ionic strength 0.05, 120 V, 40 mA, duration of electrophoresis 40 min; standards of electrophoretic mobility: B) Evans' blue, P) pyronine.

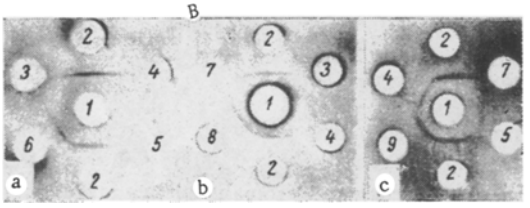

Fig. 2

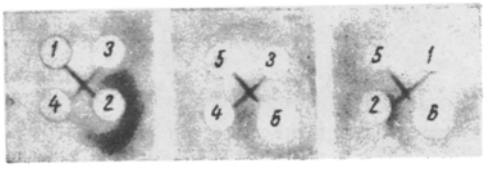

Fig. 3

Fig. 2. Immunodiffusion analysis with standard test system: 1) monospecific serum; 2) solution of standard antigen. Standard test system for identification of specific β-globulin of pregnancy: a) human, b) guinea pig, c) rat. Pregnant blood serum: 3) rat, 4) mouse, 5) guinea pig, 7) human. Extracts of placental tissues: 6) human, 8) guinea pig, 9) rat.

Fig. 3. Comparative immunodiffusion analysis using two complete test systems. Monospecific antiserum against β-globulins of pregnancy: 1) rat, 3) human, 6) guinea pig. Solution of standard antigens: 2) rat, 4) human, 5) guinea pig.

phoresis in agar gel [7], keeping to standard experimental conditions. The preparations were photographed in scattered light.

EXPERIMENTAL RESULTS

As Fig. 1 shows, specific human, rat, and guinea pig β-globulins of pregnancy occupied a relatively wide migration zone on electrophoresis in agar gel: from α_2- to β_2-globulins. These proteins differed only a little in their relative electrophoretic mobility. It will be noted that after keeping the sera for 2-4 weeks at temperatures of between +2 and −2°C, changes in the electrophoretic mobility of the specific β-globulins in the same

samples of human and animal blood sera could be observed. Similar changes in electrophoretic mobility also are characteristic of β_1-glycoprotein [8].

Immunodiffusion analysis (Fig. 2) with a standard test system showed that the β-globulins of pregnancy being studied were present in relatively high concentration in blood serum (in a titer of 1:32-1:128) and also in extracts of placental tissue (1:2-1:8), but they were never found in the blood serum or tissue extracts of nonpregnant individuals. Human BGG and the analogous animal proteins can be considered to be synthesized in the placenta, from which they are secreted mainly into the maternal blood stream.

The β-globulin of the pregnant rat was shown to have considerable immunological similarity to the corresponding protein of the pregnant mouse (Fig. 2c), whereas the analogous human, rat, and guinea pig proteins, when compared, did not form well-developed precipitation lines that could indicate positive crossed serological reactions. This antigenic individuality also was demonstrated when two complete test systems (human–rat, human–guinea pig, rat–guinea pig) were compared, when two independent precipitation lines were formed as a cross (Fig. 3).

These results can be interpreted as evidence that human BGG and the analogous rat and guinea pig proteins possess antigenic individuality, whereas the rat and mouse β-globulins of pregnancy contain many common antigenic determinants and, indeed, they possibly have a completely identical antigenic structure. It was shown previously [8] that the specific β_1-glycoprotein of human pregnancy is immunologically identical with the analogous monkey protein.

Specific β-globulins of pregnancy with marked antigenic individuality can thus be found in the blood serum of pregnant mammals that share with humans a similar hemochorial type of placenta (rat, guinea pig). Mammals of closely related species (human–monkey, rat–mouse), however, secrete immunologically similar β-globulins into the blood stream during pregnancy. The question of the origin of these proteins, the site of their synthesis, and their physiological role in man and animals requires special investigation.

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