

# IMMUNOCHEMICAL STUDY OF HUMAN TROPHOBLAST-SPECIFIC

## $\beta_1$ -GLOBULIN AND ITS ANALOGS IN ANIMALS

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Human trophoblast-specific  $\beta_1$ -globulin and specific pregnancy  $\beta$ -globulins of rabbits, rats, and guinea pigs possess immunologic similarity. Similar antigenic determinants, not detectable by immunodiffusion methods, were found by preincubation of these agents with heterologous antisera.

KEY WORDS: trophoblast-specific  $\beta_1$ -globulin; pregnancy  $\beta$ -globulins; similar antigenic determinants.

Trophoblast-specific  $\beta_1$ -globulin was first found [1] in the blood serum of pregnant women and was called  $\beta$ -globulin of pregnancy [2]. It was later found [3] that this globulin is synthesized in the trophoblast and, consequently, that it can be included in the group of trophoblast-specific proteins [4]. Proteins similar to human trophoblast-specific globulin (TBG) in electrophoretic mobility and certain physicochemical properties have also been found [5, 7, 8] in the blood serum of pregnant animals with a hemochorial type of placenta. However, partial immunologic similarity of TBG has been discovered [5, 7, 9] only between mammals of closely related species (man-monkey, rat-mouse).

The object of this investigation was to look for cross-reacting antigenic determinants in the TBG molecule in man and certain laboratory animals. The discovery of immunologic analogs of TBG in laboratory animals would enable the biological role of TBG during pregnancy and in the development of trophoblastic tumors to be studied experimentally.

### EXPERIMENTAL METHOD

Monospecific antisera against human TBG and specific pregnancy  $\beta$ -globulins of rabbits, guinea pigs, and rats were obtained by immunization of rabbits and hens [5, 7, 8]. The antisera thus obtained were verified by immunoelectrophoresis and named as follows: 1) anti-TBG — antiserum against human trophoblastic  $\beta_1$ -globulin, 2) anti-BRT — antiserum against specific pregnant rat serum  $\beta$ -globulin, 3) anti-BGP — the same for guinea pig, 4) anti-BRb — the same for rabbit. The antigens were either semipurified preparations of TBG and specific pregnancy  $\beta$ -globulins of the animals or blood sera of pregnant individuals — human (BSH), rat (BSRt), guinea pig (BSGP), and rabbit (BSRb) respectively. Anti-TBG were verified against a standard test system and by immunodiffusion by the method described previously [6].

Rocket immunoelectrophoresis was carried out by Laurell's method [10]. Before analysis the antigens for testing were mixed and incubated with monospecific chicken antisera against TBG or against specific pregnancy  $\beta$ -globulins of the animals. After incubation the mixture of antigen with heterologous antiserum was studied by rocket immunoelectrophoresis, for which the agar was mixed with rabbit antiserum against the corresponding specific pregnancy  $\beta$ -globulin. The results were recorded by photography in scattered light.

### EXPERIMENTAL RESULTS

As Fig. 1 shows, monospecific antisera gave rise to similar precipitation arcs typical of specific pregnancy  $\beta$ -globulins in the serum  $\beta$ -globulin zone of the pregnant individuals. These antisera, during immunodiffusion analysis with a standard test system, did not react

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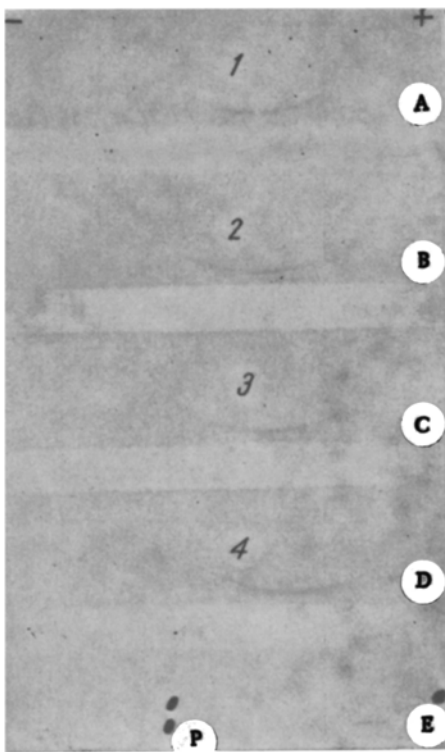


Fig. 1

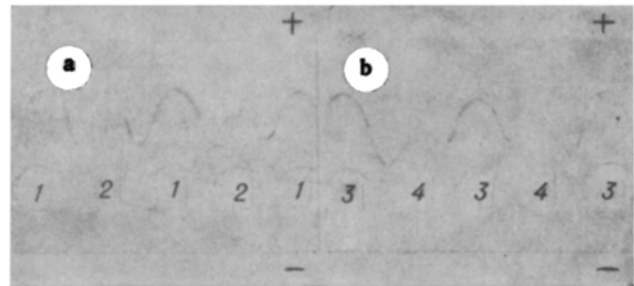


Fig. 2

Fig. 1. Immunoelectrophoretic analysis of human TBG and its analogs in animals. Blood serum from pregnant individuals: 1) human, 2) guinea pig, 3) rat, 4) rabbit. Monospecific rabbit antisera against: A) human TBG; B) guinea pig; C) rat, and D) rabbit pregnancy  $\beta$ -globulin. Reference substances: P) pyronine, E) Evans' blue.

Fig. 2. Detection of immunologic similarity of human TBG with rabbit pregnancy  $\beta$ -globulin. Electrophoresis in gel containing rabbit monospecific antiserum against: a) human TBG, b) rabbit pregnancy  $\beta$ -globulin. Serum of pregnant individuals incubated with nonimmune chicken serum: 1) human, 3) rabbit, 2) pregnant human serum incubated with chicken antiserum against rabbit pregnancy  $\beta$ -globulin, 4) pregnant rabbit serum incubated with chicken antiserum against TBG.

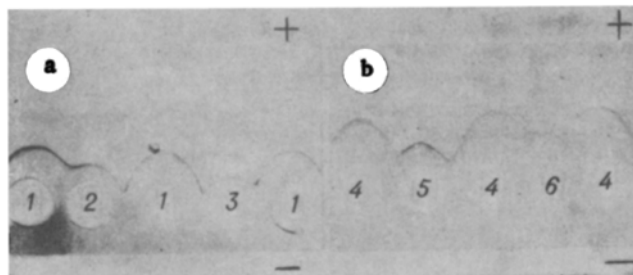


Fig. 3. Detection of immunologic similarity of rat, guinea pig, and rabbit pregnancy  $\beta$ -globulins and human TBG. Electrophoresis in gel containing rabbit monospecific antiserum against a) rat, and b) guinea pig pregnancy  $\beta$ -globulin. Serum of pregnant individuals incubated with nonimmune chicken serum: 1) rats, 4) guinea pig. Serum of pregnant individuals incubated with chicken antiserum against TBG: 2) rats, 6) guinea pig. Serum of pregnant individuals incubated with chicken antiserum against rabbit  $\beta$ -globulin: 3) rats, 5) guinea pig.

with the corresponding sera of male and nonpregnant female individuals. They likewise did not give cross reactions on counterflow immunodiffusion with heterologous antigens, i.e., "spur"-formation — the phenomenon of partial immunologic similarity between antigens compared — was not observed.

After incubation of semipurified preparations of human TBG with anti-BRb and, conversely, after incubation of BSRb with anti-TBG, a decrease in the anodal mobility both of human TBG and of specific rabbit pregnancy  $\beta$ -globulin could be observed (Fig. 2). Similar results with a change in electrophoretic mobility after incubation also were obtained in experiments with anti-BGP and anti-BRt and BSRt and BSGP respectively (Fig. 3). In the control experiments no changes in electrophoretic mobility of TBG or of its immunologic analogs were observed. There is reason to suppose that the decrease in anodal mobility of TBG and the corresponding rat, guinea pig, and rabbit pregnancy  $\beta$ -globulins was attributable to complex formation — the antigen-antibody reaction. The antigen-antibody complexes thus formed could be detected with the aid of homologous antisera against the corresponding pregnancy  $\beta$ -globulins, i.e., antigens present in the soluble complexes preserved their ability to react with specific antibodies, with the formation of an insoluble precipitate.

The results show that TBG and specific rat, guinea pig, and rabbit pregnancy  $\beta$ -globulins possess immunologic similarity. The number of similar antigenic determinants in the molecule of human TBG and its analogs in other mammals varies evidently from one to three, for during the reaction of these antigens with heterologous antibodies no insoluble precipitate was formed, only soluble complexes. Partial similarity of cross-reacting antigenic determinants is another possibility. The final conclusion regarding the number of similar antigenic determinants and the degree of their identity in molecules of TBG and its analogs belonging to animals of distantly related species can be drawn from the results of amino-acid analysis of the primary structure of these functionally related proteins.

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