

IMMUNOAUTORADIOGRAPHIC STUDY OF SPECIES SPECIFICITY OF β -GLOBULINS OF PREGNANCY

O. P. Shevchenko, I. M. Larina,
and Yu. S. Tatarinov

UDC 612.124-06:612.63-019

A comparative immunoautoradiographic analysis was made of human β_1 -G-globulin and specific pregnancy β -globulins of guinea pigs, rats, and rabbits. The antigens tested were found to possess high species specificity.

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

It was shown previously [4, 6] that specific β -globulins of pregnancy of mammals of closely related species (man-monkey, rat-mouse) possess marked immunological similarity. Human β_1 -G-globulin (BGG) has been found in the blood serum in the early period of pregnancy and also in patients with chorionepithelioma of the uterus and teratoblastoma of the ovary [1-3]. The discovery of BGG analogs in the blood serum of laboratory animals would provide an experimental model for the study of these proteins during pregnancy and the development of trophoblastic disease.

A comparative immunoautoradiographic investigation was made of human, rabbit, guinea pig, and rat β -globulins specific for pregnancy, using rabbit and hen antisera.

EXPERIMENTAL METHOD

A semipurified preparation of human BGG was isolated from retroplacental serum by electrophoresis in agar gel followed by precipitation of the β -globulin fraction with 50% saturated ammonium sulfate.

Monospecific rabbit antisera against human BGG and rat and guinea pig β -globulins of pregnancy were obtained as described previously [4]. Hen antisera against human BGG and rabbit β -globulin of pregnancy were obtained in the same way. These antisera were described as follows: 1) antiserum against human β_1 -globulin, anti-BGG; 2) antiserum against β -globulin from pregnant rat serum, anti-PRT; 3) antiserum against β -globulin from guinea pig, anti-PGP; 4) antiserum against β -globulin from rabbit, anti-PRB.

Pregnant human (PHS), rat (PRTS), guinea pig (PGPS), and rabbit (PRBS) sera were used as antigens.

Comparative analysis of the test antigens was carried out by the use of standard test systems by immunoautoradiography [2, 5].

The search for antigenic determinants common to BGG and the analogous animal proteins was carried out with rabbit antibodies against ^{125}I -labeled human BGG. The antibodies were isolated from monospecific antisera. Sepharose 4B, treated with cyanogen bromide and conjugated with the semipurified preparation of BGG, was used as the immunoadsorbent. The pregnant rat, guinea pig, and rabbit sera were incubated with ^{125}I -labeled antibodies against BGG (anti-BGG- ^{125}I) for 24 h at room temperature, and this was followed by immuno-electrophoresis [7]. After formation of precipitation arcs the preparations were photographed in scattered light, after which the same preparations were subjected to autoradiography.

EXPERIMENTAL RESULTS

The specific β -globulins of pregnancy of man and the experimental animals studied differed somewhat in their electrophoretic mobility (Fig. 1a); the rabbit antigen had the highest and the rat antigen the lowest mobility. The writers showed previously [4] that specific β -globulins of pregnant animals, like human BGG,

Department of Biochemistry, 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. 83, No. 6, pp. 721-723, June, 1977. Original article submitted December 15, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$7.50.

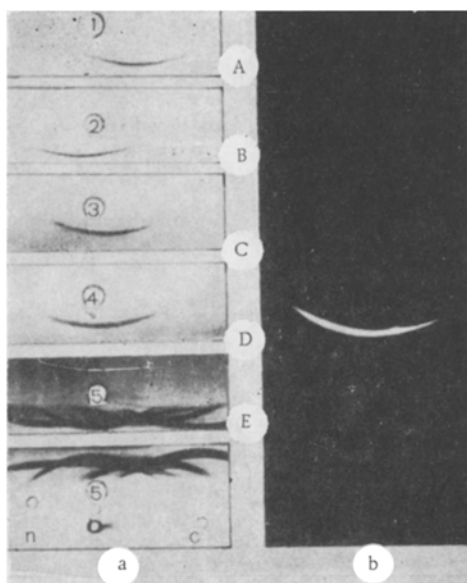


Fig. 1

Fig. 1. Immunoelectroradiographic analysis of specific human, rabbit, rat, and guinea pig antigens of pregnancy: a) immunoelectrophoretic analysis; b) results of autoradiography. Pregnant rabbit (1), rat (2), guinea pig (3), and human (4) blood sera; donor's blood serum (5). Monospecific antisera against β -globulins of pregnancy; rabbit (A), rat (B), guinea pig (C), human (D); antiserum against donors' serum (E).

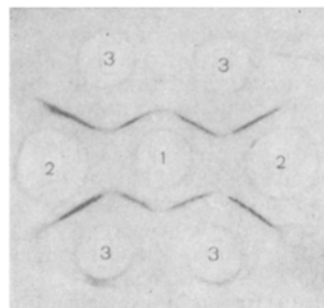


Fig. 2

Fig. 2. Comparison of test systems for human BGG with rabbit and rat antisera by immunodiffusion method. Monospecific antisera against human BGG: 1) rabbit, 2) hen, 3) pregnant human serum in standard dilution.

are characterized by small changes in the electrophoretic mobility of these antigens in the same preparations on keeping.

As Fig. 1b shows, incubation of pregnant human serum taken in excess with anti-BGG- ^{125}I led to incorporation of label into the precipitation arc. When a small quantity of labeled antibodies was incubated with excess of antigen, soluble antigen-antibody- ^{125}I complexes evidently were formed, and in the course of immunoelectrophoresis these were incorporated in the precipitation arc. Under the same conditions no incorporation of label took place into the precipitation arcs of the animal β -globulins. Possibly the antigenic determinants capable of binding rabbit antibodies against human BGG were absent from these proteins.

Comparative immunoautoradiographic analysis with standard test systems showed that specific rabbit, rat, and guinea pig pregnancy antigens and human BGG possess antigenic individuality. Hen antisera, like rabbit (Fig. 2) antisera against human BGG, on immunoautoradiographic investigation, did not give crossed serological reactions with specific animal β -globulins.

The results are evidence of the high species specificity of the β -globulins of pregnancy of man, rats, rabbits, and guinea pigs. To discover analogs of human BGG in laboratory animals a comparative analysis must be made of the properties of these proteins. For this purpose it is proposed to study the physicochemical properties of specific serum antigens of pregnant rats, rabbits, and guinea pigs.

LITERATURE CITED

1. Yu. S. Tatarinov, N. V. Mesnyankina, D. M. Nikulina, et al., *Byull. Éksp. Biol. Med.*, No. 9, 79 (1974).
2. Yu. S. Tatarinov, D. M. Falaleeva, D. M. Él'gort, et al., *Byull. Éksp. Biol. Med.*, No. 9, 86 (1975).
3. Yu. S. Tatarinov, D. M. Falaleeva, and V. V. Kalashnikov, *Akush. Gin.*, No. 7, 63 (1975).
4. Yu. S. Tatarinov, S. K. Krivososov, et al., *Byull. Éksp. Biol. Med.*, No. 10, 1223 (1976).
5. D. M. Él'gort and G. I. Abelev, *Byull. Éksp. Biol. Med.*, No. 2, 118 (1971).
6. H. Bohn and H. Ronnenberger, *Arch. Gyäk.*, 215, 277 (1973).
7. P. Grabar and C. A. Williams, *Biochim. Biophys. Acta*, 10, 193 (1953).