

IMMUNOCHEMICAL IDENTIFICATION OF ORGAN-SPECIFIC  
HUMAN PLACENTAL  $\alpha_2$ -GLOBULIN AND ITS CONTENT  
IN THE AMNIOTIC FLUIDD. D. Petrunin, I. M. Gryaznova,  
Yu. A. Petrunina, and Yu. S. Tatarinov

UDC 618.346-008.839.624:618.36-]-078.73

An organ-specific human placental  $\alpha_2$ -globulin, differing immunologically from previously known  $\alpha_2$ - and  $\beta_1$ -globulins of pregnancy,  $\alpha$ -fetoprotein, placental lactogen, and chorionic gonadotropin, was identified by immunochemical analysis. The antigen thus discovered is present in large quantities in the placental tissue and amniotic fluid in the early stages of pregnancy, but at parturition its concentration is sharply reduced.

KEY WORDS: human placenta; organ-specific antigen.

The study of the antigenic structure of the placenta at the individual antigen level is a topical problem in the immunology of pregnancy at the present time. Only scattered communications on the antigenic composition of the human placenta can be found in the literature [1, 2, 6, 10]. However, the authors of these papers, although determining the number of antigenic components, usually did not identify them with antigens characteristic of pregnancy that have previously been described. Some of the "placental" antigens were evidently typical proteins of the so-called pregnancy zone or specific globulins associated with pregnancy [3, 4, 7, 9].

The object of this investigation was the immunochemical identification of an organ-specific placental  $\alpha_2$ -globulin and its detection in some biological fluids during pregnancy.

## EXPERIMENTAL METHOD

Placental tissue with the addition (1:1) of Tris-glycine buffer, pH 8.3, containing detergents (Triton X-100 and Tween-80), was homogenized with powdered glass. The resulting homogenate was frozen and thawed three and then centrifuged at 8000 rpm. The supernatant was lyophilized. Extracts from adult persons dying from trauma and from fetuses obtained by the termination of pregnancy on medical grounds were prepared in a similar way. Extracts containing 5 g % of protein were used.

Antisera were obtained from rabbits (12 animals) immunized with extracts of placentas at different times of development and with concentrates of amniotic fluid, with the addition of potassium alum as an adjuvant. The antisera were exhausted with dried plasma and freeze-dried extracts of adult human organs under the control of immunodiffusion analysis.

Immunoelectrophoresis was carried out by the method of Grabar and Williams [8] and immunodiffusion analysis by Ouchterlony's method in the modification of Khrankova and Abelev [5].

## EXPERIMENTAL RESULTS

Immunochemical analysis of the absorbed immune sera revealed specific antibodies precipitating with an antigenic component of the placenta with the electrophoretic mobility of  $\alpha_2$ -globulins (Fig. 1). A standard monospecific test system was obtained for this antigen and used to identify it in various tissues.

Department of Biochemistry and Department of Obstetrics and Gynecology, 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. 7, pp. 803-804, July, 1976. Original article submitted December 12, 1975.

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.

TABLE 1. Results of Immunochemical Determination of Organ-Specific  $\alpha_2$ -globulin Placental Antigen during Development of Pregnancy

Tissue and biological fluid	Period of pregnancy (in weeks)	Number of individual tests	Result of determination of $\alpha_2$ -globulin placental antigen		
			positive	negative	titer
Placenta	6—12	19	19	0	1:4—1:64
"	20—30	18	18	0	1:4—1:64
"	40	24	24	0	1:2—1:8
Amniotic fluid	6—12	19	19	0	1:4—1:64
"	20—30	24	24	0	1:2—1:8
"	40	24	5	19	1:1
Fetal serum	20—30	18	0	18	
"	40	132	3	129	1:1
Maternal serum	6—12	21	7	14	1:1
"	20—30	22	0	22	
"	40	169	2	167	

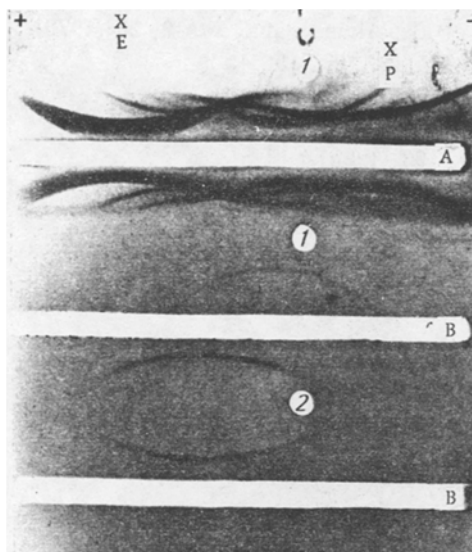


Fig. 1. Immunoelectrophoretic characteristics of organ-specific  $\alpha_2$ -globulin placental antigen: A) antiserum against human serum proteins; B) monospecific antiserum against organ-specific  $\alpha_2$ -albumin placental antigen. 1) Donor's serum, 2) placental extract. Reference substances: P) pyronin, E) Evans' Blue.

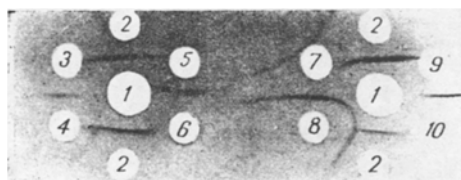


Fig. 2. Comparative immunodiffusion analysis of organ-specific  $\alpha_2$ -globulin placental antigen,  $\alpha_2$ G- and  $\beta_1$ G-globulins of pregnancy placental lactogen, and  $\alpha$ -fetoprotein. Test system for organ-specific  $\alpha_2$ -globulin placental antigen: 1) antiserum, 2) solution of antigen; test system for  $\alpha_2$ G-globulin of pregnancy: 4) antiserum, 3) solution of antigen; test system for placental lactogen: 5) antiserum, 6) solution of antigen; test system for  $\beta_1$ G-globulin of pregnancy: 8) antiserum, 7) solution of antigen; test serum for  $\alpha$ -fetoprotein: 9) antiserum, 10) solution of antigen.

As Fig. 2 shows, placental  $\alpha_2$ -globulin is not identical with  $\alpha_2$ G- and  $\beta_1$ G-globulins of pregnancy [3, 4], with placental lactogenic hormone, or with  $\alpha$ -fetoprotein. Additional experiments also showed that this  $\alpha_2$ -globulin is likewise not identical with chorionic gonadotropin.

The content of the identified antigen in various substrates during the development of pregnancy is shown in Table 1; clearly it was present in the largest amounts in the tissue of the placenta and in the amniotic fluid in the early periods of pregnancy, and its content fell steadily until parturition.

At the level of sensitivity of the test system placental  $\alpha_1$ -globulin was not found in the sera of 52 blood donors, in 93 tests on extracts of organs (heart, brain, lung, liver, kidney, adrenal, spleen, pancreas, gastric mucosa, small and large intestine, ovary, uterus) of healthy adults, or in 81 tests on the analogous organs of fetuses at different stages of gestation.

On the basis of these results the  $\alpha_2$ -globulin can be classed as an organ-specific placental antigen, which can be secreted into the amniotic fluid and, in some cases, can penetrate into the maternal and fetal blood streams.

It will be interesting to study the behavior of the identified placental antigen in various forms of pathological pregnancy, especially those of immunological nature.

#### LITERATURE CITED

1. S. D. Bulienko, V. V. Tkachishli, and P. I. Fogel, *Akush. Gin.*, No. 2, 56 (1975).
2. D. Muzhnai, *Byull. Éksp. Biol. Med.*, No. 2, 50 (1963).
3. Yu. S. Tatarinov and V. N. Masyukevich, *Byull. Éksp. Biol. Med.*, No. 6, 66 (1970).
4. Yu. S. Tatarinov, V. N. Masyukevich, N. V. Mesnyankina, et al., *Akush. Gin.*, No. 9, 25 (1970).
5. N. I. Khramkova and G. I. Abelev, *Byull. Éksp. Biol. Med.*, No. 12, 107 (1961).
6. H. Bohn, *Arch. Gynäk.*, 212, 165 (1972).
7. H. Bohn, *Blut*, 26, 205 (1973).
8. P. Grabar and G. A. Williams, *Biochim. Biophys. Acta*, 10, 193 (1953).
9. T. M. Lin and S. P. Halbert, *Internat. Arch. Allergy*, 48, 101 (1975).
10. H. Schultze-Mosgau and K. Fischer, *Arch. Gynäk.*, 210, 458 (1971).