PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 62–66 (1974)

Pregnancy-Associated Serum Antigens in the Rat and Mouse[1] (37748)

TSUE-MING LIN, S. P. HALBERT, AND D. KIEFER

University of Miami School of Medicine, Department of Pediatrics, Miami, Florida 33136

Human pregnancy is accompanied by an increase in the concentration of several known plasma proteins (1–3). Some of these are hormones (*e.g.*, chorionic gonadotropin and placental lactogen), while others are known to possess enzymatic activities (*e.g.*, oxytocinase and heat-stable alkaline phosphatase). In addition, a number of recent reports (4–7) have described plasma constituents in human pregnancy which are not detectable by the methods used in nonpregnancy female plasma. These plasma components (also found in serum) have been called "pregnancy-associated," "pregnancy-specific," or "pregnancy zone" proteins. Thus far, no known biological activity or function has been convincingly ascribed to them. Our laboratory has demonstrated four pregnancy-associated plasma proteins in women during the third trimester by various immunodiffusion methods, using hyperimmune rabbit antiserum to human pregnancy plasma that had been exhaustively absorbed with normal nonpregnant female and male human plasma (8, 9). Three of these have been partially purified and characterized (10).

It was felt that an animal model would be most helpful for investigations of the biological significance of these pregnancy-associated proteins. For that reason, a similar study of pregnant rat and mouse plasma has been carried out by our group. Analogous pregnancy-associated plasma antigens were detected during gestation in both of these species, as described in the present report.

Materials and Methods. Fifty white rats of the Sprague-Dawley (Holtzman) strain were purchased from Holtzman Co. (Madison, WI) 2 wk after impregnation. They were housed individually in air-conditioned quarters, and fed water and food *ad libitum*. Five to 10 ml of blood were drawn by heart puncture in the last 3 days of pregnancy, and the serum was harvested. Each animal was necropsied for confirmation of pregnancy. A portion of each serum specimen was incorporated into a pool, while the remainder was maintained individually. Five rats were allowed to give birth to their litters normally and serum specimens were obtained by cardiac puncture 10 days later. A pool of newborn rat sera was derived from 100 newborn rats (1–3 days old) when they were sacrificed by decapitation.

Seventy-two near term white mice of the CFW Swiss Webster strain were obtained from Carworth Farms (New City, NY). Based on the known mating dates, these animals were sacrified 1 to 3 days before the expected delivery dates. Cardiac puncture yielded 1–2 ml of blood from each mouse, and a portion of each harvested serum was pooled. Necropsies confirmed late pregnancy in each animal used.

Pools of nonpregnant female and male rat serum of the same strain were obtained by cardiac puncture. Additional supplies of nonpregnant Sprague–Dawley (Holtzman) rat plasma and Swiss Webster mouse plasma were purchased from the Pel-Freez Co. (Rogers, AR). Portions of these were lyophilized for absorption purposes. All samples were stored frozen at —20° when not in use.

For immunodiffusion, 1% agarose in 0.01 *M* phosphate-buffered saline (pH 7.1) (PBS) was used with 4- and 8-mm well distance templates (Cordis, Miami). For microim-

[1] This investigation was supported by a research grant from the National Institute of Health (No. HD 5736).

munoelectrophoresis, 1% agarose in Veronal acetate (μ = 0.025, pH 8.6) was employed. Details are described elsewhere (8, 9).

To obtain antiserum to the rat and mouse pregnancy-associated serum antigens, three New Zealand white female rabbits each (Pel-Freez Co., Rogers, AR) were immunized intradermally at weekly intervals for three doses, followed by monthly boosters. Each 1 ml dose consisted of 0.5 ml of a pregnancy serum pool homogenized with 0.5 ml of complete Freund's adjuvant, given at five widely separated sites. The rabbits were bled from the ear arteries 7 to 14 days after the third immunization, and after each of the monthly boosters. The antisera were absorbed at the rate of 200 mg or more of lyophilized nonpregnant plasma powder, for each milliliter of antiserum. After mixing and incubation at 37° for 2 hr, followed by 4° overnight, they were clarified by centrifugation in the cold. Immunoglobulin concentrates were prepared from the antisera by precipitation with ammonium sulfate at 50% saturation, the sediment being redissolved in a minimum amount of PBS, usually representing about 1.5-fold concentration over the original volume.

Human pregnancy plasma, and rabbit antibodies to human pregnancy-associated plasma proteins were obtained as described elsewhere (9).

Results. Rat pregnancy serum. Absorption of the antisera to pregnant rat sera with nonpregnant rat plasma at the rate of 200 mg (or more)/ml removed all reactivity with the nonpregnant rat plasma pools derived from female and male rats. In addition, lyophilized pooled male and nonpregnant female rat plasma dissolved at 200 mg/ml also showed no reactions. The more potent of these absorbed antisera still produced four precipitin lines with pooled pregnant rat serum; equivalent reactions were seen with individual pregnant rat sera (see Fig. 1). All of the 45 individual pregnant rat sera showed similar immunodiffusion patterns.

The immunoglobulins from pools of potent absorbed antisera could be concentrated only

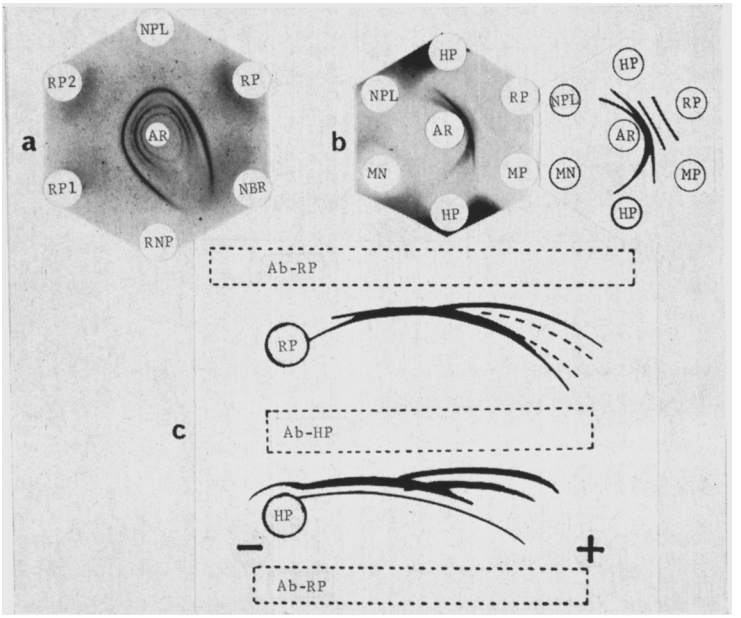

FIG. 1. Immunodiffusion (a, b) and immunoelectrophoresis (c) of rat pregnancy-associated serum antigens. (NPL) Lyophilized nonpregnant female and male rat plasma (200 mg/ml); (RP) pool of rat late pregnancy serum; (RP1, RP2) individual rat late pregnancy serum; (NBR) newborn rat serum; (RNP) pooled nonpregnant female and male rat serum; (HP) pool of third trimester human pregnancy plasma; (MP) pool of mouse late pregnancy serum; (MN) pool of nonpregnant male and female plasma; (AR, Ab-RP) absorbed antiserum to rat pregnancy serum; (Ab-HP) absorbed antiserum to human pregnancy serum.

1.5- to 1.7-fold, due to the large quantity of absorbing nonpregnancy rat plasma protein required. Although the absorbed antiserum itself revealed the 4 pregnancy-associated rat antigens, the immunoglobulin concentrates also showed only 4, but somewhat more crisply. In an electrophoretic field, the rat pregnancy-associated serum antigens migrated almost to the same extent as the analogous human proteins, but they spread into larger precipitin areas (see Fig. 1c), and two of them were only faintly seen.

As controls, two rabbits were similarly immunized with pooled nonpregnant rat female and male plasma. The unabsorbed antisera showed complex reactions with the various rat sera, but, after similar absorption with nonpregnant rat plasma, all immunoreactivity against pregnant or nonpregnant rat sera was eliminated.

The sera obtained from five rats 10 days postpartum failed to react with the immunoglobulin concentrates of potent absorbed antisera to pregnant rat serum. The newborn rat sera were also nonreactive with this reagent (Fig. 1a).

Mouse pregnancy serum. When the antisera to mouse pregnancy serum were completetly absorbed with nonpregnant mouse plasma, some of them also revealed up to 4 antigens in mouse pregnancy sera, none being seen in nonpregnant mouse sera (see Fig. 2). Individual pregnant mouse sera showed reactions equivalent to the pooled specimens. In immunoelectrophoresis, only two precipitin arcs were detected. These were elongated and were located as in rat systems (Fig. 2b).

Relationship between human, rat, and mouse pregnancy-associated plasma proteins. When tested against potent rabbit antibodies to human pregnancy-associated proteins, none of the rat or mouse pregnancy serum pools showed any reactions by immunoelectrophoresis (see Figs. 1 and 2), or by immunodiffusion (see Fig. 3). The same was true when the antiserum to human pregnancy plasma was tested against 45 individual pregnant rat sera and 72 pregnant mouse sera. Reciprocally, rabbit antibodies to rat and mouse pregnancy-associated antigens were

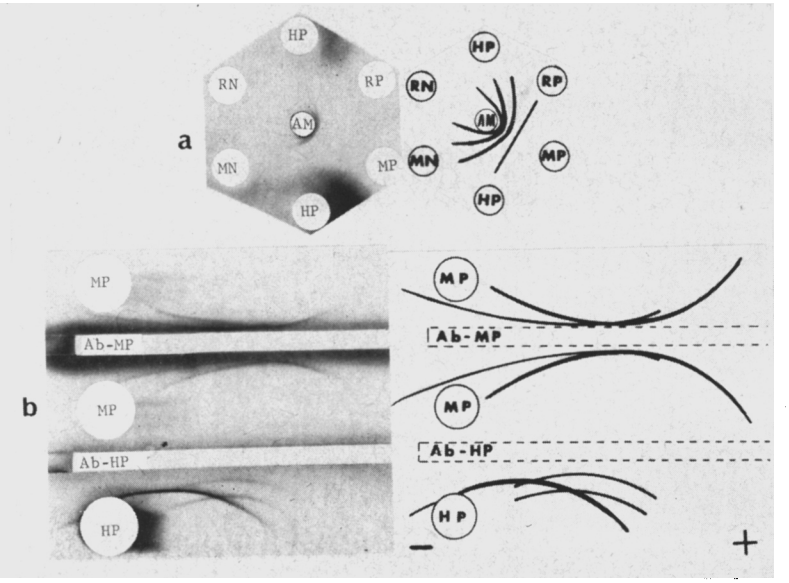

FIG. 2. Immunodiffusion (a) and immunoelectrophoresis (b) of mouse pregnancy-associated serum antigens. (HP) Pool of third trimester human pregnancy plasma; (RP) pool of rat late pregnancy serum; (MP) pool of mouse late pregnancy serum; (MN) pool of nonpregnant female and male mouse plasma; (RN) pool of nonpregnant female and male rat plasma; (AM, Ab-MP) absorbed antiserum to mouse pregnancy serum; (Ab-HP) absorbed antiserum to human pregnancy plasma.

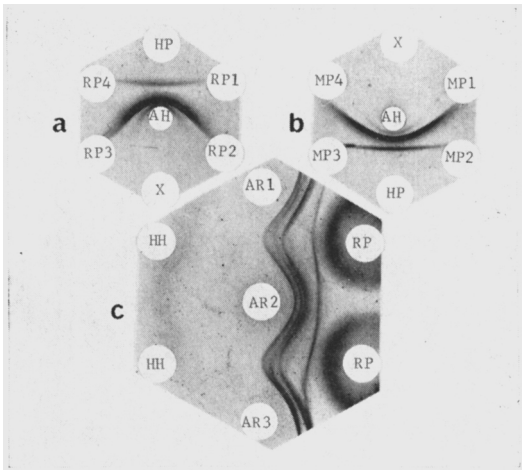

FIG. 3. Immunodiffusion comparison among rat, mouse, and human pregnancy-associated antigens. (HP) Pool of third trimester human pregnancy plasma; (RP1, RP2, RP3, RP4) individual rat late pregnancy serum; (X) empty; (MP1, MP2, MP3, MP4) individual mouse late pregnancy serum; (RP) pool of rat late pregnancy serum; (AH) absorbed antiserum to third trimester human pregnancy plasma; (AR1, AR2, AR3) absorbed antiserum to rat pregnancy serum from three rabbits.

nonreactive against human pregnancy plasmas (Figs. 1c, 2b and 3c).

Antibodies to one of the rat pregnancy-associated antigens partially cross-reacted with the analogous mouse pregnancy antigen. On the other hand, none of the antibodies to mouse pregnancy-associated antigens reacted reciprocally with rat pregnancy serum. It is thus apparent that rather strict species specificity of these pregnancy associated serum antigens was observed in the human, rat, and mouse with the reagents and technics used. Only one of these pregnancy proteins in the rat and mouse were immunochemically related.

In agreement with other investigators (11, 12) several cross-reactions were observed between the normal serum proteins of these three species when unabsorbed antisera were tested. The cross-reactions were most numerous and intense between rat and mouse sera, as might be anticipated, and at least 4 to 5 proteins were involved.

Discussion. Employing the same procedures used in our earlier studies on human pregnancy-associated plasma proteins (8, 9), up to 4 different antigens were also detected in the sera of pregnant rats and pregnant mice, which were not seen in the sera of nonpregnant animals. In the rat, these pregnancy-associated antigens had decreased to nondetectable levels at 10 days postpartum.

Bohn (13) and Hofmann (14) have described several human pregnancy-associated serum proteins with antisera to placenta and/or pregnancy plasma, and they also found that antibodies to the human proteins did not cross-react with pregnancy serum from the rat or dog. One pregnancy-associated plasma protein was found in mink by Larsen, Porter and Porter (15), which could represent an analogous constituent.

The precipitin lines between these antibody concentrates and rat or mouse pregnancy sera in immunodiffusion and immunoelectrophoresis were usually fainter than those seen in the human systems. It is not clear whether this was due to lower antibody levels, or to the difficulties experienced in removing antibodies to normal plasma proteins from these specimens by absorption with nonpregnancy plasmas. At least 200 mg of lyophilized normal nonpregnant plasma were usually required for complete absorption. The immunoglobulin fractions of such specimens could not readily be concentrated subsequently more than twofold, as compared to the three- to fivefold concentrations feasible with antiserum to human pregnancy plasma. More prolonged immunization intensifies this problem, and it is apparent that alternative methods should be established to increase the specificity of the antibody responses. Use of purified pregnancy-associated proteins, or of harvested immune complexes from immunodiffusion plates are possibilities. It also might be feasible to render newborn rabbits tolerant to normal nonpregnancy plasma proteins, followed by immunization with pregnancy plasma.

Since considerable progress has been made in characterizing the human pregnancy-associated plasma antigens as proteins of widely differing molecular weights, isoelectric points and other properties (6, 9, 13, 14) it seems likely that the rat and mouse pregnancy-associated plasma antigens are also

protein in nature. Since it is quite probable that they serve similar functions in all mammalian species, studies are therefore now possible to determine their biological significance experimentally.

Summary. Rabbits were hyperimmunized with late rat and mouse pregnancy serum. After thorough absorption of the antisera with nonpregnancy plasma of the same strains of animal, up to 4 pregnancy-associated serum antigens were revealed in both rat and mouse pregnancy sera by immunodiffusion methods. They were mostly species-specific, no cross-reactions being seen with human pregnancy-associated plasma proteins. Only a partial cross-reaction was found between one of the rat and mouse pregnancy antigens. The rat pregnancy-associated serum antigens were not detected 10 days after parturition, nor were they found in the serum of newborn rats.

1. Song, C. S., Merkatz, I. R., Rifkind, A. B., Gillette, P. N., and Kappas, A., Amer. J. Obstet. Gynecol. **108**, 227 (1970).

2. Spellacy, W. N., Teoh, E. S., Buhi, W. C., Birk, S. A., and McCreary, S. A., Amer. J. Obstet. Gynecol. **109**, 588 (1971.)

3. Ances, I. G., Amer. J. Obstet. Gynecol. **113**, 291 (1972).

4. Afonso, J. F., and DeAlvarez, R. R., Amer. J. Obstet. Gynecol. **86**, 815 (1963).

5. Studd, J. W. W., Blainey, J. D., and Bailey, D. E., J. Obstet. Gynaecol. Brit. Commonw. **77**, 42 (1970).

6. Bohn, H., Arch. Gynaekol. **210**, 440 (1971).

7. Hofmann, R., Friemel, H., and Brock, J., Arch. Gynaekol. **208**, 187 (1969).

8. Gall, S. A., and Halbert, S. P., Int. Arch. Allergy Appl. Immunol. **42**, 503 (1972).

9. Lin, T. M., Halbert, S. P., Kiefer, D., Spellacy, W. N., and Gall, S. A., Amer. J. Obstet. Gynecol. In press.

10. Lin, T. M., Halbert, S. P., and Kiefer, D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **32**, 623 (1973).

11. Clausen, J., and Heremans, J., J. Immunol. **84**, 128 (1960).

12. Bauer, K., Humangenetik **7**, 76 (1969).

13. Bohn, H., Blut **24**, 292 (1972).

14. Hofmann, R., Straube, W., Klausch, B., Friemel, H., and Gunther, J., Arch. Gynaekol. **212**, 246 (1972).

15. Larsen, A. E., Porter, D. D., and Porter, G. H., Proc. Soc. Exp. Biol. Med. **136**, 430 (1971)

Received June 7, 1973. P.S.E.B.M., 1974, Vol. 145.