Comparison of the pregnancy-associated murine protein-2 and human pregnancy-specific β_1 -glycoprotein

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Summary. Immunological cross-reaction between human pregnancy-specific β_1 -glycoprotein (SP-1) and pregnancy-associated murine protein-2 (α_2 -PAMP, PAMP-2) was demonstrated by line immunoelectrophoresis using antibodies against the proteins raised in hens. The pattern of change of PAMP-2 and SP-1 values in serum during pregnancy was very similar. The murine placental growth-rate corresponded to PAMP-2 levels in maternal serum during pregnancy, indicating placental origin.

We suggest that the mouse and its PAMP-2 can be used to study human SP-1.

Introduction

Human pregnancy-specific β_1 -glycoprotein (SP-1 or PS β G) was originally described as a β -mobile glycoprotein present in serum of pregnant women (Bohn, 1971). The protein appears to be synthesized by the placenta (Lin & Halbert, 1976), but ectopic production has been described (Tatarinov & Sokolov, 1977; Rosen, Javadpour, Calvert & Kaminska, 1979). Molecular heterogeneity of SP-1 has been reported (Teisner, Westergaard, Folkersen, Husby & Svehag, 1978) and two proteins with SP-1 determinants have been isolated (Teisner, Folkersen, Hindersson, Jensenius & Westergaard, 1979); one with β_1 -electrophoretic mobility and a molecular weight of 90 000 and the other with α_2 -electrophoretic mobility and a molecular weight of approximately 400 000.

The function and biological significance of SP-1 are unknown. Attempts to establish an animal model for the study of the protein have so far only been successful using non-human primates (Bohn & Ronneberger, 1973). The present report compares the pregnancy-associated murine protein-2 (PAMP-2) (Hau, Svendsen, Teisner & Svehag, 1978; Hau, Svendsen, Teisner & Thomsen Pedersen, 1980) to human SP-1 to see whether mice could be used to study the biological function of SP-1.

Materials and Methods

Animals and antiserum production

Experiments were performed with outbred NMRI-mice. Conception time was determined by the presence of a vaginal plug, and designated Day 1 of pregnancy. Blood samples were obtained by periorbital puncture (Hau *et al.*, 1980). On each day of pregnancy 10 mice were killed and the placental tissue was weighed.

Partial hysterectomy was performed in 5 adult females by ligating the left horn of the uterus a few millimetres from the bifurcation. The surgery was performed under general anaesthesia induced by i.p. injection of 0.3 mg pentobarbitone sodium.

A monospecific antiserum against PAMP-2 was produced in a rabbit as previously described (Hau *et al.*, 1978, 1980). The previous nomenclature α_2 -PAMP has been changed to PAMP-2 because 4 pregnancy-associated proteins have now been detected in mice. A rabbit anti-serum to SP-1 immunoglobulins (code A 131, lot 018c) was obtained from Dako Immunoglobulins, Copenhagen, Denmark.

Hen antiserum against PAMP-2 was obtained after 3 injections, at 2-week intervals, of partly purified PAMP-2 and equal amounts of Freund's incomplete adjuvant. The purification procedure consisted of separation by size chromatography in G 150 superfine gel (Pharmacia) on a Pharmacia K 26/100 column, followed by preparative zone electrophoresis and concentration by vacuum evaporation. The antigen was administered subcutaneously in doses of 500 μ g protein. Hen antiserum against SP-1 was obtained after a similar immunization procedure, using SP-1 purified from human late pregnancy serum by $(\text{NH}_4)_2\text{SO}_4$ precipitation, size chromatography and preparative zone electrophoresis as previously described (Teisner *et al.*, 1979).

Samples from pregnant women

A total of 453 serum samples was obtained from 84 normally pregnant women from the 6th week of pregnancy until 1 week *post partum*. The samples were stored at -20°C until needed for testing.

Quantitative rocket immunoelectrophoresis

Rocket immunoelectrophoresis for quantification of PAMP-2 and SP-1 was performed on 11×20 cm glass slides in a 1% agarose gel (Indubiose A 37, L'Industrie Biologique Francaise) with a Tris-barbital buffer, ionic strength 0.020, pH 8.5, at 2.5 V/cm for 18 h (Laurell, 1972). The gel contained 1.5 μ l rabbit anti-PAMP-2 preparation/cm² or 0.8 μ l rabbit anti-SP-1/cm². The amounts of PAMP-2 or SP-1 were measured in terms of arbitrary units (AU). As recommended by W.H.O., a value of 100 AU/ml refers to the concentration of protein in pools of late pregnancy serum.

Line immunoelectrophoresis

The electrophoresis was performed as indicated by Krøll (1973) with the same gel-buffer system as for rocket immunoelectrophoresis. Four gel strips were moulded on 10×10 cm glass slides. These were, from the cathodic side; (A) a neutral gel ($10 \times 1.5 \times 0.15$ cm), (B) a line gel ($10 \times 0.5 \times 0.15$ cm), containing 15 μ l of a pool of human late pregnancy serum, (C) a neutral gel ($10 \times 1 \times 0.15$ cm) with 12 wells cut in the gel and (D) an antibody-containing gel ($10 \times 7 \times 0.15$ cm) containing the antiserum to rabbit SP-1 immunoglobulins at a concentration of 1.8 μ l/cm² agarose (Pl, 1, Fig. 1). During the electrophoresis SP-1 present in the line gel migrated through the neutral gel into the antibody-containing gel and a line precipitate (rabbit anti SP-1/SP-1) was formed, parallel to the line gel. The distance from the line gel to the precipitate was proportional to the antigen/antibody ratio.

The wells in the neutral gel, placed on the anodic side of the line gel, received 5 μ l samples of different types of antiserum from hens and rabbits, and samples of different types of antigen, consisting of serum samples from pregnant women and mice, as well as control sera from normal rabbits and hens. If a well contained antiserum with reactivity against SP-1, the antigen/antibody ratio was diminished, resulting in a negative deflection of the precipitation line above this well. When a well received a sample containing SP-1, the antigen/antibody ratio was increased and this resulted in a positive deflection of the precipitation line above this well.

Results

Rocket immunoelectrophoresis showed that SP-1 was detectable in women from Week 6 of pregnancy. Circulating SP-1 then increased in concentration and reached a maximum at Week 36 which lasted until Week 40. The post-partum decline was rapid with a half-life of approximately 30 h (Text-fig. 1a).

Text-fig. 1. Rocket immunoelectrophoresis measurements of (a) SP-1 in 453 serum samples from 84 normal pregnant women, and (b) PAMP-2 in 318 serum samples from 30 primigravid mice. Values are mean $\pm$ 1 s.e.m.; in (a) each point represents mean values of the 11–60 samples obtained at different times within each 2-week period, and in (b) the samples were from 9–28 mice on each day. (c) Total placental weights of 90 mice throughout pregnancy. Each value represents the mean $\pm$ s.e.m. for 10 animals. P = parturition.

The PAMP-2 in mice was detectable by rocket immunoelectrophoresis from Day 8 of pregnancy, increased until Day 14, and remained at a maximum level until Day 17. Serum level decreased before parturition and the half-life of the protein was 13 h, determined by the changes of PAMP-2 concentration after delivery of the placenta (Text-fig. 1b).

In the partly hysterectomized mice the mean ($\pm$ s.e.m.) PAMP-2 level at Day 17 was 108 ± 18 AU/ml, whereas the normal level was 194 ± 12 AU/ml. The total placental weight of 17-day-pregnant females was 1.440 ± 0.12 g compared with 0.609 ± 0.07 g for the semi-hysterectomized females.

In intact females the placental weight increased until Day 17 of pregnancy and then decreased until parturition (Text-fig. 1c).

Immunological cross-reaction between SP-1 and PAMP-2 was demonstrated by line immunoelectrophoresis. The precipitation line was formed by a complex between human SP-1 and rabbit anti SP-1. Hen antiserum against both SP-1 and PAMP-2 reacted, when placed in wells in the neutral gel, by negative deflection of the precipitation line, whereas serum from non-immunized rabbits and hens caused no deflection. SP-1 antigen present in a pool of serum from women caused a positive deflection, whereas PAMP-2 antigen present in serum of pregnant mice caused no deflection (Pl. 1, Fig. 1).

Discussion

It has previously been demonstrated that neither SP-1 (Bohn, 1971) nor PAMP-2 (Hau *et al.*, 1980) are detectable in serum of healthy non-pregnant individuals. The molecular weights of the two proteins are very similar (Bohn, 1971; Hau *et al.*, 1978) and both proteins consist of at least two different forms with corresponding electrophoretic migration velocities but with different concanavalin A-binding properties (Teisner *et al.*, 1978; Hau *et al.*, 1980). The different Con A-binding ability of PAMP-2 and SP-1 is probably due to a higher degree of glycosylation of SP-1 than PAMP-2, but might be a result of differences in configuration of the proteins, with a shielding of the carbohydrate of PAMP-2, resulting in a retardation in migration velocity instead of complete precipitation. The isoelectric points of SP-1 and PAMP-2 are in the same range (Lin, Halbert, Kiefer, Spellacy & Gall, 1974; Bohn, 1976; Hau *et al.*, 1980) (Table 1).

Table 1. Physico-chemical and biological characteristics of SP-1 and PAMP-2

Characteristic	SP-1	PAMP-2
Considered pregnancy-specific*	Yes (1)	Yes (5)
Molecular weight	90 000 (1)	70 000 (5)
Isoelectric point	3.8–4.15 (2)	4.0 (6)
Molecular forms	2 (3)	2 (6)
Half-life	30 h	13 h
Electrophoretic mobility of the main component	β_1 (3)	α_2 (6)
Con A-binding properties of the two molecular forms†	++, ++ (4)	–, + (6)

* Not found in healthy non-pregnant individuals.

† Precipitation by Concanavalin A in the first dimensional gel in crossed affinity electrophoresis: –, no affinity; +, retardation in migration velocity; ++, complete precipitation.

(1) Bohn, 1971; (2) Lin *et al.*, 1974; Bohn, 1976; (3) Teisner *et al.*, 1978; (4) Westergaard *et al.*, 1979; (5) Hau *et al.*, 1978; (6) Hau *et al.*, 1980.

Serum concentrations of SP-1 and PAMP-2 during pregnancy appear similar in pattern (Text-figs 1a and 1b), except that in the mouse PAMP-2 values begin to fall before parturition. This may be because the last mean value for SP-1 before parturition is based on samples obtained at different times in the last 2 weeks of pregnancy, whereas the PAMP-2 mean values represent daily samples taken within the same hour each day. However, a more likely explanation is the difference in the growth pattern of the placenta between women and mice. As

Line immunoelectrophoresis with rabbit anti-SP-1 in the antibody-containing gel (D), serum from pregnant women in the line gel (B) and two neutral gels (A, C).

The contents of the wells were: (1) rabbit anti-SP-1 (Dako) diluted 1:50; (2) rabbit anti-SP-1 (Dako) diluted 1:100; (3) hen anti-PAMP-2, undiluted; (4) serum of control hen, undiluted; (5) hen anti-SP-1, undiluted; (6) serum of pregnant women, diluted 1:8; (7) serum of pregnant mice, undiluted; (8) serum of pregnant mice, diluted 1:5; (9) serum of pregnant mice, diluted 1:10; (10) serum of non-pregnant mice, undiluted; (11) serum of normal rabbit, undiluted; (12) buffer.

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seen in Text-fig. 1c, the placental mass in mice decreases before parturition, whereas this is not the case in women. Concentrations of both proteins decline rapidly after parturition, SP-1 with a half-life of 30 h and PAMP-2 with a half-life of 13 h. The close correlation between serum PAMP-2 values and the placental weight (Text-figs 1b and 1c) indicates that PAMP-2, like SP-1 (Lin & Halbert, 1976), is a product of the placenta. This is confirmed by the reduction of the PAMP-2 concentration in the 17-day-pregnant partly hysterectomized mice.

Immunological cross-reactivity between the proteins cannot be demonstrated using rabbit antisera. However, by using the phylogenetically distant hen in the production of antibodies, it was possible to demonstrate immunological determinants which were common to the two proteins. An explanation of the fact that a cross-reaction was observed between PAMP-2 and SP-1 when using hen antibodies against the proteins may be because there is no pregnancy in the hen. The lack of a hen SP-1 analogue suggests that the hen antibodies are produced against determinants which PAMP-2 and SP-1 have in common, whereas the rabbit, likely to possess an analogue to SP-1, immunologically reacts only against the determinants which differ between SP-1, PAMP-2 and the rabbit's own SP-1 analogue.

The immunological cross-reaction and the close relationship between the physico-chemical properties and the serum levels of SP-1 and PAMP-2 during pregnancy suggest that mice can be used to study the function of human SP-1.

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