Development of a Laboratory Animal Model for Functional Studies of three Human Pregnancy Proteins: α-Foetoprotein (AFP), β_1-Glycoprotein (SP-1) and Pregnancy Zone Protein (PZP)

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Introduction

Some serum proteins exhibit very small differences from species to species, and antisera raised against human proteins e.g. albumin or α_2-macroglobulin are often useful when studying the analogous proteins in various animal species. By contrast, a number of serum proteins are highly species specific with different physico-chemical properties in different species. These are probably phylogenetically rather new proteins. The biological significance of many human serum proteins is presently unknown and this has urged scientists to develop laboratory animal models for functional studies. In primate species most proteins cross-react readily with human proteins, which means that antisera produced against the human proteins can be used in various immunochemical analyses. Using phylogenetically more distant species such as rodents major problems occur because the rodent proteins most often do not cross-react with the human analogues. Consequently specific antisera against the animal proteins must be produced when demonstrating immunological cross-reaction with human proteins. This demonstration of cross-reaction is desirable in order to verify assumptions of analogy between proteins of different species, because the proteins differ from species to species making it difficult to determine for certain which proteins are analogous.

The biological function of the pregnancy-associated serum proteins pregnancy zone protein (PZP, α_2-PAG), pregnancy specific β_1-glycoprotein (SP-1, PSβG) and α-foetoprotein (AFP) is unknown. A murine model for the study of these proteins has been developed (Hau et al. 1978, 1980ab, 1981ab). The analogue to PZP has been termed pregnancy-associated murine protein-1 PAMP-1, the analogue to SP-1 PAMP 2, and the analogue to AFP PAMP-4 or murine AFP.

Physico-chemical properties of PAMP-1, PAMP-2 and murine AFP

The physico-chemical properties of the three human proteins and their murine analogues are not identical (Table I–III) and where the AFP molecules of the two species are quite closely related, marked differences exist between PZP and PAMP-1.

Table I. Physico-chemical and biological properties of PZP and PAMP-1.

	PZP	PAMP-1
Occurrence — male serum	(+)	—
Non-pregnant female serum	(+)	+
Molecular weight	360,000	150,000
Isoelectric point	4.7	4.2
Electrophoretic mobility	α_2	α_1
Surface properties	heterogeneous — 1 form hydrophilic 1 form amphiphilic	heterogeneous — 1 form hydrophilic 1 form amphiphilic
Sialic acid contents	47 %[a]	69 %[a]
Reaction with Con A	++[b]	++[b]
Intracellular localization	hepatocytes	hepatocytes
Inductor in males	oestrogen	oestrogen
Inductor in females	oestrogen	oestrogen
Half-life	140 h	26 h

a) Migration velocity in crossed immunoelectrophoresis following neuraminidase treatment in % of original migration velocity.

b) Complete precipitation of protein in crossed affino immunoelectrophoresis with free Con A in the first dimension gel.

Table II. Physico-chemical and biological properties of SP-1 (main component with β_1-mobility) and PAMP-2.

	SP-1	PAMP-2
Occurrence — in males	—	—
In non-pregnant females	—	—
Molecular weight	90,000	78,000
Isoelectric point	3.8 ± 0.2	4.0
Electrophoretic mobility	β_1	α_2
Surface properties	heterogeneous — 1 form hydrophilic 1 form amphiphilic	heterogeneous — 1 form hydrophilic 1 form amphiphilic
Sialic acid contents	91 %[a]	32 %
Reaction with Con A	++[b]	—/+[b]
Intracellular localization	syncytiotrophoblast	spongiotrophoblast
Half-life	30 h	13 h

a) Migration velocity in crossed immunoelectrophoresis following neuraminidase treatment in % of original migration velocity.

b) Reaction with Con A in crossed affino immunoelectrophoresis. — = no affinity, + = retardation in migration velocity and ++ = complete precipitation.

Immunological cross-reaction with human proteins

AFP of humans and mice cross-reacts readily using antisera raised in mammals, but no immunological cross-reaction could be detected between PAMP-1 and PAMP-2 and any of the human proteins listed in Table IV using antisera raised in goats and rabbits. However, using antisera raised in hens immunological cross-reaction between PAMP-1 and

Table III. Physico-chemical and biological properties of human and murine AFP.

	Human AFP	Murine AFP
Occurrence — in males	—	—
In non-pregnant females	—	—
Molecular weight	70,000	72,500
Isoelectric point	4.9—5.2	4.4
Electrophoretic mobility	α_1	α_2
Sialic acid contents	13 %[a])	12 %[a])
Surface properties of protein in foetal tissue	heterogeneous — 1 form hydrophilic 1 form amphiphilic	heterogeneous — 1 form hydrophilic 1 form amphiphilic
Con A, reaction with	heterogeneous — 1 form not binding (o) 1 form binding (1)	heterogeneous — 1 form not binding (o) 3 forms binding (1,2,3)
Dissociation constants of Con A-AFP complex	5.0×10^{-6}	4.5×10^{-6}
Mol. form-1		
Mol. form-2	absent	3.3×10^{-6}
Half-life	5 days	15 h
Affinity for oestrogens	+	+

a) Migration velocity in crossed immunoelectrophoresis following neuraminidase treatment in % of original migration velocity.

human PZP and between PAMP-2 and human SP-1 was demonstrated employing line immunoelectrophoresis.

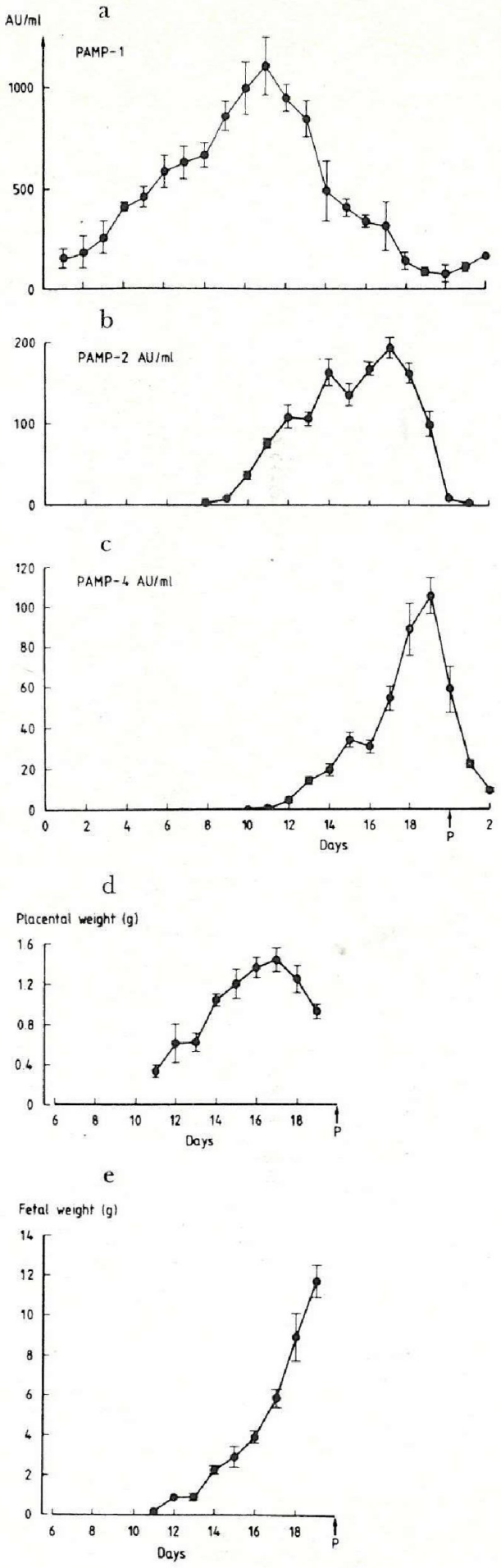
Since goats and rabbits must be expected to have pregnancy-associated proteins similar to those of humans, it was presumed that antibodies raised in these species would have specifities against the different antigenic determinants only and not to common surface structures. Consequently antibodies against human and murine pregnancy-associated proteins were raised in hens, a species unlikely to possess analogues to mammalian pregnancy proteins.

Biological properties of PAMP-1, PAMP-2 and murine AFP

Like human PZP, PAMP-1 is a pregnancy-associated protein also being synthesized by non-pregnant individuals (Table I). The protein is undetectable in normal adult mouse serum but the protein appears following castration or estrogen treatment of males (Hau et al. 1982a). Testerone treatment of females and castrated males results in the elimination of PAMP-1 from the circulation (Hau et al. 1983a). During pregnancy the serum concentration of PAMP-1 increases to reach a maximum shortly after mid-pregnancy (Fig. 1a). Prior to parturition the level decreases to pre-conception values. PAMP-1 is present intracellularly in the liver parenchymal cells, and the protein has affinity for the trophoblastic cell surface lining the maternal blood spaces in the placenta (Hau et al. 1983b).

Unlike PAMP-1, PAMP-2 is not detectable in serum of non-pregnant mice (Table II). The protein appears in the maternal circulation during

Fig. 1



the first half of pregnancy (Fig. 1b) and it is located intracellularly in the trophoblastic cells (Chemnitz et al. 1982). Like the human analogue SP-1, PAMP-2 thus seems to be of placental origen, and the correlation between PAMP-2 serum level and placental mass during pregnancy is highly significant (Hau et al. 1982b). Fig. 1d shows the total placental mass during pregnancy.

AFP is produced mainly by the yolk sac and foetal liver. During pregnancy the protein increases in concentration in maternal circulation (Fig. 1c). Fig. 1e shows the total foetal mass during pregnancy. There is a highly significant correlation between the circulating level of maternal AFP and foetal mass during pregnancy (Hau et al. 1981b).

Discussion

Because of the differences in physico-chemical properties between human and murine pregnancy proteins, immunological cross-reaction between the proteins was a most welcome indication of analogy.

The gonadal hormonal balance seems to determine the PAMP-1 level in non-pregnant animals, but the cause for the rise in serum during pregnancy is at present unknown. We have shown that the PZP level in the African green monkey is suppressed by estrogen (Teisner et al. 1979) and that there is a negative correlation between estriol and human PZP in twin pregnancies (We-

Daily serum levels of PAMP-1, PAMP-2 and PAMP-4 (m-AFP) and placental and foetal weight during pregnancy. Each value represents the mean ± s.e.m. for 10 animals. P = parturition.

stergaard et al. 1982). It is thus doubtful that increased levels of estrogen alone is the reason for the elevated level of PAMP-1 observed during pregnancy.

The highly significant correlation between the maternal serum level of PAMP-2 and placental mass and between serum AFP and foetal mass during pregnancy points at the mouse as a useful tool when assessing the mode of action of teratogenic substances. Quantitative measurements of the serum proteins may reveal whether the toxic substance leads to foetal growth retardation via placental insufficiency or not.

Summary

A murine model for the study of the function of three human pregnancy-associated serum proteins has been developed. The human proteins are pregnancy zone protein (PZP) of maternal origin, pregnancy-specific β_1-glycoprotein (SP-1) of placental origin and α-foetoprotein (AFP) of foetal origin. The maternal serum levels of murine SP-1 (PAMP-2) and murine AFP during pregnancy reflect the growth of the placenta and foetuses respectively, indicating the usefulness of the model in teratogenic studies.

Sammendrag

En musemodel til studiet af funktionen af tre humane graviditets-associerede serumproteiner er blevet udviklet. De humane proteiner er pregnancy zone protein (PZP) produceret af moderen, pregnancy specific β_1-glycoprotein (SP-1) produceret af placentaen og α-foetoprotein (AFP) produceret af fosteret. De maternelle serumniveauer af murint SP-1 (PAMP-2) og murint AFP er nøje korreleret til væksten i henholdsvis placenta- og fostermassen, hvilket indicerer forsøgsdyrmodellens brugbarhed i teratogeniske undersøgelser.

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