Pregnancy Associated Glycoproteins (PAGs) in Bos taurus taurus and Bos taurus indicus


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Introduction

In 1991, Zoll et al. purified a pregnancy-associated glycoprotein from bovine fetal cerebrospinal fluid (PG-2) and in 1992, they identified and sequenced it (Zoll et al., 1992). In the same year, molecular biology studies showed that PAGs belong to the asialoglycoprotein family, a large group of proteins with a common moiety on the cell surface (Fesik et al., 1995). The PAGs can be divided into three main categories: the first category includes PAGs that are induced by pregnancy, the second category includes PAGs that are induced by infectious agents, and the third category includes PAGs that are induced by both pregnancy and infectious agents. The PAGs can be detected in the maternal circulation by week 3 of pregnancy and have been used as a gestation marker in cows (Sassler and Zoll, 1991; Zoll et al., 1992).

Aim

The aim of this study was to compare crude placental extracts of B. taurus taurus and B. taurus indicus and purified PAG1 against two different antisera by means of Double Radial Immunodiffusion (DRID).

Materials and Methods

1. Antigens and antisera:

1.1. Antigens:

A) Placental extracts and purified PAG1:

Approximately 5 months after conception, placental tissues were collected from B. taurus taurus and B. taurus indicus cows. The tissue was minced and homogenized with a hand mixer in 50 mM phosphate buffer containing PMSF (0.2 mM) and EDTA (1 mM) to a final concentration of 1:1 (w/v). The homogenate was stirred overnight. It was then centrifuged at 20,000 × g for 1 h, and the pellet was dispersed. The supernatant was dialyzed against 5 mM ammonium bicarbonate (pH 7.8) and lyophilized and the final powder stored at 4°C. The antigen preparation step was summarized in Figure 1. The powder from placental extracts was reconstituted in a concentration of 20 mg/mL in phosphate buffer in order to use all the antibodies.

B) Albinions:

The bovine and porcine albumins (BSA and PSA) were used to control the specificity of the antisera. These proteins were dissolved in phosphate buffer containing 1% methanol.

1.2. Antisera:

1.2.1. Antiserum specific to B. taurus taurus and B. taurus indicus:

Two groups of antisera were stored in the Department of Reproduction of University of Liege in the usual conditions (Table 1). The first group of antisera (anti-PAG1) was raised in rabbits previously vaccinated with purified PAG1. The second group of antisera (anti-PAG2) was produced against semi-purified PAG2 antigens. All the antisera were obtained in rabbits according to the method of Vatsyayana (1971). The rabbits received injections of 500 mg of antigen, divided over 16 weeks and bled 3 weeks after the first immunization.

1.2.2. Antiserum against B. taurus taurus and B. taurus indicus:

Antiserum against B. taurus taurus and B. taurus indicus was used to verify the reactions between the PAGs and crude placental extracts were not due to the albumin contamination in the extract with the antisera. These antisera were obtained by the same protocol described above.

2. Double Radial Immunodiffusion (DID):

An agarose solution of 2% in veronal buffer was poured onto clean microscope slides, and after solidification, 0.5 mm diameter wells were punched into the agarose layer. After filling the samples in the wells, the slides were left to stand for 24 h in the wet chamber. The soluble proteins were then washed away by 2 changes in 0.9% NaCl solution and 2 changes in distilled water. The slides were stained with Coomassie Blue (P20) solution for 30 min and the excess stain was washed out by repeated changes in ethanol/acidic distilled water (1:1 v/v).

2.1. Double Immunodiffusion- Quanti-quantum Test:

This test was used to verify the specificity of the antisera against the antigen. The antisera (anti-PAG1, anti-PAG2, anti-B. taurus taurus, anti-B. taurus indicus and anti-placental extracts with their following dilutions 1:1, 1:2, 1:4, 1:16, 1:64, 1:256, 1:1024) were placed in the peripheral wells. The central well was filled by the bovine albumin (BSA) or the porcine albumin (PSA) (Figure 2).

2.2. Double Immunodiffusion- Quali-quantum Test:

This test was used to compare purified PAG1 and crude placental extracts of B. taurus taurus and B. taurus indicus against the groups of antisera (Figure 3).

Results

1. Control of antisera specificity:

BSA and PSA (data not shown) did not cross-react with any of the antisera (anti-PAG1 and anti-PAG2) (Figure 4A) but they cross-reacted clearly with anti-BSA and anti-PSA (Figure 4B).

2. Comparison of placental extracts and PAG1:

In the PAG1 system (Figure 5A), there was one clear precipitation line between the purified PAG1 protein and anti-PAG1. This line was also observed with placental extracts of B. taurus taurus and B. taurus indicus. One more concentric line closer to the central well was also observed for both extracts. In the PAG2 system (Figure 5B) no precipitation line appeared with the pure PAG2 and anti-PAG2 confirming the immunological difference between the two PAG2. When placental extracts were compared, B. taurus indicus gave one precipitation line and B. taurus taurus gave two lines.

Conclusions

In conclusion, B. taurus indicus seemed to be similar to B. taurus taurus in the PAG1 system, while appearing different in the PAG2 system. The clear difference concerning the PAG2 is worthy for further investigations using the SDS-PAGE and Western blotting methods.

References


