Validation of two homologous radioimmunoassays for measuring pregnancy-associated glycoproteins (PAGs) in ewe

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Abstract

We have recently produced different antisera against PAGs isolated from placenta collected at days 80 to 100 of gestation (R780 to R805) and at later (>14 day) stages of pregnancy (R776 and R786). The aim of this study was to validate two homologous RIA systems. Antiserum R780 (against ovPAG G5+6) and R805 (against ovPAG G1+2), were used in RIA and RIA-2, respectively. The specific activity of the tracer was 14,900 Ci/mmol. The minimal detection limits for RIA-1 and RIA-2 were 0.2 ng/ml and 0.3 ng/ml, respectively. The inter-assay CV of samples with low (1.3 ng/ml), medium (2.5 ng/ml) and high (4.0 ng/ml) PAG concentrations were 13%, 12% and 7% for RIA-1 and 12%, 11% and 6% for RIA-2, respectively. The intra-assay CV was 3%, 6% and 9% for RIA-1 and 8%, 9% and 5% for RIA-2, in samples with 1.3, 2.5 and 4.0 ng/ml of PAGs, respectively. The recovery ranged from 96% to 112%. No cross-reactivity was observed with albumin and other members of aspartic proteinase family (renn, cathepsin D and pepsin). The sensitivity of RIA-1 was 0.2 ng/ml and 0.3 ng/ml, respectively. The specificity of RIA-2 was evaluated by assaying for ovPAGs in solution of various proteins of protease family and pools of sera from pregnant and non-pregnant ewes. The following aspartic proteinases were tested: pepsin, peptic, renn, cathepsin D and pepsin.

Introduction

Pregnancy-associated glycoproteins (PAGs) are synthesized by mono and/or binucleate trophoblastic cells. They constitute a large family of aspartic proteinases, showing a greatest sequence identity with pepsinogen.

PAGs are interesting for both scientific and economic reasons in the framework of livestock breeding programs. The determination of PAG concentrations in the maternal bloodstream is the basis of tests for pregnancy diagnosis or follow-up.

Production of new antigens isolated from sheep placenta at two different stage of gestation can be useful for the development of homologous radioimmunoassay (RIA) systems.

Aim

The present study describes the development of two sensitive and specific double-antibody RIA's for ovine PAGs previously isolated from foetal cotyledon.

Materials and Methods

Antigen production

Purification scheme involved sequential DEAE-cellulose chromatography, gel filtration and CM ceramic [1,2]. Two fractions were proven to be interesting by means of Western blot. They were used to produce two different antisera.

Antiserum

The origin and the characteristics of the antigens used to produce the antisera are summarized in Table 1. Three adult rabbits (3.4 kg) were immunized according to the method of Vaitukaitis et al. [3]. The proteins were reconstituted in distilled water, emulsified in an equal volume of Freund's adjuvant, and then injected into a rabbit. Injections were given intradermally at multiple sites along the back, which had previously been shaved. The rabbit received boosts of fresh antigen at one week intervals. Three weeks after the first injection, blood was collected from the marginal ear vein and allowed to clot for 12-24 h at room temperature. The sera obtained were removed and stored at -20°C until assayed.

Tracer

A purified preparation of ovPAG G1+8 was used as tracer after iodination [4].

Table 1: Antiserum used in RIA-1 and RIA-2

<table>
<thead>
<tr>
<th>System</th>
<th>Antigen</th>
<th>N-terminal sequence</th>
<th>Accession number</th>
</tr>
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<tbody>
<tr>
<td>R780</td>
<td>ovPAG G5+6</td>
<td>ISRVSXKTLPLNLMDL</td>
<td>P39250</td>
</tr>
<tr>
<td>R805</td>
<td>ovPAG G1+2</td>
<td>RGGSNHLPRLNIRD</td>
<td>P38441</td>
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<td>R780</td>
<td>ovPAG G1+2</td>
<td>RGGSNHLPRLNIRDY</td>
<td>P398441</td>
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<tr>
<td>R805</td>
<td>ovPAG G1+2</td>
<td>RGGSNHLPRLNIRDY</td>
<td>P398441</td>
</tr>
</tbody>
</table>

RIA validation (cont'd)

Inter-assay precision was assessed by determining the coefficient of variation of repeated measurements in seven different assays of three serum pools with low, medium and high concentrations of ovPAGs. Intrassay precision was determined by repeated measurements (n = 10) of these three ovine serum pools within one assay.

Results

1. The tracer specific activity was 14,900 Ci/mmol, allowing a highly sensitive RIA with a non-specific binding lower than 3%.
2. The final antisera dilution were 160,000 and 1/50,000 respectively for R780 (RIA-1) and R805 (RIA-2).
3. The minimal detection limits of RIA-1 and RIA-2 were 0.2 ng/ml and 0.3 ng/ml, respectively.
4. The inter-assay CV of samples with low (1.0 ng/ml), medium (2.5 ng/ml) and high (4.0 ng/ml) PAG concentrations were 13%, 12% and 7% for RIA-1 and 13%, 11% and 6% for RIA-2, respectively.
5. The intra-assay CV were 3%, 6% and 9% for RIA-1 and 8%, 9% and 5% for RIA-2, in samples with 1.0, 2.5 and 4.0 ng/ml of PAGs, respectively.
6. The recovery ranged from 96% to 112%.
7. Concerning the specificity, no cross-reaction was observed with albumin and other members of aspartic proteinase family (renn, cathepsin D, pepsinogen and pepsin) tested till 10,000 ng/ml. In non-pregnant animals, PAG concentrations were lower than 0.2 and 0.3 ng/ml, respectively for RIA-1 and RIA-2.

Conclusion

In conclusion, the two homologous RIA systems developed in this study were sensitive and specific for the detection of ovine PAGs.

Perspectives

Further investigations are in progress to evaluate these two RIA systems for early pregnancy diagnosis in blood and milk samples and for prediction of single or multiple gestations.

Bibliography