IMMUNOSUPPRESSIVE SUBSTANCES IN SEXUAL ORGAN FLUIDS OF CATTLE

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SUMMARY

Bull seminal ribonuclease (AS RNase) and phospholipid binding protein-haemolytic factor (PBP) are proteins exhibiting immunosuppressive activity isolated from seminal vesicle fluid. Both proteins are synthesized in ductus deferens and seminal vesicles and AS RNase also in cauda epididymidis under the effector influence of androgens. AS RNase has no cytotoxic effect on cattle lymphocytes, while PBP is lytically active. PBP can lyse all types of blood cells, but only in the absence of serum. Individual differences in the lytic sensitivity of cattle erythrocytes are not correlated with the lytic sensitivity of leukocytes. The lytic activity of PBP against leukocytes is increased in the surroundings of oestrous cervical mucus. Seminal plasma has lytic activity, too.

Oestrous cervical mucus and ovarian follicular fluid have also immunosuppressive activity demonstrated by the inhibition of mitogenically stimulated lymphocytes.

INTRODUCTION

Spermatozoa ejaculated or inseminated into the female sexual organs are foreign cells for a carrier. For this reason they could be immunologically easily damaged. Some protective immunosuppressive substances have been therefore supposed. Immunosuppressive factors in seminal plasma have been studied mainly in humans (for a review see James and Hargreave, 1984). In farm animals some substances with immunosuppressive activity have been investigated in bulls (for a review see Matoušek, 1985). As regards female sexual fluids in farm animals the immunosuppressive features have been studied predominantly with regards to pregnancy status (for a review see Fisher et al., 1985).

The aim of the present study was to investigate the occurrence of immunosuppressive activity associated with the probable protection of spermatozoa in cattle male and female sexual fluids.

PRINCIPAL METHODS

Bull seminal ribonuclease (AS RNase) and phospholipid binding protein-haemolytic factor (PBP) were isolated from the bull seminal vesicle fluid (Dostál and Matoušek, 1973; Kysilka, 1973). Seminal plasma (SP) was obtained by means of artificial vagina at the University of Agriculture, Insemination Centre in Stochov. Cervical mucus (CM) and ovarian follicular fluid (FF) were collected in Prague slaughterhouse and from cows bred at the Institute of Cattle Breeding in Rapotín. All fluids were stored at -20°C. The plate cytotoxic macrotest (Gorer and O’Gorman, 1956) was used for CM and FF investigation. Lytic activity of PBP and SP in vari-
ed dilutions was studied in Takacsy plates without complement. The number of lytically undestroyed leukocytes was determined microscopically in the counting chamber. All isolated substances and freeze drying fluids were studied by the blastic transformation test (Staňek et al., 1978; Pearson et al., 1979). The synthesis of AS RNase and PBP in bull sexual organs was investigated using indirect immunofluorescence (Batty and Walker, 1967; Matoušek and Kysilka, 1980). Testosterone and LH in blood plasma were determined by RIA (Thibier and Rolland, 1976).

**EFFECT OF IMMUNOSUPPRESSIVE SUBSTANCES OF CATTLE SEXUAL FLUIDS**

**Bull seminal plasma**

There are several papers devoted to seminal plasma immunosuppressive substances in humans (for a review see James and Har-greave, 1984). As regards bulls, the first isolated immunosuppressive substances from SP were seminal ribonuclease (BS RNase or AS RNase) (Floridi and D'Alessio, 1967; Dostál and Matoušek, 1973) and phospholipid binding protein - haemolytic factor (PBP) (Kysilka, 1973). AS RNase was studied firstly in our laboratory as aspermatogenic, anti-embryonic and cancerostatic factor (for a review see Matoušek, 1985). In recent years we have found its inhibition activity of blastic transformation of human, cattle and pig lymphocytes (Cinatl et al., 1977; Staněk et al., 1978; Matoušek et al., 1979; Souček et al., 1981; 1983). AS RNase had no effect on mitogenically unstimulated lymphocytes. PBP has been responsible for erythrolysis firstly observed by Millar (1956). After its isolation we have proved, in addition to lysis of erythrocytes, also its responsibility for removing of cytoplasmatic droplets from homologous and also heterologous spermatozoa (Matoušek and Kysilka, 1980; 1984). But only recently we have also proved its lytic activity on cattle leukocytes isolated from the blood (Table 1). The lytic effect of gradually diluted SP on unstimulated leukocytes is noted in Table 1, too. There are individual differences of cattle erythrocytes to lysis by PBP. This sensitivity studied with the use of seminal vesicle fluid as a source PBP is probably genetically controlled (Matoušek and Staněk, 1972). Individual differences to lysis by PBP exist also in leukocytes, but are not of the same character as in erythrolysis. PBP and SP titrated in bovine serum had no lytic effect. It is supposed that some substances of serum, probably protein(s) inhibit the lysis.

Both immunosuppressive substances isolated from SP (AS RNase and PBP) are synthesized in seminal vesicles and ductus deferens, AS RNase also in cauda epididymidis. The genes responsible for this synthesis are under the effector action of androgens (Matoušek et al., 1980; 1981).

**Female sexual organ fluids**

Our knowledge of immunosuppressive substances of cow sexual organ fluids is even more limited then that of bull SP and spermatozoa. Up to now only the immunosuppressive effect of uterine secretions in non-pregnant and pregnant cows has been published (Roberts, 1977; Segerson et al., 1984; Fisher et al., 1985).
The results in immunosuppressive activity of ovarian follicular fluid are noted in Table 2.

Table 1. Lytic effect of PBP and SP on bovine leukocytes titrated in PBS (phosphate buffer solution) and CM (cervical mucus)

<table>
<thead>
<tr>
<th>Lytic agent</th>
<th>Titration</th>
<th>No of leukocyte solutions used for titration</th>
<th>Titer of lytic agent which destroyed more than 50 per cent of leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBP 1% PBS</td>
<td>24</td>
<td>8 - 64</td>
<td></td>
</tr>
<tr>
<td>PBP 1% CM</td>
<td>20</td>
<td>16 - 256</td>
<td></td>
</tr>
<tr>
<td>SP (28 bulls) PBS</td>
<td>24</td>
<td>8 - 256</td>
<td></td>
</tr>
<tr>
<td>SP (28 bulls) CM</td>
<td>20</td>
<td>16 - 512</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Incorporation of $^3$HdR in per cents into DNA of human (HS) and cattle stimulated (CS) lymphocytes incubated with freeze dried bovine ovarian follicular fluid (FF) - 400 μg/ml and cervical mucus (CM) - 250 μg/ml.

<table>
<thead>
<tr>
<th>Stimulating mitogens</th>
<th>Incorporation without (0) and with freeze-dried fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control (PBS)</td>
<td>100</td>
</tr>
<tr>
<td>PHA (100 μg/ml)</td>
<td>100</td>
</tr>
<tr>
<td>Con A (20 μg/ml)</td>
<td>100</td>
</tr>
<tr>
<td>PWM (10 μg/ml)</td>
<td>100</td>
</tr>
</tbody>
</table>

+ $P < 0.01$

In last time we have also studied the immunosuppressive activity of oestrual cervical mucus. The results in the plate cytoxic macrotest were mostly negative. However, the inhibition activity on DNA synthesis of blastic transformed human lymphocytes was very strong (Table 2). The stimulative interaction of CM in the mixture with SP (Table 1) shows that this oestrual fluid helps in the cooperation with SP to destroy the homologous female immune cells.

GENERAL DISCUSSION

Spermatozoa present in female sexual organs are foreign
cells for female animals and for this reason must be protected against humoral and cellular immunological attacks. This protection is probably secured by immunosuppressive substances investigated and discussed mainly in humans for man (for a review see James and Hargreave, 1984). In farm animals these immunosuppressive factors were studied predominantly in bull seminal plasma where two such substances were isolated up to date (for a review see Matoušek, 1985). From these two immunosuppressive substances PBP has been shown very strong against all types of leukocytes. The lytic activity against leukocytes exhibits not only isolated PBP but also crude SP. Up to date, very little attention was devoted to female sexual organ fluids from this aspect. Our first observation of specific cytotoxic effect of ovarian follicular fluid against autologous and homologous leukocytes in about 50% of cows (Matoušek et al., 1985) and the common inhibition activity of this dialyzed and freeze-drying fluid on DNA incorporation into blastic transformed lymphocytes (Table 2) evokes an idea that also females can protect sperm in their sexual organs. The inhibition of blastogenesis by CM documented here support this idea. The stimulative effect of CM in the lysis of leukocytes by SP shows that probably other substances in CM help to damage the homologous and autologous female immune cells during oestrus in the protection of spermatozoa present in female sexual organs.

CONCLUSIONS

Special substances that probably protect spermatozoa in cow sexual organs against autologous immune cells are present in sexual organ fluids of bulls and cows. Seminal ribonuclease and phospholipid binding protein isolated from bull seminal vesicle fluid have this immunosuppressive character. There are probably other immunosuppressive substances in bull seminal plasma. The immunosuppressive activity is present also in cattle ovarian follicular fluid and cervical mucus.

REFERENCES


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