IMMUNE RESPONSE TO GENITAL MYCOPLASMOSIS IN BULLS

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ABSTRACT

This study was undertaken to determine whether a specific anti-
mycoplasmal immune response could be detected in the male bovine genital
tract and to better define mechanisms of immunity at that site. Specific
Mycoplasma agalactiae subsp. bovis agglutinins were titrated in the serum,
semen and preputial mucus extracts of two bulls with M. agalactiae
induced chronic seminal vesiculitis and of one normal control bull. Titer
from infected bulls averaged 64 for serum, 1024 for semen and <8 for
preputial mucus extracts whereas the control bull titers were 16 for serum,
<8 for semen, and <8 for preputial mucus extracts. Because of the high
semen agglutinin titers from infected bulls it was proposed that semen
titers may be more useful diagnostically than serum titers.

Studies of immunoglobulin levels in semen revealed that IgA,
IgG1 and IgG2 levels were all much higher in infected bulls than in
the control bull. These high semen IgA levels together with the high
semen agglutinin titers indicated a local secretory immune response
in genital tracts of infected bulls.

INTRODUCTION

Mycoplasmas are known to infect genital tracts of cows and bulls
(1) as well as people (2). Mycoplasma agalactiae subsp. bovis has been
associated with infertility in both male (1,3) and female (1,4)
cattle. Other mycoplasmas have been reported as possible causes of
reproductive failure in men (5,6) and women (2). In order to under-
stand the pathogenesis of mycoplasma infections specifically and
genital infections in general, it is of importance to study the local
and systemic immune responses of infected animals. Once information
is available on host-parasite relationships in the male genital tract
it may be possible to improve diagnostic, prophylactic and therapeutic
procedures for such genital infections. To this end a study of the
local and systemic immune response in genital mycoplasmosis of bulls
was undertaken.
Materials and Methods

Animals consisted of three normal bulls which were two to three years old at the beginning of the study. Each of two bulls (883 and 960) were infected with a pure culture of *Mycoplasma agalactiae* subsp. *bovis* by injection of 3 ml of a broth culture (approximately 100 colony forming units/ml) into one seminal vesicle. Mycoplasmas were consistently recovered from the semen thereafter. The colonies were identified as *M. agalactiae* subsp. *bovis* by immunofluorescence (7). Thirty three months later (when the semen from both animals contained many inflammatory cells) serum, preputial mucus and semen were collected from both infected bulls and a noninfected control bull (33). On palpation at this time both infected bulls were determined to have bilateral seminal vesiculitis and bull 883 had developed a unilateral epididymitis and periorchitis. The control bull was normal on palpation. Serum, semen and preputial mucus samples were collected from bull 960 again 38 months post infection, at which time he had a chronic ampullitis and seminal vesiculitis but no palpable enlargement of the epididymis.

Preputial mucus was aspirated, weighed, and extracted in 5 ml of saline at 4°C overnight after vortexing with glass beads. Then the extracts were vortexed again, centrifuged at 48,000 x g for 30 minutes and the supernatant was stored at -70°C. Semen was centrifuged at 500 x g for 20 minutes to remove cells and the supernatant stored at -70°C also.

When all the samples had been collected, they were thawed for simultaneous quantitative determinations. Immunoglobulin class and albumin levels were measured in semen and preputial mucus by radial immunodiffusion (RID) as described by Duncan et al (8). If levels were too low to detect, the samples were concentrated 10 times by ultrafiltration (Amicon Corporation, Lexington, MA) and RID tests were repeated. Mycoplasma agglutinin titers were determined by the tube test using organisms cultivated from an isolate of bull 960. The organisms were suspended in phosphate buffered saline containing 0.02% sodium azide at an OD of 1.0 at 525 nm. Serum, semen or preputial mucus extract were diluted in formalized saline. After addition of equal volumes of antigen suspension and incubation at 37°C for 48 or 72 hours, the agglutination was read by resuspension of sediments.

Results

Mycoplasmas, identified as *M. agalactiae* subsp. *bovis* by immunofluorescence, were cultured from the semen and preputial mucus of bulls 883 and 960. All samples collected from the control bull (33) were negative for *M. agalactiae* subsp. *bovis*.

Preputial mucus extract, semen, and serum *M. agalactiae* agglutinin titers are given in Table I. It is noteworthy that in this primarily local infection of the genital tract serum titers of the infected bulls were elevated but the titers in their semen were much higher. Agglutinins were not detected in any preputial mucus samples.
Table I

*M. agalactiae* subsp. *bovis* agglutinin titers in genital secretions and serum

<table>
<thead>
<tr>
<th>Bull number</th>
<th>Months post infection</th>
<th>Agglutinin titers</th>
<th>Preputial mucus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>semen</td>
<td>serum</td>
</tr>
<tr>
<td>883</td>
<td>33</td>
<td>512</td>
<td>64</td>
</tr>
<tr>
<td>960</td>
<td>33</td>
<td>2048</td>
<td>64</td>
</tr>
<tr>
<td>960</td>
<td>38</td>
<td>1024</td>
<td>ND*</td>
</tr>
<tr>
<td>33</td>
<td>--*</td>
<td>&lt;8</td>
<td>16</td>
</tr>
</tbody>
</table>

*(--), not infected; ND, not done.

Since antibody titers were high in semen and inflammatory cells were present, quantitative studies on immunoglobulin and albumin levels are reported for semen (Table II). Preputial mucus extract levels were studied also, but values for both the infected animals and the control fell within the normal range as determined in this laboratory on a separate series of bulls (P. Bier, unpublished data). Semen immunoglobulin and albumin levels, on the other hand, were clearly higher in infected animals than in the control (Table II). Levels of IgA and IgG were five to ten times higher than those in the control sample (Table II) or in a series of samples from normal bulls (P. Bier, unpublished data). There was little difference in the semen IgM levels in all 3 bulls.

Table II

Immunoglobulin and albumin levels in semen

<table>
<thead>
<tr>
<th>Bull number</th>
<th>Months post infection</th>
<th>Ig protein/ml semen plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>albumin</td>
</tr>
<tr>
<td>883</td>
<td>33</td>
<td>.56</td>
</tr>
<tr>
<td>960</td>
<td>33</td>
<td>1.10</td>
</tr>
<tr>
<td>960</td>
<td>38</td>
<td>.80</td>
</tr>
<tr>
<td>33</td>
<td>--*</td>
<td>.09</td>
</tr>
</tbody>
</table>

*(--), not infected; UD, undetectable; T, trace.
DISCUSSION

The finding of positive antibody titers in semen and serum of infected animals but not in their preputial mucus extracts suggests that the antigenic stimulus in this disease was not across the preputial epithelium. *M. agalactiae* subsp. *bovis* was cultured from preputial mucus but in a more extensive investigation of this disease negative cultural results were reported (3). Therefore, it is probable that the few mycoplasmas cultured from the preputial mucus of bulls 883 and 960 represents semen contamination rather than preputial colonization.

The presence of agglutinins in semen and serum and evidence of seminal vesiculitis on palpation suggests that the antigenic stimulation came from the infection in the upper tract. Ernø (9) and Lein (3) previously demonstrated increases in serum antibody titers in genital mycoplasmosis of bulls. In these studies semen titers were not reported so it is not known whether the local immune response was greater than the systemic response. Other workers have reported higher (10) or slightly increased levels (11) of IgA in human semen as compared with serum and have suggested that local production of IgA takes place in the male genital tract. In normal bovine semen both Mach (12) and Bier (unpublished data) found approximately equal amounts of IgA, IgG1, and IgG2. Since serum IgA levels in cattle are approximately 1 percent of the IgG levels (8,12) it could be concluded from these studies that local secretion of IgA took place in the male bovine genital tract. In the above studies neither changes in the immunoglobulin levels with infection of the upper male genital tract nor specific antibody responses in semen were investigated. The present study gives some insight into the response to infection in this area. Elevations in IgM were not detected (Table II), probably because these bulls had been infected for more than two years and IgM antibodies are known to characterize the early local (13) and systemic immune responses. The high albumin levels in semen from infected bulls (Table II) along with inflammatory cells in semen and swelling of the seminal vesicles imply that an exudative process was occurring. Therefore, serum antibody must have been entering the seminal fluid. However, the elevated IgA levels in semen from infected bulls and the high agglutinin titers in the same samples indicate that a major portion of the antibody in the semen was the result of a specific local immune response.

Detection of antibodies to *M. agalactiae* subsp. *bovis* in bulls with mycoplasmal seminal vesiculitis is of interest because the incidence of bovine genital mycoplasmosis is unknown (1). Serum antibody titrations are reported to be useful in the diagnosis of this disease in bulls (3,9). However, in the present study semen titers in infected animals were much higher than serum titers and semen titers in the control bull were lower than serum titers (Table I). Therefore, we propose that semen agglutinin titers would be more useful diagnostically than serum titers.
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REFERENCES


