Experimental Mycoplasma Mastitis in Mice

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Thirteen strains of mycoplasma representing six different species, Acholeplasma laidlawii, Mycoplasma dispar, M. bovirhinis, M. bovigenitalium, M. agalactiae subsp. bovis, and M. mycoides subsp. mycoides, were inoculated into the mammary glands of mice, and the number of mycoplasmas present in the glands three days after inoculation was determined. The predominant response included involution of inoculated glands and a neutrophil infiltration. With the exception of M. dispar, the pathogenicity of the six species for mice was found to be similar to their pathogenicity for cattle.

MATERIALS AND METHODS

Mycoplasmas. The sources of Acholeplasma laidlawii strains BM1 and 1305/68, Mycoplasma bovirhinis strains C56R and C155, M. agalactiae subsp. bovis strain NCTC 10131, and M. bovigenitalium strains M338/70 and M981/70 have been published (12) as have the sources of M. dispar strains Grl226 and 462/2 (NCTC 10126) (13). M. agalactiae subsp. bovis Ab/1 was isolated from the lung of a calf that died during an outbreak of pneumonia (18); it was purified by picking, on three occasions, single colonies grown from filtered (450-nm pore size; Millipore Corp.) broth cultures (16). Details of M. mycoides subsp. mycoides strains KH,J and Gladysdale have been reported (3, 7). The Iriri strain was isolated from an outbreak of contagious bovine pleuropneumonia in Iriri, Karamoja, Uganda in 1964.

Media. All strains for inoculation were grown at 37°C in glucose-calf serum (GS) broth containing ampicillin (2) and stored at −70°C. The number of viable organisms per milliliter was determined as color change units (17) for all the species except M. bovigenitalium and M. agalactiae subsp. bovis, which were determined as colony-forming units. For inoculation, mycoplasma cultures were diluted in 0.15 M phosphate-buffered saline, pH 7.2.

Mice. Lactating mice of the BSVS strain bred at Compton were used 4 to 7 days after parturition. The offspring were removed 1 h before inoculation.

Inoculation. The mycoplasma suspension was inoculated (0.1 ml) into mammary glands R4 and L4 of each mouse (fourth gland from anterior on right and left sides) as described by Chandler (4), and 3 days later the mice were killed. Five mice were inoculated with each strain of mycoplasma and a control group was inoculated with undiluted broth or phosphate-buffered saline containing 10% (vol/vol) or 1% (vol/vol) GS broth. At autopsy both inoculated glands were removed.

Necropsy procedures. L4 was cut in two, and one-half was fixed in 12% neutral buffered formalin and processed for histological examination. The other half of L4 and the whole of R4 were ground in GS broth with sterile sand, and the number of mycoplasmas present in each homogenate was determined as color change units or colony-forming units as appropriate. The number present per gland was then calculated. Geometric means were used throughout, and for statistical analysis the t-test was applied to logarithms to base 10 of mycoplasma numbers.

Percentage of lactation tissue, i.e., alveoli and blood vessels, was calculated from histological sections by counting the number of points on a 100-point graticule, at ×63 magnification, that fell on such tissue; 25 fields were counted for each group of five mice and an average was obtained. Comparisons were made by applying the t-test to these values.

RESULTS

In Table 1 the strains of mycoplasmas are arranged in the order indicated by the average decrease in difference between the number of organisms inoculated into the mammary gland...
and the number recovered 3 days later. Only mammary glands inoculated with M. agalactiae subsp. bovis strain Ab/1 yielded as many mycoplasmas as were inoculated. The percentage of lactation tissue and the neutrophil response in the mammary gland are also indicated for each strain in Table 1. Mammary glands inoculated with A. laidlawii or M. dispar were significantly reduced in lactation tissue when compared to control glands ($P < 0.001$), although there was no neutrophil response in the ducts and no infiltration of macrophages and plasma cells in either the perilvascular area or the interalveolar fat. No difference between the strains of both these species was revealed by recovery of mycoplasmas or by histopathological examination.

Both strains of M. bovirhinis, M. bovigenitalium strain M991/70, M. agalactiae subsp. bovis strain NCTC 10131, and M. mycoides subsp. mycoides strains Iriri and Gladysdale induced histopathological changes in the mammary gland that were similar in appearance, despite differences in the numbers of mycoplasmas recovered. There was a reduction in lactation tissue relative to control mammary glands, and there were neutrophils in the larger alveoli and ducts; most of these neutrophils showed pyknotic changes. Few inflammatory cells were seen in the interalveolar fat, but there was a mild infiltration of mononuclear cells around the involuted alveoli and degenerative changes in the epithelium of the alveoli and ducts. The involution changes, as indicated by the percentage of lactation tissue, in the glands infected with strain M338/70 of M. bovigenitalium were not significantly greater than those in the control group ($0.2 > P > 0.1$), although the number of mycoplasmas recovered was comparable to that from glands inoculated with strain M991/70. The number of M. mycoides subsp. mycoides strain KH$_{10}$J recovered was low, the neutrophil response was mild, and involution changes were only slightly greater than in control glands ($0.050 > P > 0.025$).

A greater number of M. agalactiae subsp. bovis strain Ab/1 were recovered from inoculated glands than of strain NCTC 10131 ($0.025 > P > 0.010$), and the histopathological changes were more severe, although there was no difference in involution caused by the two strains ($0.5 > P > 0.4$). The neutrophil response in the ducts of glands inoculated with strain Ab/1 was intense and most of the cells were pyknotic.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Inoculum per gland (log$_{10}$)</th>
<th>No.$^b$ recovered per gland (log$_{10}$)</th>
<th>Difference between inoculum and no. recovered per gland (log$_{10}$)</th>
<th>% Lactation tissue</th>
<th>Neutrophil response$^c$</th>
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<tr>
<td>A. laidlawii</td>
<td>1305/68</td>
<td>8.0</td>
<td>&lt;1</td>
<td>&gt;7</td>
<td>45</td>
<td>-</td>
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<td>A. laidlawii</td>
<td>BN1</td>
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<td>&lt;1</td>
<td>&gt;6</td>
<td>49</td>
<td>-</td>
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<td>KH$_{10}$J</td>
<td>8.6</td>
<td>3.5</td>
<td>5.1</td>
<td>52</td>
<td>+</td>
</tr>
<tr>
<td>M. dispar</td>
<td>462/2</td>
<td>6.0</td>
<td>1.6</td>
<td>4.4</td>
<td>44</td>
<td>-</td>
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<td>M. bovirhinis</td>
<td>C56R</td>
<td>7.0</td>
<td>2.6</td>
<td>4.4</td>
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<td>+++</td>
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<td>2.3</td>
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<td>2.7</td>
<td>3.3</td>
<td>44</td>
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<td>5.2</td>
<td>1.6</td>
<td>44</td>
<td>+++</td>
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<td>6.5</td>
<td>0.5</td>
<td>44</td>
<td>+++</td>
</tr>
<tr>
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<td>45</td>
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<td>6.7</td>
<td>0.3</td>
<td>68</td>
<td>++</td>
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<td>6.4</td>
<td>+0.2</td>
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<td>++++</td>
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<tr>
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<td></td>
<td></td>
<td>74</td>
<td>-</td>
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$^a$ The mycoplasmas are arranged in the order indicated by the decreasing difference between number of organisms inoculated into the mammary gland and the number recovered after 3 days, with associated histopathological changes.

$^b$ Geometric mean of 10 glands from 5 mice.

$^c$ + , Neutrophil response, majority morphologically normal; +++, intense neutrophil response, majority morphologically normal; ++++, neutrophil response, majority pyknotic; ++++, intense neutrophil response, majority pyknotic.
Degenerative changes in the alveolar epithelium together with reduction in alveolar diameter gave the epithelium a hyperplastic appearance. In areas where there was perialveolar cellular infiltration, the alveolar architecture was indistinct, although there was little fibrosis (Fig. 1a).

A neutrophil response was not observed in the mammary glands inoculated with GS broth or 10 or 1% GS broth in phosphate-buffered saline, and 74% of the gland was lactation tissue with a normal epithelium (Fig. 1b).

**DISCUSSION**

In natural outbreaks of mycoplasma mastitis in cattle there is a marked reduction in milk production and an elevated milk cell count, and mycoplasmas can be isolated from the milk (14).

There were three significant consequences of intramammary inoculation of mycoplasmas in mice. First, all the strains of mycoplasma, except *M. bovigenitalium* strain M338/70, caused involution of the gland when compared with uninfected controls and this included glands inoculated with *A. laidlawii* and *M. dispar*, although with these two species none or very few mycoplasmas were recovered. Second, intramammary inoculation of mycoplasmas caused a neutrophil response in the larger alveoli and ducts; this was seen in response to all the species except *A. laidlawii* and *M. dispar*. It was observed that, as in infection of the mouse mammary gland by ureaplasmals (11) and in contrast to staphylococcal infection (1), the neutrophil response occurred in the absence of abscess formation or of an intense interalveolar cellular infiltration with fibrosis. Third, with the exception of *A. laidlawii*, mycoplasmas of all the strains were recovered from the mammary glands 3 days after inoculation. The experimental design did not allow a distinction between multiplication and persistence of the inoculated mycoplasmas nor did it indicate whether multiplication was necessary for induction of the pathological response observed. In both bacterial and ureaplasmal mastitis in mice multiplication has been demonstrated (1, 11), and in the present study the failure of *A. laidlawii* to persist and the wide range in the numbers of other mycoplasmas recovered indicated that the mammary gland did not merely allow persistence of mycoplasma but that survival depended on properties of the myco-

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**Fig. 1.** (a) Mammary gland of mouse 3 days after inoculation of *M. agalactiae* subsp. *bovis* strain Abil. The alveolar structure is lost due to involution and perialveolar cell infiltration, and the duct (D) contains cell debris. Giemsa, ×200. (b) Mammary gland of mouse 3 days after inoculation of GS broth. The alveoli are large and there are no cells in the duct (D). Giemsa, ×200.
plasma that allowed colonization despite host defense mechanisms. The more fastidious nature and relatively slow growth rate of *M. dispar* (9) may have resulted in this species being less pathogenic for mice over 3 days than for cattle over a longer period (J. Brownlie, C. J. Howard, and R. N. Gourlay, Res. Vet. Sci., in press). Some or all strains of the remaining species, *M. bovirhinis, M. bovigenitalium, M. agalactiae* subsp. *bovis*, and *M. mycoides* subsp. *mycoides*, were found to be pathogenic for mice and were recovered from the mammary gland. Thus, with the exception of *M. dispar*, the pathogenicity of the six species of mycoplasma for mice approximated that for cattle (6, 8; Brownlie et al., Res. Vet. Sci., in press).

No histopathological evidence was found for there being any major differences in the mechanism of disease production with different mycoplasma species, although in cattle, at least, the lung lesion produced by *M. mycoides* subsp. *mycoides* is characteristic. This lack of differences between mycoplasma species may be due to the short duration of infection in mice and because of the limited number of ways in which the mammary gland can respond to infection. When comparisons were made within a species, differences in the virulence of strains were detected. *M. agalactiae* subsp. *bovis* strain NCTC 10131 did not multiply to the same extent or cause such a severe neutrophil response as strain Ab/1, which is a recent isolate, and there were even more marked differences in virulence between the avirulent vaccine strain KH₀₂J and the highly virulent Gladsdale strain of *M. mycoides* subsp. *mycoides*, which paralleled their virulence for cattle.

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**LITERATURE CITED**


