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The effect of an intramammary infusion of endotoxin on experimentally induced mycoplasmal mastitis

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SUMMARY

The infusion of 10 μg of endotoxin lipopolysaccharide from *Escherichia coli* into the mammary gland of four cows 16 h before inoculation with ureaplasmas did not prevent, or even diminish, the subsequent ureaplasmal mastitis. There was no reduction in the severity or duration of the inflammatory cell response in milk or in the clinical appearance of the resulting mastitis. Also, the excretion of ureaplasmas was not reduced.

A similar experiment with *Mycoplasma dispar* in two cows demonstrated that endotoxin was again ineffective in preventing the mastitis. Furthermore, there was some indication that the proliferation and excretion of this mycoplasma was enhanced in endotoxin-treated quarters.

INTRODUCTION

Intramammary infusion of endotoxin from *Escherichia coli* has been reported to cure chronic *Pseudomonas aeruginosa* mastitis in cattle (Schalm & Ziv-Silberman, 1968). Furthermore, the toxic effect observed with high doses of endotoxin can be reduced by using microgram amounts, which still retain their effectiveness to prevent bacterial mastitis (Brownlie, 1979). The *in vivo* action of endotoxin is complex but a characteristic effect of intramammary infusions is the massive neutrophilia in milk (Schalm & Ziv-Silberman, 1968; Brownlie, 1971). These cells, being phagocytic, are generally reported to have a protective effect (Blobel & Katsube, 1964; Derbyshire, 1964) and the presence of 'digested' bacteria intracellularly in milk neutrophils has been recorded (Paape & Wergin, 1977). As we have previously reported on the pathogenicity of various mycoplasmas in the bovine udder (Gourlay, Howard & Brownlie, 1972; Brownlie, Howard & Gourlay, 1976), we have examined, in this paper, the effect of endotoxin infusion in preventing ureaplasma and *Mycoplasma dispar* mastitis.

MATERIALS AND METHODS

Animals

The four cattle used were 3–5-year-old Friesians in the first 8–12 weeks of their second to fourth lactation. They were housed in isolation units and fed a con-

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ventional diet of hay, concentrates and water. They had no history of mastitis and their milk cell counts were below 100000 cells/ml, of which the neutrophils were less than 10%. No obvious pathogenic bacteria or mycoplasmas were isolated from milk samples that had been taken with full hygienic precautions.

Mycoplasmas

Two species of mycoplasma were used. The *Ureaplasma* sp., strain Vic 9, had previously been shown to induce mastitis in cows (Howard, Gourlay & Brownlie, 1973) and likewise the *M. dispar* strain Gri 226 (Brownlie *et al.* 1976). The ureaplasma was grown in urea-broth (Howard *et al.* 1975) and *M. dispar* in glucose-serum broth (Gourlay & Leach, 1970) containing ampicillin (Andrews *et al.* 1973). The number of mycoplasmas per ml of milk was determined as colour change units (c.c.u.) per ml as previously reported (Gourlay *et al.* 1972; Brownlie *et al.* 1976). In all cows, the number of mycoplasmas inoculated into each quarter was 10^6 for the ureaplasma or 10^8 for *M. dispar*.

Endotoxin

Ten μg lipopolysaccharide from the endotoxin of *Escherichia coli* O55:B5 (Difco Laboratories, Detroit, U.S.A.) was dissolved in 20 ml of pyrogen-free distilled water and infused by a sterile cannula through the teat canal of individual quarters.

Cell counting

Total and differential cell counts were made on milk smears fixed and stained by the 'single dip' technique of Broadhurst & Paley (1939).

Production of experimental mastitis

The experimental design was the same in all cattle. Two quarters, the right fore and right hind, were infused with endotoxin after completion of the afternoon milking. After the next morning's milking, 16 h after endotoxin treatment, either ureaplasmas or *Mycoplasma dispar* were infused into the left and right hind quarters. The left fore quarter acted as an uninoculated control, having had neither endotoxin nor mycoplasmas.

Fore-milk samples were taken, with full sterile precautions, from all quarters at the morning and afternoon milking. Sampling started 2 days before endotoxin treatment and continued for the course of the infection. Total bacteria present in the milk were counted on ox-blood agar plates but in all experiments they were $< 10^2$/ml.

RESULTS

Ureaplasma – *Vic* 9

There was an inflammatory response in all the quarters inoculated with ureaplasmas and they were reisolated in the milk for 7 to at least 90 days after inoculation (Table 1). Clinical evidence of mastitis was observed in three of the four inoculated quarters (Table 2); in one case, the mastitis had not resolved after

Table 1. *The isolation of mycoplasmas from milk following intramammary inoculation of cow's milk with endotoxin and either* Ureaplasma *sp. or* Mycoplasma dispar

Cow no.	Mycoplasmas inoculated	Days that mycoplasmas were isolated from quarters of the mammary gland inoculated with: Control	Endotoxin	Endotoxin* and mycoplasma	Mycoplasma
106	*Ureaplasma* sp.	0	0	6 (6·0)†	7 (6·7)
117‡	*Ureaplasma* sp.	0	0	90 (6·7)	90 (6·7)
182	*Ureaplasma* sp.	0	0	23 (6·0)	23 (5·0)
189	*Ureaplasma* sp.	0	0	8 (5·7)	8 (5·7)
106	*M. dispar*	0	0	15 (8·0)	13 (8·0)
182	*M. dispar*	0	0	15 (5·0)	5 (2·7)

* 10 μg *Escherichia coli* endotoxin lipopolysaccharide was injected 16 h before mycoplasma inoculation.

† ( ) Number of mycoplasmas (10^n), isolated at peak response.

‡ This cow was culled at 90 days as the mycoplasma mastitis had not resolved.

Table 2. *The cellular response in milk following intramammary inoculation of endotoxin and either* Ureaplasma *sp. or* Mycoplasma dispar

Cow no.	Mycoplasmas inoculated	Days with 10^6 cells per ml milk from quarters of the mammary gland treated with: Control	Endotoxin	Endotoxin* and mycoplasma	Mycoplasma
106	*Ureaplasma* sp.	0	4 (6·7)†	19 (> 8·0)c	27 (7·4)c
117‡	*Ureaplasma* sp.	0	2 (6·5)	90 (> 8·0)c	90 (> 8·0)c
182	*Ureaplasma* sp.	0	6 (7·6)	27 (7·3)	20 (7·3)
189	*Ureaplasma* sp.	0	4 (7·3)	14 (> 8·0)c	12 (7·1)y
106	*M. dispar*	1	5 (6·7)	20 (> 8·0)c	20 (> 8·0)c
182	*M. dispar*	0	6 (7·1)	8 (7·0)	7 (7·1)

c = clots in milk. y = milk yellow.

* 10 μg *Escherichia coli* endotoxin lipopolysaccharide was injected 16 h before mycoplasma inoculation.

† ( ) No. cells (10^n) per ml of milk at peak of cell response.

‡ This cow was culled at 90 days as the mycoplasma mastitis had not resolved.

90 days and the cow, No. 117, had to be culled, despite treatment by two courses of intramammary terramycin.

In those quarters receiving only endotoxin there was a transitory cell response lasting 2–6 days (Table 2) but no gross changes in the milk were observed.

The quarters treated with endotoxin 16 h before ureaplasma inoculation, responded similarly to quarters inoculated with ureaplasmas alone. The duration of the cell responses and the ureaplasma excretion corresponded closely, although there was an indication that the mastitis in endotoxin-treated quarters was marginally more severe.

Mycoplasma dispar – *Gri* 226

Both cows inoculated with *Mycoplasma dispar* became infected. The inflammatory cell response in milk was not affected by endotoxin treatment (Table 2) but the duration of mycoplasma excretion was three times as long in the endotoxin-treated as in the untreated quarters of cow 182. Similarly, in cow 106 the excretion of mycoplasmas stopped after 8 days although a further single isolation was recorded at 13 days (Table 1). These results demonstrate that endotoxin treatment does not diminish and may even enhance *M. dispar* infection.

DISCUSSION

Microgram doses of endotoxin can prevent experimental bacterial mastitis (Brownlie, 1979) and therefore the possible presence of a similar protective effect against mycoplasmal mastitis was examined, particularly as mycoplasmal mastitis often responds poorly to antibiotics (Jasper, Bushnell & Dellinger, 1974). However, the experiments reported here have shown that pre-treating the mammary gland with endotoxin did not prevent ureaplasma mastitis. The mastitis produced by the ureaplasma strain – Vic 9, was similar to the experimental disease already reported (Howard *et al.* 1973).

In the two cows infected with *M. dispar* the endotoxin again had no effect on reducing the cellular response (Table 2). Moreover, it may have enhanced the duration and titre of mycoplasma excretion.

It is interesting to recall a previous observation (Brownlie *et al.* 1976) of a pre-existing leucocytosis, which had been noted retrospectively and not induced experimentally; this actually enhanced a *M. dispar* mastitis. Jain, Jasper & Dellinger (1969) also noted that a leucocytosis in milk did not protect against *M. bovis* mastitis. These observations and the present results suggest that a bacterial mastitis and the resulting leucocytosis could increase susceptibility to mycoplasma mastitis. The neutrophils, which comprise over 90% of the cells in a sterile leucocytosis of the mammary gland, were shown not to kill *M. dispar* or *M. bovis* unless specific antibody was present (Howard *et al.* 1976).

Other *in vitro* studies on normal bovine milk and whey demonstrated a dialysable factor capable of killing a variety of mycoplasma species, including ureaplasma and *M. dispar* (Brownlie, Howard & Gourlay, 1974; Howard *et al.* 1975). However, this activity was considerably reduced following inoculation of endotoxin into the mammary gland (Brownlie *et al.* 1974). In that paper, we suggested that there are differences between the bactericidal and mycoplasmacidal systems and the present *in vivo* studies confirm this suggestion.

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