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Selective Adhesion of Microorganisms to the Ductular Epithelium of the Bovine Mammary Gland

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Streptococcus agalactiae, Streptococcus faecalis, Staphylococcus aureus, Escherichia coli, and *Corynebacterium bovis* were examined for their ability to adhere to the ductular epithelial cells of the bovine udder. *S. agalactiae* and *S. aureus* adhered readily and in large numbers, whereas the other organisms adhered poorly or not at all. The organisms showing the ability to adhere are those which frequently cause mastitis. These data suggest that selective adherence to the ductular epithelium may be the first stage in the pathogenesis of streptococcal and staphylococcal mastitis.

There is now evidence that selective attachment to mucosal cells is critical in determining the distribution of some bacteria in the mouth and gastrointestinal tract (4, 7). It becomes reasonable to suggest that selective adhesion is the first stage in the pathogenesis of infections gaining access to the body via mucosal or other surfaces. One such disease currently under intensive investigation, and in which selective attachment appears to be the first stage in pathogenesis, is gonorrhoea (8, 9). This paper describes preliminary experiments that show the ability of mammary gland pathogens to adhere to the cells of the udder ductular epithelium.

MATERIALS AND METHODS

Cells. The udder was removed intact from freshly slaughtered lactating cows. Within 2 h the skin was removed and the lactiferous sinus was opened. Excess milk was rinsed off with 0.01 M phosphate-buffered saline (PBS). Major lactiferous ducts were gently brushed with a fine pipette brush (4 by 0.5 cm), and the cells removed in this way were gently suspended in PBS in a volume of approximately 10 ml. Cells were washed three times and resuspended to approximately 10^5 cells per ml in PBS.

Organisms. Freshly isolated strains were grown in Todd-Hewitt broth for 20 h. Their origins were: *Staphylococcus aureus* and *Streptococcus agalactiae* from subclinical mastitis; *Corynebacterium bovis* from normal milk; and *Streptococcus faecalis* and *Escherichia coli* from bovine feces. Cultures were then centrifuged, washed once in PBS, and resuspended to 10^7 organisms per ml.

Adherence of organisms to ductular epithelial cells. Aliquots of 1 ml each of epithelial cell suspension and culture were mixed in test tubes and rotated at 37 C for 1 h to allow contact between organisms and cells. The suspension was then centrifuged and unattached organisms were removed by washing three times with PBS. Smears were made, air dried, and stained with Gram crystal violet for 15 to 30 s. Some preparations were examined unstained by phase-contrast microscopy. The number of organisms attaching to the cells was estimated by counting the number attached to 50 cells. Control samples were incubated with buffer instead of bacteria.

Attachment of S. aureus and S. agalactiae to the ductular epithelium in vivo. Lactating cows destined for slaughter after experimental surgery were used. Quarters selected were shown free of pathogens by culture of milk 2 days before and confirmed at the time of inoculation. The quarters were milked out, and as much of the remaining secretion as possible was washed out with PBS. Then 10 ml of washed organisms prepared as described above was infused and the udder was massaged gently to facili-

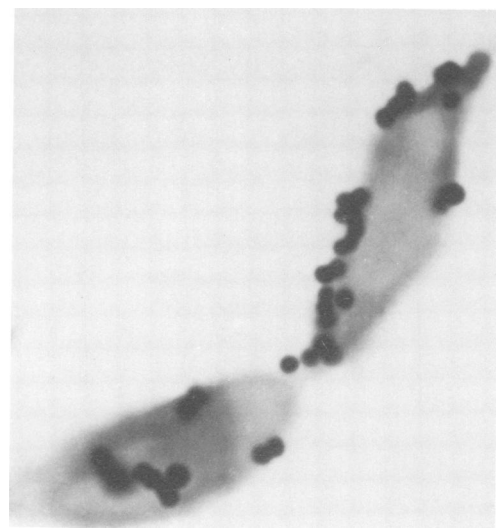

FIG. 1. *S. aureus adhering to ductular epithelial cells from the bovine udder after incubation for 1 h. Mixture contained 10^5 epithelial cells and 10^7 bacteria. Crystal violet stain.*

tate distribution into the ductular area. After 1 h the cow was destroyed, the udder was removed, and the cells were collected, washed, and examined as described above.

RESULTS

The attachment of *S. agalactiae* and *S. aureus* was significant (Fig. 1). When viewed under phase contrast, careful focusing on the different cell surfaces was necessary, as the cells were often encased in bacteria. This was most obvious with *S. agalactiae,* where chains of cocci would surround the cell (Fig. 2).

The relative attachment of the organisms used is shown in Table 1. In some preparations, cells were detached in groups or sheets making the attachment less per cell, and whenever possible, organisms attaching to single cells were counted. The absence of background, unattached organisms was considered evidence of successful removal of unattached cells. Control preparations showed only an occasional organism attaching to cells.

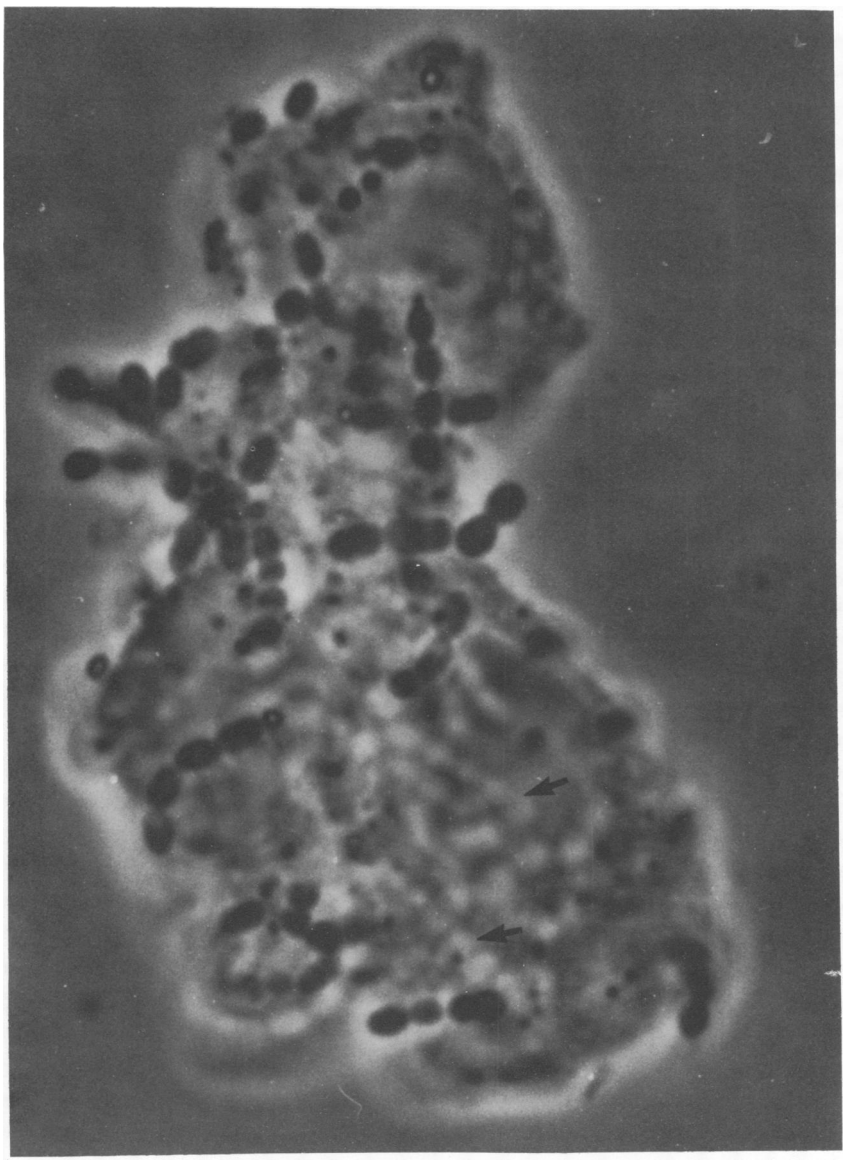

FIG. 2. *S. agalactiae adhering to an epithelial cell, prepared as in Fig. 1, except that the mixture contained 10^5 eptihelial epithelial cells and approximately 10^8 bacteria. One of a series of exposures focused at different planes. Arrows indicate chains of cocci not in focus at this plane. Phase-contrast microscopy.*

TABLE 1. *Relative attachment of organisms to cells of the ductular epithelium of the udder*

Organism	Total no. of organisms attaching to 50 cells[a]
Staphylococcus aureus	511
Streptococcus agalactiae	443
Streptococcus faecalis	2
Escherichia coli	7
Corynebacterium bovis	7

[a] Average of six similar experiments.

Two strains of *S. aureus*, two of *S. agalactiae*, and two of *E. coli* were used, with results similar to those in Table 1.

Attachment of *S. agalactiae* and *S. aureus* were also demonstrated in vivo, although the number of organisms per cell (average of five and six, respectively), was lower than seen in the in vitro preparations.

DISCUSSION

These preliminary experiments throw considerable light on the nature of mammary gland infection. *S. agalactiae*, *S. aureus*, micrococci, and *C. bovis* are the most common organisms isolated from the bovine udder (2). *S. agalactiae* and *S. aureus* are the main pathogens, and the distribution of the lesions in diseased quarters suggests spread via the teat canal and the ductular tissue. Micrococci are in general weakly pathogenic, inducing only minor microscopic lesions in the lower parts of the gland (6). *C. bovis* is considered nonpathogenic for the udder and there is some evidence that it is confined to the teat canal (1). It did not adhere to the ductular epithelium as well as did the udder pathogens; preliminary observations suggest it adheres better to cells lining the teat sinus than to the ductular epithelium.

It has been postulated that the chronic, progressive form of staphylococcal mastitis may be explained by a persistent focus of infection, usually in the lower part of the gland, resulting in the natural flow of infected milk into the large ducts or to the lactiferous sinus. This is followed by mechanical transfer of the organisms to other ducts and hence to other sections of the gland (3). Adherence to the ductular epithelium, as demonstrated in these studies, would be a likely mechanism for localization after this intramammary transmission, and would prevent the flushing away of the organisms during the milking process.

E. coli and *S. faecalis* are common organisms which readily contaminate udder and teats and presumably gain ready access to the interior of the udder. Since specific attachment to the intestinal mucosa is an important characteristic of some enteropathogenic *E. coli* in piglets (5), a similar property may be associated with those *E. coli* strains which cause mastitis. The strains of *E. coli* that failed to attach to the epithelial cells in this study were freshly isolated from feces and may not represent the characteristics of udder pathogenic *E. coli*.

It therefore seems likely that the first stage in pathogenesis of bovine mastitis, at least with *S. agalactiae* and *S. aureus*, is selective tissue attachment to the cells of the ductular epithelium; subsequent events would depend on factors such as the virulence of the organism and the host defense mechanisms.

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