Chemotactic Effect of *Staphylococcus aureus* on Neutrophils Isolated from the Bovine Mammary Gland

**Abstract**

*Staphylococcus aureus* suspended in milk from mammary glands pretreated with endotoxin were chemotactic for neutrophils from the same quarters. The milk or the organisms individually had little effect.

**Introduction**

There is an increased influx of neutrophils from the blood into the mammary gland during inflammation (7). Phagocytosis by neutrophils both in vivo and in vitro has been demonstrated and shown to restrict multiplication of certain pathogens infecting the mammary gland (4, 5, 12). The phagocytic activity of neutrophils from bovine mammary glands has been studied, but the mechanisms involved in their infiltration into the gland are yet to be explored. The directional movement of neutrophils up a chemical concentration gradient towards a “target” is referred to as chemotaxis (8-10). Since bovine mastitis is usually bacterial in origin and since certain bacteria can induce chemotaxis (9), it was of interest to study chemotaxis in relation to mastitis with a common pathogen, *Staphylococcus aureus*.

**Materials and Methods**

A Boyden apparatus as modified by Comely (3) was used. The apparatus contained two chambers separated by a 3.0-µm millipore filter. Neutrophils were obtained from a quarter previously inoculated with *Escherichia coli* endotoxin, and washed three times in 199 tissue culture fluid. *Staphylococcus aureus* was isolated from a natural case of bovine mastitis. The organism was classified as an α and β toxin producer by bovine blood agar plates according to the method of Jasper and Jain (6), and as Group III phage type 7 according to the nomenclature established by the World Health Organization (1). It was cultured in beef heart infusion broth for 24 hours and washed three times in 199 tissue culture fluid before use. A known number of neutrophils was put into the top chamber and the solutions under test were put into the bottom chamber. After 3 hours of incubation at 37°C the filter was removed, fixed, and stained using the method described by Boyden (2). The filter was mounted on a glass slide so that the side previously in contact with the test solutions was facing upward. Neutrophils appearing on this side of the filter were considered to have responded to the chemotactic stimulus. The number of neutrophils in 20 microscopic fields on each filter was counted.

**Results and Discussion**

From the results presented in Table 1, it can be seen that milk from the endotoxin-treated quarter in the presence of *Staphylococcus aureus* exhibited a chemotactic stimulus for neutrophils, whereas normal milk from the same quarter collected before treatment with endotoxin and *Staphylococcus aureus* alone had little effect. Therefore, it appeared that the stimulus was the result of an interaction between the staphylococci and a system present in the milk from the treated quarter and to a much lesser extent in milk from the normal quarter. It also appeared that the system supplied by the milk from the treated quarter was partially destroyed by heating at 56°C for 30 minutes. Others have shown that antibody antigen aggregates in the presence of complement (11, 15, 16), trimolecular complexes of C'5, C'6, and C'7 complement components (15, 16), and cleavage products of C'5 and C'3 (13, 14) are chemotactic for neutrophils. Thermal susceptibility in this test system suggests a role for complement; however, it is yet to be determined what factors are involved in the system supplied by milk from the endotoxin-treated quarter. Further work on the characterization of the chemotactic factor in this milk is in progress.

**Summary**

*Staphylococcus aureus* in milk from an endotoxin-treated quarter exhibited a marked chemotactic stimulus for bovine neutrophils. The bacterium or the milk from the treated quarter tested individually had little effect. The organisms suspended in normal milk had

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1 Millipore Corporation, Bedford, Massachusetts.
2 Lipopolysaccharide B. *E. coli* 026: B6. Commercial preparation obtained from Difeo Laboratories, Detroit, Michigan. This endotoxin is prepared by trichloroacetic acid Bolvin method according to the procedure of Webster et al. J. Immunol., 74: 455 (1955).
TABLE 1. Chemotactic activity of different systems in the presence or absence of *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Series number</th>
<th>Substances tested for chemotactic power</th>
<th>Number of neutrophils per 20 microscopic fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal milk, centrifuged at 3,000 rpm for 10 minutes and fat removed.</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Milk from endotoxin-treated quarter centrifuged at 3,000 rpm for 10 minutes and fat removed.</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td><em>Staphylococcus aureus</em> suspended in 199 Tissue Culture fluid.</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>199 Tissue Culture medium.</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Normal milk centrifuged at 3,000 rpm for 10 minutes plus $2 \times 10^6$ <em>S. aureus</em> bacteria per milliliter.</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>Milk from endotoxin-treated quarter centrifuged at 3,000 rpm for 10 minutes plus $2 \times 10^6$ <em>S. aureus</em> bacteria per milliliter.</td>
<td>215</td>
</tr>
<tr>
<td>7</td>
<td>Milk from endotoxin-treated quarter centrifuged at 3,000 rpm for 10 minutes and heated at 56 C for 30 minutes plus $2 \times 10^6$ <em>S. aureus</em> bacteria per milliliter.</td>
<td>114</td>
</tr>
<tr>
<td>8</td>
<td>Milk from endotoxin-treated quarter centrifuged at 3,000 rpm for 10 minutes plus $2 \times 10^6$ <em>S. aureus</em> bacteria incubated at 37 C for 30 minutes and heated at 56 C for 30 minutes.</td>
<td>258</td>
</tr>
</tbody>
</table>

* These results represent the means of duplicate determinations from one experiment on one cow. They are in agreement with results obtained in other experiments, details of which will be presented at a later date.

Little chemotactic activity. Milk from the endotoxin-treated quarter heated to 56 C for 30 minutes before inoculation with the organisms showed a decreased chemotactic effect.

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References

Liver Lipids of Lactating Bovine: Fatty Acid Composition

Abstract

The quantity and composition of the hepatic lipids of two groups of lactating cows, maintained on low-protein, low-energy, and high-protein diets were compared in the initial and sixth week of lactation. The lipid of livers from animals on the inferior diet was almost twice that of animals on the high-protein diet in the first week of lactation. However, within six weeks the lipids of livers from both groups of cows were comparable. The fatty acid analyses revealed that the lipids from cows on the low-protein, low-energy diets contained higher quantities of saturated and short-chain fatty acids than from cows maintained on the high-protein corn diet.

Introduction

Despite the knowledge that the liver performs a vital role in mammalian lipid metabolism, surprisingly little is known about the regulatory role of the bovine liver in relation to milk lipid synthesis. It is now well established that the triglycerides and possibly other lipids associated with the low-density lipoproteins serve as the precursors of significant quantities of the milk fat produced by the ruminant (3). It is assumed that this lipoprotein complex is elaborated mostly in the liver, as in other animals (12, 13). The ability of orotic acid to depress ruminant serum lipoproteins and the alterations of lipoproteins during ketosis corroborate this assumption (5, 9). Hence, it seems plausible to suggest that the liver can significantly influence the lipids supplied to the mammary gland and that the role of the liver, in turn, may be regulated by the appropriate quantity and quality of available lipids.

During a study of the effects of diets on bovine liver enzymes and their relationship to the ketogenic state, the fat content and fatty acid composition of liver samples was also examined. Results of the analyses of the liver samples obtained from two groups of lactating cows are presented in this paper.

Methods

The cows in Group A, comprising six animals, had received only hay during pregnancy and into the first week of lactation. Following the first biopsy these animals were given hay ad libitum and 1 lb of grain (15% protein) per 5 lb of milk produced. Group B (four animals) were receiving 9.1 kg of hay and grain (24% protein) ad libitum at calving and these animals were supplied with hay and grain ad libitum after calving. Liver samples were obtained by biopsy in the first and sixth week of lactation (1). Samples of the livers, each weighing approximately 1 g, were finely minced with surgical scissors and the lipids extracted by the method of Folch et al. (2). The extracted lipids were analyzed by thin-layer and gas-liquid chromatography (6, 7).

Results and Discussion

The quantity of total lipid in the livers of the various animals showed considerable variation. However, the animals in Group A possessed greater quantities of lipid than those in Group B, when the first biopsy was obtained (Week 1). The higher lipid content in Group A livers may have been related to their protein-energy deficient diets. Conceivably, such a dietary state limited the availability of the appropriate apoproteins required to transport the intracellular lipids from the liver to the adipose and mammary tissue as lipoprotein complexes. Significantly, the animals in Group A possessed high ketone and low blood glucose titers (1) indicative of a ketotic state at the sampling period. An accumulation of lipids in the bovine liver is usually associated with the ketotic condition and McCarthy et al. (9) have stated that the lipid transport system is se-