Effects of Staphylococcus aureus on Bovine Mononuclear Leukocyte Proliferation and Viability: 
Modulation by Phagocytic Leukocytes

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ABSTRACT
In vitro effects of killed Staphylococcus aureus cells on bovine blood mononuclear leukocytes from uninfected cows or cows with chronic staphylococcal mastitis were assessed using a lymphocyte proliferation assay and a [51Cr] release cytotoxicity assay. Killed S. aureus cells cultured with mononuclear leukocytes caused a concentration-dependent decrease in lymphocyte proliferation that was associated with a commensurate decrease in mononuclear leukocyte viability. Responses of mononuclear leukocytes from uninfected and infected cows to killed S. aureus were similar, indicating effects were independent of the infection status of the animal. Addition of blood polymorphonuclear leukocytes to blood mononuclear leukocyte cultures without S. aureus cells did not alter mononuclear leukocyte viability but suppressed lymphocyte proliferation at the highest polymorphonuclear leukocyte:mononuclear leukocyte ratios (4:1 and 8:1) tested. When S. aureus cells and polymorphonuclear leukocytes were cultured with mononuclear leukocytes, both blood and milk polymorphonuclear leukocytes protected against the loss of viability compared with leukocytes cultured with S. aureus cells alone but did not consistently restore proliferative responses of the lymphocytes. These observations demonstrate that lymphocyte proliferation and mononuclear leukocyte viability are detrimentally affected by S. aureus cells, an effect that can be modulated by blood or milk polymorphonuclear leukocytes.

INTRODUCTION
Chronic staphylococcal mastitis in dairy cows is characterized by viable bacteria in the milk and elevated leukocytes, predominantly polymorphonuclear leukocytes (PMNL) in mammary tissue and milk. The early development of staphylococcal mastitis depends on the interaction between invading bacteria and milk PMNL. Studies of chronic mastitis have demonstrated several defects in the phagocytic function of milk PMNL including indiscriminate ingestion of casein and fat, reduced effectiveness of protein-mediated and peroxidase-mediated bactericidal systems, reduced glycogen reserves, and lower phagocytic indices (1).

The role of the lymphocyte in the development of chronic staphylococcal mastitis is poorly defined. Targowski and Berman (12) demonstrated that the leukocytic response to the bovine mammary gland to infused staphylococcal antigens is determined by previous exposure to antigen. They suggested that during chronic staphylococcal mastitis the continual presence of staphylococcal antigens in the udder promote lymphocyte-mediated immune responses to these antigens. Quantitative cytological examination of bovine mammary parenchyma experimentally infected with S. aureus indicated lymphocytes were the predominant infiltrating cell type in infected tissue (7). Plasma cells were prevalent in infected stroma and became more numerous as infection progressed. Milk lymphocytes from glands chronically infected with staphylococci had reduced proliferative responses to mitogens compared...

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with lymphocytes from uninfected glands and peripheral blood, suggesting in vivo lymphocyte function is compromised by infection (8). Milk mononuclear leukocyte (MNL) populations from chronically infected glands produced a soluble factor(s), which suppressed in vitro proliferation of normally responsive populations of lymphocytes (5). Other factors that might contribute to the hyporesponsiveness of milk lymphocytes are the viable bacteria and elevated number of PMNL present in infected glands. Staphylococci produce several factors that affect leukocyte viability and function (6, 13). The myeloperoxidase-\( \text{H}_2\text{O}_2\)-halide system of activated PMNL is cytotoxic for human MNL (3). Human lymphocyte mitogenesis is also suppressed by reactive oxygen species produced by activated PMNL (16).

The present study evaluated the in vitro effects of killed \textit{S. aureus} cells on the proliferation and viability of peripheral blood MNL from uninfected cows and cows with chronic \textit{S. aureus} mastitis and whether these effects could be modulated by autologous blood or milk PMNL.

**MATERIALS AND METHODS**

**Animals**

Six multiparous lactating Holstein-Friesian cows from the National Animal Disease Center dairy herd were used. Chronic mastitis was established in a single gland of each of three animals by infusion of 2000 cfu of \textit{S. aureus} (ATCC #27940, a strain isolated from bovine mastitis) into a single gland during the 1st mo of the second lactation. Infected animals were in their fourth lactation during the period of study. The infection status of individual glands was determined by culturing quarter milk samples weekly during the study period. Viable staphylococci were present in milk from infected glands from the establishment of infection to the conclusion of the study. Three age-matched cows not experimentally infected and free of naturally occurring mastitis served as controls.

**Isolation of Polymorphonuclear and Mononuclear Leukocytes**

Blood PMNL were isolated by a modification of a previously described method (2). Briefly, 200 ml of peripheral blood were collected into acid-citrate dextrose solution and centrifuged (1000 x g for 20 min). The plasma layer was discarded, and 5 ml of buffy coat layer removed, diluted in 25 ml of phosphate-buffered saline solution (PBS, \( \text{pH} 7.2\)), containing 100 units/ml penicillin G, 100 \( \mu \text{g/ml} \) streptomycin), and used for MNL recovery described herein. The remaining red cell pack was reduced to 50 ml and then subjected to two hypotonic lysis steps to remove erythrocytes. Pelleted leukocytes were resuspended in PBS, washed (250 x g for 10 min), and subsequently resuspended in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10 mM HEPES buffer, 2 mM L-glutamine, and antibiotics as described. This procedure yielded a cell population of 90 to 95% PMNL with viability >95%.

Milk PMNL were recovered from 750-ml milk samples from each infected gland. Milk samples were centrifuged (400 x g for 20 min), the pelleted cells resuspended in 150 ml (3 x 50 ml tubes) PBS, and washed (250 x g for 10 min). Washed cells were resuspended to a minimum volume of 50 ml from which 12 ml volume aliquots were layered over 3 ml of Percoll (1.084 specific gravity) (Pharmacia Fine Chemicals, Uppsala, Sweden) in 15-ml conical bottom plastic centrifuge tubes. These were centrifuged (400 x g for 40 min), and contents of each tube removed to the cell pellet. Pellets were resuspended, pooled, washed in PBS (250 x g for 10 min), and subsequently resuspended in RPMI 1640 medium. More than 95% of recovered cells were PMNL with viability >95%.

Peripheral blood MNL were used as a target cell population because they are easily enriched, have excellent viability after enrichment, and respond consistently to mitogenic stimulation. In contrast, milk MNL from uninfected glands are difficult to enrich, have reduced viability, and have reduced responses to mitogenic stimulation when compared with autologous peripheral blood MNL (8).

Peripheral blood MNL cells (of which the lymphocyte is a subpopulation) were isolated from buffy coat layers removed during the blood PMNL enrichment procedure. Twelve milliliters of diluted buffy coat cells were layered over 3 ml of ficoll-diatrizoate, specific gravity 1.084 (Sigma Chemical Company, St.
Louis, MO), in 15-ml conical tubes and centrifuged (400 × g for 40 min). Mononuclear leukocyte bands containing lymphocytes were removed, pooled for each cow, subjected to two lysis steps to remove erythrocytes, and washed (250 × g for 10 min) in PBS. Washed cells were resuspended in RPMI 1640 medium. Mononuclear leukocytes comprised >99% of recovered cell populations. Cell viability ranged from 85 to 90%. Total numbers of viable leukocytes and differential counts were determined using previously described procedures (9).

Selected MNL and PMNL populations were treated with cytochalasin B (Sigma) (10). Briefly, each population was split into two 10-ml volumes (in RPMI 1640 medium), and adjusted to 5.0 × 10^6 cells/ml. One set was supplemented with 0.1% dimethylsulfoxide and 20 μg/ml cytochalasin B, and the control set remained unsupplemented. Both sets were incubated for 20 min at 39°C in 5% CO_2-humidified air. Cells were pelleted, washed (250 × g for 10 min) twice in PBS, and resuspended in RPMI 1640 medium. Treatment did not alter leukocyte viability.

**Lymphocyte Proliferation Assay**

Wells of 96-well flat bottom tissue culture plates (Falcon 3070, Becton-Dickinson, Oxford, CA) were seeded with 10^5 blood MNL in a total volume of 200 μl/well. Medium was RPMI 1640 with the addition of heat-inactivated (56°C for 30 min) autologous serum or fetal calf serum to give a final concentration of 10% in the well. In addition, some wells contained one or more of the following components suspended in medium: Concanavatin A (Con A, 7.8 μg/ml), heat-killed and washed *S. aureus* cells (at 10^6, 10^7, or 10^8 bacteria/well), blood PMNL, or milk PMNL (1.0 to 8.0 × 10^5 cells/well). The total volume of each well was maintained at 200 μl. Plates were incubated for 72 h at 39°C in 5% CO_2-humidified air. At 72 h, 0.5 μCi of [methyl-^3H]thymidine, specific activity 67 Ci/mmol, Amersham Corporation, Arlington Heights, IL) in 50 μl of RPMI 1640 medium was added to each well and the plates incubated an additional 18 h. Well contents were harvested onto glass fiber filters using a cell harvester (Cambridge Technology, Cambridge, MA) and incorporated radioactivity measured by liquid scintillation counting. Assays were prepared in triplicate sets and the mean counts per minute (cpm) determined for each assay. Percent proliferation of blood lymphocytes in test systems was determined using the formula: Percent proliferation of blood lymphocytes = [(mean response (cpm) to Con A, with *S. aureus* and/or PMNL)/(mean response (cpm) to Con A alone)] × 100.

**Cytotoxicity Assay**

The effect of heat-killed *S. aureus* cells or leukocytes on the viability of peripheral blood MNL was determined by a 51Cr release assay. Mononuclear cells (2.5 × 10^7/ml) in RPMI 1640 medium were exposed to 200 μCi of sodium [51Cr]chromate (specific activity 14.2 megabecquerel/μg/ml of cell suspension and incubated 1 h at 39°C in 5% CO_2 air. Cells were subsequently washed (250 × g for 10 min) thrice and resuspended to 2.0 × 10^6/ml in medium. Wells of 96-well V-bottom tissue culture plates were seeded with 10^5 labeled cells. Medium was RPMI 1640 with 10% heat-inactivated autologous serum or fetal calf serum. Wells contained labeled MNL alone (medium control) or with combinations of the following components suspended in medium: heat-killed *S. aureus* cells (10^7, 10^5, 10^6, or 10^8 bacteria/well), blood or milk PMNL (1.0 to 8.0 × 10^5 cells/well), unlabeled mononuclear leukocytes (1.0 to 8.0 × 10^5 cells/ml), Con A (7.8 μg/ml), opsonized zymosan (0.2 mg/ml, Sigma), or Triton X-100 (as a measure of total release). Wells were maintained at a total volume of 250 μl. Assays were run in triplicate on 3 separate d. Plates were incubated for 15 h at 39°C, and then centrifuged (400 × g for 20 min). The amount of 51Cr released was determined by harvesting 125 μl of supernatant from each well and measuring the radioactivity using a gamma counter (Packard Auto-Gamma #5780, Packard Instrument Company, Downers Grove, IL). Percent specific cytotoxicity release was calculated using the formula: Percent specific cytotoxicity = [(mean cpm test − mean cpm medium control)/(mean cpm total release − mean cpm medium control)] × 100. Lymphocyte proliferation and cytotoxicity assays associated with a specific experiment were performed simultaneously using leukocyte populations enriched from the same blood sample.
Statistical Analysis

All data are expressed as the mean (± SD) response, and differences in the means were evaluated using Student's t test (P < .05) without assumption of equal variances.

RESULTS

During the period of study, intramammary infection remained isolated in the experimentally infected glands with >10³ viable S. aureus cells/ml of milk from mastitic glands. Infected animals remained clinically normal except for elevated leukocyte numbers and presence of viable bacteria in milk from infected glands.

Effects of killed S. aureus cells on in vitro proliferation of blood lymphocytes and viability of blood MNL populations from control and infected cows are in Figure 1. A decrease in proliferation of blood lymphocytes to mitogen and a parallel decrease in viability of MNL occurred as the ratio of bacteria to MNL increased. Proliferative responses to lymphocytes from infected cows were more inhibited than those of lymphocytes from uninfected cows at a bacteria:leukocyte ratio of 10¹:1. When the ratio was increased, differences in the inhibition of these lymphocyte populations were not significant. Inhibition of lymphocyte proliferation was greatest when the bacteria:leukocyte ratio was 10³:1. Unstimulated lymphocytes (not cultured with mitogen) demonstrated a similar loss in [3H]thymidine incorporation as the bacteria:leukocyte ratio was increased.

Mononuclear leukocytes from both control and infected cows progressively lost viability as numbers of bacteria were increased in the culture (Figure 1). Differences in viability of MNL from control and infected cows were not different at any bacteria:leukocyte ratio evaluated. The greatest changes in both lymphocyte proliferation and MNL viability occurred when bacteria:leukocyte ratios changed from 10¹ to 10²:1.

Prior to determining whether PMNL could modulate effects of bacteria to MNL, experiments were undertaken to determine the effect of blood PMNL addition to MNL cultures (Table 1). Addition of blood PMNL to MNL cultures at PMNL:MNL ratios of 1:1, 2:1, 4:1, and 8:1 did not alter viability of MNL from control or infected cows. However, proliferative responses of blood lymphocytes from control and infected cows were progressively and significantly reduced at PMNL:MNL ratios of 2:1, 4:1, and 8:1 (control cow MNL) and 4:1 and 8:1 (infected cow MNL). Differences in the degree of inhibition of the proliferation of lymphocytes from control and infected cows did not differ significantly at the PMNL:MNL ratios evaluated.

Reduced lymphocyte proliferation without an associated loss in MNL viability in PMNL supplemented cultures suggested ⁵¹Cr released by dying MNL might have been ingested or acquired by PMNL in culture, masking cytotoxic effects of PMNL for MNL. To test this hypothesis, blood PMNL (alone or with bacteria) from control or infected cows were cultured with ⁵¹Cr (equivalent to the total amount released by 10⁵ ⁵¹Cr-labeled MNL). After incubation for 15 h, the amount of ⁵¹Cr remaining in supernatant from PMNL cultures was not significantly different from wells containing only ⁵¹Cr. Supplementation of cultures with bacteria (10² killed S. aureus per PMNL) did not alter the amount of ⁵¹Cr present in supernatants.

TABLE 1. Effect of autologous blood polymorphonuclear leukocytes (PMNL) on the viability of blood mononuclear leukocytes (MNL) and proliferative responses of blood lymphocytes. 1

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Controls</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{51}$Cr-released</td>
<td>Percent proliferation</td>
</tr>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>SD</td>
</tr>
<tr>
<td>1:1</td>
<td>95</td>
<td>4</td>
</tr>
<tr>
<td>2:1</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td>4:1</td>
<td>98</td>
<td>6</td>
</tr>
<tr>
<td>8:1</td>
<td>92</td>
<td>7</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts differ (P<.05).

1 Data from control (n=3) and infected (n=3) cows; each sampled on 3 separate d.

2 Ratio of PMNL to $^{51}$Cr-labeled MNL (in cytotoxicity assay) or unlabeled MNL (in lymphocyte proliferation assay): MNL number fixed at $10^5$ per well.

3 $^{51}$Cr-released expressed as mean (± SD) percent of spontaneous release (Concanavalin A at 7.8 μg/ml in all assays).

4 Percent proliferation (see text for formula); mean (± SD) shown.

Addition of blood PMNL to MNL cultures containing a fixed number of bacteria altered effects of bacteria on the MNL population (Figure 2, Table 2). These data also indicated that effects of PMNL supplementation were similar when cultures contained either autologous or fetal calf serum. A general trend was enhancement or partial restoration of lymphocyte proliferation when PMNL:MNL ratios were 1:1 and 2:1 with the greatest restoration at 2:1. However, PMNL:MNL ratios of 4:1 and 8:1 inhibited proliferation to a greater extent than when no blood PMNL were present. The degree of the inhibition of proliferative responses of lymphocytes from control or infected cows was not different at specific amounts of PMNL supplementation. Cytotoxicity assays indicated a progressive reduction in the cytotoxic effect of bacteria with increasing numbers of PMNL added to cultures.

Effects of milk PMNL from infected cows on MNL function and viability in the presence of S. aureus were comparable to those seen with blood PMNL. At PMNL:MNL ratios of 1:1 and 2:1, no significant change in the proliferative responses occurred; however, at ratios of 4:1 and 8:1, proliferative responses were more inhibited (Figure 3). These effects were similar using either milk or blood PMNL. The cytotoxicity of bacteria for MNL was progressively reduced by increasing the proportion of either milk or blood PMNL in culture. At PMNL:MNL ratios of 4:1 and 8:1, both milk and blood

![Figure 2](image-url)
TABLE 2. Effect of peripheral blood polymorphonuclear leukocytes (PMNL) on viability and proliferative responses of mononuclear leukocytes (MNL) cultured with *Staphylococcus aureus* cells in medium containing fetal calf serum.\(^1\)

<table>
<thead>
<tr>
<th>Ratio(^2)</th>
<th>Percent specific cytotoxicity(^3)</th>
<th>Percent proliferation(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Infected</td>
</tr>
<tr>
<td></td>
<td>(\bar{X}) SD</td>
<td>(\bar{X}) SD</td>
</tr>
<tr>
<td>No PMNL</td>
<td>45 4(^a)</td>
<td>56 15(^a)</td>
</tr>
<tr>
<td>1:1</td>
<td>29 2(^b)</td>
<td>32 10(^{a,b})</td>
</tr>
<tr>
<td>2:1</td>
<td>25 2(^b)</td>
<td>30 3(^b)</td>
</tr>
<tr>
<td>4:1</td>
<td>11 2(^c)</td>
<td>16 4(^c)</td>
</tr>
<tr>
<td>8:1</td>
<td>2 1(^d)</td>
<td>6 2(^d)</td>
</tr>
</tbody>
</table>

\(a,b,c,d\) Means within columns with different superscripts differ (\(P<.05\)).

\(^1\) Data from control (n=3) and infected (n=3) cows; each sampled on 3 separate d; all assays employed fetal calf serum (10%).

\(^2\) Ratio of PMNL to MNL; MNL number fixed at 10\(^5\) per well, *S. aureus* number fixed at 10\(^7\) per well.

\(^3\) Defined in text, data expressed as mean (± SD) percent specific cytotoxicity or proliferation.

PMNL reduced the cytotoxic effects of the bacteria. Cytochalasin B treatment of blood or milk PMNL did not alter their capacity to reduce the cytotoxic effect of killed *S. aureus*.

Data from experiments performed to determine if the reduction in the cytotoxic effect of bacteria by PMNL was due to the presence of a second leukocyte population in the culture, not specifically PMNL, as shown in Table 3. No reduction in cytotoxicity was shown when MNL (unlabeled) were added; however, when blood or milk PMNL were added at ratios of 8:1, reduction in cytotoxicity was significant.

**DISCUSSION**

In the present study, lymphocyte proliferation and cytotoxicity assays were used to evaluate the effects of a mastitis-causing strain of *S. aureus* and PMNL on the function and viability of bovine MNL. Killed *S. aureus* cells cultured with blood MNL from uninfected and mastitis cows caused a concentration-dependent decrease in blood lymphocyte proliferation that was accompanied by concomitant decrease in the viability of blood MNL, indicating that *S. aureus* cells have a cytotoxic effect. The reduction in proliferative responses of blood lymphocytes may not have been entirely due to the cytotoxicity of the bacteria. Particulate peptidoglycan, a component of the cell wall of *S. aureus*, has been shown to induce immunosuppressive cells capable of inhibiting anti-

![Figure 3. Modulation of effects of killed *Staphylococcus aureus* cells on peripheral blood lymphocyte proliferation and blood mononuclear leukocyte viability by autologous milk (○--○) and blood (○--○) polymorphonuclear leukocytes. Data are from proliferation and cytotoxicity assays using cells from infected cows. Mononuclear leukocytes (MNL) were cultured at 10\(^5\) cells/well in 10% autologous serum. The *S. aureus* cells were at 10\(^7\) bacteria/well.](image-url)
TABLE 3. Effect of polymorphonuclear leukocytes (PMNL) or mononuclear leukocytes (MNL) on viability of blood MNL cultured with *Staphylococcus aureus* cells.  

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Blood PMNL</th>
<th>Milk PMNL</th>
<th>Blood MNL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X SD</td>
<td>X SD</td>
<td>X SD</td>
</tr>
<tr>
<td>1:1</td>
<td>28 ± 6a, b</td>
<td>29 ± 8a</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>2:1</td>
<td>24 ± 5a</td>
<td>28 ± 6a</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>4:1</td>
<td>22 ± 12a</td>
<td>22 ± 6a</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>8:1</td>
<td>8 ± 6b</td>
<td>13 ± 4b</td>
<td>26 ± 4</td>
</tr>
</tbody>
</table>

a, b Means within columns with different superscripts differ (P<.05).  
1 Data from infected cows sampled on 3 separate d.  
2 Ratio of PMNL to 51-Cr-labeled MNL, MNL number fixed at 10^5 per well, *S. aureus* number fixed at 10^7 per well.  
3 Expressed as mean (± SD) percent cytotoxicity. Mean cytotoxicity of *S. aureus* without PMNL present was 32 ± 5.

body production to T-cell dependent antigens (11). Conceivably, immunosuppressive cells induced by *S. aureus* may have inhibited responses of lymphocytes to polyclonal stimulation. Alternatively, staphylococcal components not removed during washing of the killed *S. aureus* cells may have been toxic, reducing the viability and proliferative responses of the MNL population. Use of killed versus viable bacteria in vitro was necessary to avoid rapid overgrowth of the culture systems by bacteria during extended incubation; however, in the environment of the infected mammary gland, viable *S. aureus* cells and their membrane-damaging extracellular toxins (13) might have a greater potential for a cytotoxic effect on milk leukocytes.

Milk and mammary gland parenchymal tissue from cows with staphylococcal mastitis contain greatly elevated numbers of PMNL (4, 7, 8). Mature PMNL function primarily in the phagocytosis and destruction of microorganisms, but when activated, became secretory with the potential to damage cells or tissues of the host (3, 15, 16). In the present study, addition of blood PMNL to cultures containing a fixed number of blood MNL and Con A (mitogen for lymphocytes and PMNL activator) did not alter the viability of MNL, but did suppress proliferative responses of lymphocytes to mitogen in a PMNL concentration-dependent fashion. Both lymphocytes and PMNL have cell membrane receptors for Con A and might compete for lectin, influencing lymphocyte proliferation. However, supplementation of cultures with excess Con A (15.6 and 31.2 µg/ml) failed to restore lymphocyte proliferation. When opsonized zymosan (.2 mg/ml) was substituted for Con A as a PMNL activator (10), no loss of MNL viability occurred. Inhibition of lymphocyte proliferation without loss of MNL viability may indicate production of a noncytotoxic factor by PMNL capable of regulating lymphocyte function. These data suggest that the elevated number of PMNL in milk and tissue of chronically infected mammary glands may be deleterious to lymphocyte function.

Addition of blood PMNL (at PMNL:MNL ratios of 1:1 and 2:1) to a culture system containing blood MNL and *S. aureus* cells prevented the detrimental effects of *S. aureus* cells on lymphocyte proliferation. Higher ratios, which effectively reduced the cytotoxic effect of bacteria, failed to prevent suppression of lymphocyte proliferation. These data, with those showing that the PMNL (at high PMNL:MNL ratios) alone inhibit lymphocyte proliferation, indicate suppression of lymphocyte proliferation may also be affected by the number of PMNL present. However, under these conditions, PMNL apparently benefited the lympho-
cyte by abrogating the cytotoxic effects of S. aureus cells. Cytochalasin B pretreatment of PMNL, which interferes with the function of cytoplasmic actin and actin-binding protein, preventing complete closure of the phagocytic vacuole, did not alter the capacity of PMNL to reduce the toxicity of S. aureus cells, indicating that intact phagocytic processes were not necessary for the effect. Antibody present in autologous serum or cytophilic antibody on blood vacuole, did not alter the capacity of PMNL to the PMNL plasma membrane, removing the bacteria from the immediate environment of the MNL.

Effects of milk PMNL from infected glands on S. aureus-induced alteration of lymphocyte function and MNL viability were not different from those of autologous blood PMNL, suggesting a similarity in the in vitro functional capacities of both cell populations. Washing and resuspending milk PMNL in culture medium prior to incorporation into assays may have eliminated milk-associated factors, such as casein and fat, considered detrimental to the phagocytic function of milk PMNL (1). In vivo, milkborne PMNL may be compromised by these factors. Milk PMNL from uninfected glands were present in insufficient quantity for testing.

In conclusion, it is possible to evaluate in vitro interactions of different leukocyte populations and their responses to S. aureus. Killed S. aureus cells exerted a cytotoxic effect on blood MNL which was manifested by a reduction in lymphocyte proliferation. This effect was reduced by PMNL supplementation of cultures, although high concentrations of PMNL exerted a negative effect on lymphocyte proliferation. Initial observations (8) that milk lymphocytes from glands with persistent S. aureus mastitis were less responsive to mitogenic stimulation in vitro may be attributable to the high number of PMNL or viable S. aureus cells present in the infected gland.

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REFERENCES
