**Staphylococcus aureus** Mastitis: Pathogenesis and Treatment with Bovine Interleukin-1β and Interleukin-2

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**ABSTRACT**

Polymorphonuclear leukocytes play a central role in the pathogenesis of bovine mastitis. Intramammary challenge with *Staphylococcus aureus* was shown to induce both quantitative and qualitative changes in mammary gland polymorphonuclear leukocytes. Intramammary infusion of recombinant bovine interleukin-1β and interleukin-2 elicited a similar cellular response. *Staphylococcus aureus*, interleukin-1β, and interleukin-2 all increased the number of somatic cells after intramammary infusion and activated the inducible superoxide production in milk polymorphonuclear leukocytes. Interleukin-2 also activated phagocytosis of these cells, and their activation was maintained for 3 to 5 d after intramammary administration. Interleukin-1β and interleukin-2 were moderately effective in the therapy of experimental *S. aureus* mastitis. Approximately 54% of the glands treated with interleukin-1β responded to therapy by transiently clearing the milk of *S. aureus*, 30% of which relapsed, and a total of 38% of the treated glands remained cured. In contrast, 83% of glands treated with interleukin-1β responded to therapy, but 50% of these quarters relapsed. A total of 42% of the quarters treated with interleukin-1β remained cured. Homologous recombinant cytokines are effective immunomodulators that augment natural defensive mechanisms similar to the normal response to pathogens and may prove to be suitable alternatives to, or may be used in combination with, antibiotics as effective mastitis therapeutic agents.

(Key words: cytokines, therapy, mastitis)

Abbreviation key: BRM = biological response modifiers, IL = interleukin, O₂⁻ = superoxide anion, PBS = phosphate-buffered saline, PMA = phorbol 12-myristate 13-acetate, PMN = polymorphonuclear cell, rb = recombinant bovine.

**INTRODUCTION**

Subclinical and clinical mastitis is a major cause of loss in milk production to the dairy industry; economic losses are estimated at 2 to 4 billion dollars annually in the US (4). One of the dominant etiological pathogens contributing to this economic loss is *Staphylococcus aureus* (23). Although antibiotic therapy can be effective in the control of mastitis from a variety of organisms, it is only moderately efficacious for *S. aureus* infections and requires a 3- to 5-d milk withdrawal (2, 5). This milk discard is the major source of economic loss to the dairy farmer and is a cause of some resistance to therapy in lactating cows, especially in light of the often poor response to therapy. Improved mastitis therapeutics remain a major unfilled need of the dairy industry.

*Staphylococcus aureus* mastitis infection is characterized by viable bacteria in the milk and an elevated number of somatic cells in the mammary tissue and milk (1, 7, 9, 14). In an infected gland, these host cells are >95% polymorphonuclear leukocytes (PMN). The development of chronic nature of *S. aureus*...
mastitis depends on the interaction between invading bacteria and these cells of the defense system (10, 28). Although the defense system of the mammary gland can be effective in eliminating as much as 3 to 4 logs of bacteria, it has been suggested that the less activated PMN may in fact contribute to the relapse of an existing infection and chronic cyclic nature of *S. aureus* mastitis (9). Furthermore, once organisms have been phagocytosed, they may be protected from the effects of antibiotic therapy (7, 19, 34).

The defense mechanisms of the host can be stimulated by pathogens and their by-products (16) and various biological response modifiers (BRM) (38), including cytokines (26, 35). Cytokines regulate the activity of the host's defense system in response to pathogens. For example, interleukin (IL)-1, although pleiotropic in many of its effects, can activate B cells, T cells, macrophages, and other primary and secondary cells of the defense system (27, 36). Interleukin-2 is a lymphocyte-derived glycoprotein that primarily induces the clonal expansion of activated T and B cells (15), but, in so doing, it can also initiate the secondary secretion of a variety of other cytokines by the activated lymphocytes. Both bovine IL-1 and IL-2 have recently been cloned and expressed (3, 20) and have been shown to potentiate the biological activity of vaccines as well as a natural killer-like activity (32, 33).

Given that normal regulation of PMN in response to infections can be mediated by cytokines (8, 11, 12, 13), the availability of recombinant bovine (rb) IL-1β and rbIL-2 allows for the first time an evaluation of the effects of exogenous administration of cytokines on the potentiation of normal defense mechanisms of the mammary gland. Enhancement of natural defense mechanisms might minimize or eliminate the need for antibiotics in the treatment of mastitis infections. However, a key to utilizing these recombinant cytokines optimally for the prevention and therapy of bovine mastitis (because these cytokines are part of a highly integrated regulatory network), lies in a thorough understanding of their role in the normal host response to a particular pathogen. The following study was initiated to identify the biological response of the mammary gland to the initiation of *S. aureus* infection and then to attempt to mimic or potentiate this response through the administration of exogenous cytokines.

**MATERIALS AND METHODS**

**Animals and Experimental Model**

Three normal uninfected mammary quarters from three separate dairy cows were used to examine the normal host response to challenge with 100 to 200 cfu of *S. aureus*. At 16, 40, 64, 112, and 160 h after intramammary infusion, the number of somatic cells and the ability of these cells to produce superoxide (O2-) after induction with phorbol 12-myristate 13-acetate (PMA) was measured. For trials studying the response of cytokines to chronic *S. aureus* infection, a total of 32 lactating Holstein-Friesian dairy cows were used. Experimental infections were established with *S. aureus* strain Newbould 305 (25). All mammary glands to be inoculated were free of bacteria on five consecutive a.m. milkings prior to infection and had individual quarter SCC less than 200,000 cells/ml of milk. Inocula were prepared from a 4-h culture grown in trypticase soy broth and diluted in sterile distilled water to contain 50 to 300 cfu/ml. In vivo inoculations were made by infusion of 1 ml of diluted culture of *S. aureus* into the teat cistern of a milked out mammary quarter. Experimental mammary quarters were cultured daily to determine the presence of *S. aureus*. All infections were established for a minimum of 3 wk before being used for therapeutic treatment. Quarters were considered to respond to therapy if the a.m. milk sample was free of *S. aureus* for a minimum of one milking immediately following the last intramammary infusion. Infections were considered cured if milk samples remained free of *S. aureus* for 14 successive daily samplings of milk. Fourteen days of continuous sampling were sufficient to eliminate most false negatives for cures, because even with antibiotic therapy >95% of all quarters that relapse do so within 14 d [(9), unpublished observations on over 1200 treated experimental infections].
Microbiology

Individual quarter milk samples were collected prior to each a.m. milking and cultured by swirling 1 ml in duplicate petri dishes containing trypticase soy agar supplemented with 5% whole ovine blood. Plates were incubated aerobically at 37°C and examined after 24 h. Staphylococcus aureus isolates were counted and identified by colony morphology and hemolytic patterns. Periodically, isolates were also tested for coagulase activity (24) and antibiotic sensitivity.

Somatic Cell Determination

Somatic cell counts were determined by Coulter counting (9, 30) and performed on 1-ml aliquots of fresh milk samples collected immediately prior to the a.m. or p.m. milking. Cells were pelleted initially at 2200 × g for 10 min, and the resulting cellular pellet was washed twice in phosphate-buffered saline (PBS) at 1200 × g for 5 min. The cellular pellet was diluted 1:50 or 1:500 in ISOTON® (Coulter Diagnostics, Hialeah, FL). The fresh cell preparation was counted using a gate for exclusion of less than 6.8 μm in diameter on a Coulter ZM (Coulter Electronics, Hialeah, FL). The cell population was also profiled on a Coulter C256 Channelizer (Coulter Electronics). Only viable somatic cells, which would be the only cells retaining biological activity, were counted using this criterion.

Quantitation of Phagocytosis by Flow Cytometry

Ten- to 250-ml samples of milk were collected immediately prior to the a.m. or p.m. milking and passed through cheesecloth to remove clumped leukocytes. Milk was then centrifuged at 4°C, 1200 × g for 30 min. Fat and skim milk were removed, and cell pellets were washed twice and resuspended in PBS and counted on a Coulter counter. Cells were brought to a final concentration of 1 × 10^6 viable cells/ml with cold RPMI-1640 (Gibco, Grand Island, NY) supplemented with 5% heat-inactivated fetal calf serum (Gibco). Then 1.6- to 2-μ fluorescent beads (Polysciences, Warrington, PA) were added to a concentration of 5.52 × 10^7 beads/10^6 cells (37). This mixture was incubated at 37°C for 30 min and then analyzed at the Flow Cytometry Laboratory, Princeton University, using a Coulter EPICS 753 (Coulter Electronics) as previously described (9). Greater than 90% of the cells from infected glands were PMN, and a total of 5000 PMN were analyzed by flow cytometry for each individual phagocytosis determination.

Whole Cell Superoxide Assay

The NADPH-dependent O_2 generation was assayed as an indication of PMN activation (22). Briefly, milk from quarters were centrifuged at 1200 × g for 15 min to pellet milk PMN. Cell pellets were washed thrice in PBS, resuspended, and counted. Fifty microliters of 5 × 10^6 milk PMN/ml of PBS supplemented with glucose, magnesium, and calcium were added to each microtiter well containing 200 μl of PBS with glucose and a final concentration of 75 μM cytochrome c. Plates were incubated for 10 to 15 min at 37°C. To each well, PMA was added to a final concentration of 100 ng/ml to activate O_2 formation. The specific rate of reduction of cytochrome c was measured using a narrow band 550-nm filter on a ThermoMax® kinetic plate reader (Molecular Devices Corporation, Menlo Park, CA) as an indirect measure of O_2 formation. Data were expressed as the mean rate of O_2 generation in nanomolar O_2 per minute per 10^6 cells.

Recombinant Cytokines and Intramammary Infusions

Recombinant cytokines (rbIL-1β and rbIL-2) were prepared and tested for in vitro biological activity by IMMUNEX Corporation, Seattle, WA (20, 31). The specific activity of rbIL-2 was 20,000 U/μg of biological activity and 1.4 ng of endotoxin/mg of protein. The specific activity of rbIL-1β was 30,000 U/μg of biological activity with 43 ng of endotoxin/mg of protein. Endotoxin levels at doses administered were 100-fold below the dose required to elicit any biological response in vivo with purified endotoxin. Cytokines were diluted to give the required dose to be delivered in 10 ml of sterile PBS. Doses were administered in syringes fitted with single use teat cannulas, and cytokines were infused through the teat.
canal of the treated mammary quarter. For all therapeutic trials, rbIL-1/3 and rbIL-2 were administered three times after the p.m. milking at either 24- or 48-h intervals into quarters that had been infected for a minimum of 3 wk. A total dose of 10 mg of rbIL-2 or 200 μg of rbIL-1/3 in 10 ml of sterile PBS was administered in each of the three infusions into each infected quarter. Sodium cephalixin (Sigma Chemical Co., St. Louis, MO) was administered after consecutive p.m. and a.m. milkings. Similarly, Cefa-Lak® (Bristol-Myers, Evansville, IN) was administered after consecutive p.m. and a.m. milkings according to package insert.

Analysis

All data were analyzed by a two-tailed Student's t test, except for efficacy data for which the Mantel-Haenszel-corrected chi-square analysis was used.

RESULTS

Activation of Host Polymorphonuclear Activity After Intramammary Challenge with Staphylococcus aureus

Normal uninjected quarters were first challenged with S. aureus to examine the normal host response to a pathogen. Figure 1A represents the total number of somatic cells and the rate of O2 generation (Figure 1B) at various time points after S. aureus challenge. At time 0, no significant O2 could be generated upon addition of PMA, suggesting that milk PMN are significantly suppressed in function. By 40 h after S. aureus challenge, the SCC increased 20-fold over the prechallenge SCC. At the same time, PMA-induced O2 increased to over 15 nM O2/min per 107 milk PMN, a level approximately equal to the activity of peripheral blood PMN in the bovine (5 to 30 nM O2/min per 107 PMN, unpublished observations). The number of cells (as indicated by asterisk) and the generation of O2 (all time points) after S. aureus challenge were significantly different from prechallenge levels (P < .05) by Student's t test.

Cyclic Nature of Host Cell Activation in Experimental Staphylococcus aureus Mastitis Infections

In the experimental S. aureus mastitis model, a cyclic rise and fall in colony-forming units per milliliter of milk (or viable S. aureus per milliliter) is observed (Figure 2A). Cycling of colony-forming units concomitantly occurred with an asynchronous cycling of the somatic cells (>90 to 95% PMN by flow cytometry). In addition to the SCC, biological activity, as measured by PMA-induced O2 production, was also measured (Figure 2B). The ability to induce O2 fluctuated almost 20-fold over the 5-d sampling period and at each sampling was greater than the activity of PMN from uninfected quarters. Statistically, d 1 and 4 of the cycling infection were different (P < .05) from d 2, 3, and 5 by Student's t test.

Effect of rbIL-1/3 and rbIL-2 on Activation of Milk PMN from Mastitic Dairy Cows as Measured by Superoxide Induction and Phagocytosis

Nine mastitic quarters from four different animals were untreated or infused with 200 μg of rbIL-1/3 or 2 mg of rbIL-2 based upon previous dose-response data (Daley et al., unpublished data). Superoxide formation by milk PMN was monitored at 0, 16, 24, and 48, 64, and 72, and 112 and 120 h postinfusion. By 48 h, the SCC increased 170-fold for the rbIL-1/3 infusion (Figure 3A) and 400-fold for the rbIL-2 infusion (Figure 3D) over the pretreatment SCC (P < .01 and P < .005, respectively). Although administration to normal uninjected glands has been shown to enhance inducible O2 production and phagocytosis (Daley et al., unpublished data), stimulation of PMN from infected quarters was only moderate and quite variable after cytokine therapy compared with pretreatment levels. Polymorphonuclear cells from mastitic glands treated with rbIL-1/3 or rbIL-2 demonstrated two- to fourfold stimulation (P < .01 for IL-1/3 for time 0 vs. other time points, and P < .01 for IL-2 for time 0 vs. 112 h) or O2 production over the pretreatment-infected gland control cells (Figure 3, B and E). Increased stimulation of O2 production was seen within 16 h postinfusion and continued through 88 h for the rbIL-1/3 treatment.
The glands treated with rbIL-2 demonstrated a significant induction of $O_2^-$ ($P < .05$) over the PMN from infected, untreated quarters (time 0) at 112 h after the last intramammary infusion of rbIL-2. After rbIL-2 administration, inducible $O_2^-$ production initially decreased and then increased to over 14 nM $O_2^-$/min per 10^7 milk PMN. Unlike rbIL-1β administration, which showed a suppression of phagocytosis after infusion ($P < .01$ for time 0 vs. 18 h), phagocytosis remained enhanced (no statistical difference) at all times in the rbIL-2-treated group (Figure 3, C and D).

**In Vivo Efficacy of rbIL-1β and rbIL-2 Formulations**

A total of 12 to 13 quarters per treatment were infused with rbIL-1β, rbIL-2, Na cepaharin in saline, or Cefa-Lak®. The number of infected quarters that cleared their infection for a minimum of one milking after the last therapy (response) and the number that remained infection free for 14 consecutive daily milkings after the last intramammary infusion (cures) were monitored (Table 1). Although the overall cure rate was equivalent for the rbIL-2 treatment (38%) and rbIL-1β treatment (42%), the percentage of quarters responding that went on to cure was different, 70% versus 51%, respectively (not statistically different). Therefore, the rbIL-2 quarters that respond remained free of bacteria more frequently than the quarters treated with rbIL-1β.

**Correlation of Superoxide Induction of Milk PMN and Therapeutic Efficacy After rbIL-2 Infusion**

Somatic cells were collected from mastitic quarters after cytokine therapy, and their cells were assayed for PMA-induced $O_2^-$ formation. Superoxide induction was also monitored at 16, 40, 64, and 136 h after the last infusion, and the quarters were separated into cured (Figure 4A) or relapsed (Figure 4B). Both groups had equivalent starting levels at 24 h posttreatment of inducible $O_2^-$ (not statistically different). Cured quarters at the 136-h time point demonstrated a 60% decrease in inducible $O_2^-$ formation ($P < .05$), but relapsed quarters only demonstrated a 7% decrease compared with the pretreatment levels. This would suggest that the endogenous PMN population of an infected gland requires a continuous source of pathogen to maintain the biological activation of the resident cells.

**DISCUSSION**

In a study of the pathogenesis of experimental *S. aureus* bovine mastitis, it was shown that the host exhibits a cyclic rise and fall of host phagocytic cells (9) in the milk (>90 to 95% PMN). Concomitant with, but asynchronous to, this host cellular response is a cyclic rise and fall of detectable *S. aureus* in the milk of the infected gland. Not only does the quantity of PMN change during this cycling, but the quality, as measured by phagocytosis and intracellular bacterial killing efficiency, also changes (9). The present study has extended this observation to include cycling of oxidative metabolism as well as the activation of phagocytic cells on in vivo bacterial challenge. This clearly demonstrates that the host responds to a challenge from most pathogens by enhancing the quantity and quality of the phagocytic cells in the mammary gland. Although partial cellular activation does occur after bacterial challenge and during a cycling infection, inefficient phagocytosis and killing may contribute to the relapse of the infection and failure of therapy by antibiotics (6). Furthermore, antibiotics can inhibit the normal defense mechanisms of the host by decreasing phagocytic function (40) and thereby further exacerbate the frequency of relapsing infections.

Exogenous administration of rbIL-1β and rbIL-2 appears to potentiate the normal response of the host to infection. Recombinant bovine interleukin-1β induced a quantitative influx of host PMN after an intramammary infusion. These cells were partially activated in that the inducible $O_2^-$ formation was enhanced over resident milk PMN from uninfected glands, but only modest effects of rbIL-1β administration were seen on PMN from infected glands. This might suggest that once PMN are activated by *S. aureus*, further activation by cytokines is only modest. However, maintenance of concomitant activation of the bactericidal components of the PMN, as well as phagocytosis by cytokine therapy, is necessary to eliminate bacteria efficiently (18, 21).
Figure 1. Induction of phagocytic cell activation after challenge with Staphylococcus aureus intramammary infusion. The polymorphonuclear cells from three mammary glands from three cows challenged with S. aureus were collected at the indicated time periods. Somatic cell counts and phorbol 12-myristate 13-acetate (PMA)-inducible superoxide anion ($O_2^-$) production were assayed in triplicate. Data are expressed as the mean stimulation index for SCC (posttreatment SCC divided pretreatment SCC) (A), or the mean rate of PMA-induced $O_2^-$, SEM, expressed as nanomolar $O_2^-$ per minute per $10^7$ viable somatic cells, (B). The asterisk in panel A indicates statistically significant SCC from pretreatment levels ($P < .05$). In panel B, time points 40, 64, 112, and 160 h are all statistically different by Student's $t$ test ($P < .01$) from the pretreatment levels.

Figure 2. Phagocytic cell activation in established Staphylococcus aureus infections. The SCC, colony-forming units (Staphylococcus aureus), and rate of superoxide anion (O$_2^-$) of milk polymorphonuclear were monitored from a cycling S. aureus mastitis infection for 5 consecutive d. The SCC per milliliter of milk (•) and colony-forming units per milliliter of milk (○) (A), and phorbol 12-myristate 13-acetate (PMA)-inducible O$_2^-$ formation (B) are shown on each day. Data are expressed as the mean of duplicate samples for SCC × 10$^3$/ml and for colony-forming units × 10$^3$/ml of milk; and mean rate of oxygen radical generation is expressed in nanomoles of O$_2$ per 10$^7$ milk cells after PMA induction (± SD) for triplicate assays. The asterisks represent statistically significant difference (P < .05) compared with d 1 by Student's t-test.
Dissociation of activation of phagocytosis without activation of bactericidal activity could, in fact, contribute to a source of *S. aureus* for the relapsed infection. Consistent with this observation, treatment of infected glands with rbIL-1β transiently cleared the milk of bacteria in 83% of the mammary glands treated; only 42% of the treated quarters remained free of infection after 14 d. This high relapse rate is represented in the low ratio of cures to responding quarters, 51%, and is similar to that of Na cepahirin in saline treatment.

Treatment with rbIL-2 resulted in a slightly lowered quantitative influx of cells, but these cells were activated for their inducible $O_2^-$ at

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Activation of milk polymorphonuclear cells from mastitic glands after intramammary infusion with recombinant bovine interleukin-1β (rbIL-1β) and interleukin-2 (rbIL-2). Three to four infected mammary glands from dairy cows were infused with rbIL-1β or rbIL-2 at each of three successive a.m. or p.m. milkings. Somatic cell counts, phagocytosis, and phorbol 12-myristate 13-acetate (PMA)-induced superoxide anion $O_2^-$ production were monitored at 16 and 24, 40 and 48, 64 and 72, and 112 and 120 h after the last infusion. The geometric mean number ± SEM of somatic cells per milliliter of milk is shown (A: rbIL-1β and D: rbIL-2). The mean rate (± SEM) of PMA-inducible $O_2^-$ formation, expressed as nanomoles of $O_2^-$ min per $10^7$ viable cells, is also shown (B: rbIL-1β and E: rbIL-2). The mean phagocytic index is shown (C: rbIL-1β and F: rbIL-2). The mean phagocytic index is expressed as the mean percentage of cells phagocytozing 1 or more beads of time (%x) divided by the mean percentage of cells phagocytozing 1 or more beads at time 0 (prior to treatment). All posttreatment time points that were significantly different from pretreatment time points are indicated by asterisks (*P < .05; **P < .001).

TABLE 1. Therapeutic efficacy of intramammary infusion of recombinant bovine interleukin (rbIL)-1β and rbIL-2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg)</th>
<th>Regimen</th>
<th>Number of glands</th>
<th>% Response</th>
<th>% Cures</th>
<th>Cure:response</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbIL-1β</td>
<td>.2</td>
<td>3x, a.m.</td>
<td>12</td>
<td>83</td>
<td>45^5</td>
<td>.51</td>
</tr>
<tr>
<td>rbIL-2</td>
<td>10</td>
<td>3x, p.m.</td>
<td>13</td>
<td>54</td>
<td>39^5</td>
<td>.70</td>
</tr>
<tr>
<td>Na Cephapirin</td>
<td>200</td>
<td>2x, p.m./a.m.</td>
<td>12</td>
<td>86</td>
<td>42^5</td>
<td>.48</td>
</tr>
<tr>
<td>Cefa-Lak®</td>
<td>200</td>
<td>2x, p.m./a.m.</td>
<td>13</td>
<td>92</td>
<td>77^5</td>
<td>.83</td>
</tr>
</tbody>
</table>

1Treatments were administered after the a.m. milking (rbIL-1β, Cefa-Lak®, or Na cephapirin) or after the p.m. milking (rbIL-2, Cefa-Lak®, or Na cephapirin) as indicated. Cytokines were administered three times at 24-h intervals, and antibiotics were administered on consecutive p.m. and a.m. milkings.

2Number of treated quarters with milk samples free of *Staphylococcus aureus* for 1 d or more after the last treatment, divided by the total number of quarters treated.

3Number of treated quarters free of *S. aureus* for 14-d consecutive milk samples after the last treatment, divided by the total number of quarters treated.

4Cure:response ratio = Percentage of cured quarters divided by percentage of responding quarters.

5None of the groups were shown to be statistically different from one another by Mantel-Haenszel corrected chi-square analysis with \( P > .1 \). All groups were similarly compared with 50 untreated control quarters followed over a 7-d period (0/50 = cured) and were shown to be significantly different at \( P < .05 \).

Several time points after administration (three to four greater than even the optimal time point for rbIL-1β). Unlike the rbIL-1β treatment, phagocytosis did not decrease but remained at an activated level following treatment of infected glands, thereby suggesting that the new cells being recruited into the gland continued to be activated. It is indeed surprising that intramammary administration of rbIL-2 elicited an influx of PMN, because PMN have not been described to express the IL-2 receptor (17). Presumably, rbIL-2 must be initiating a secondary cytokine cascade that mediates the observed effects in vivo.

The treatment of infected quarters with rbIL-2 resulted in only 54% of the mammary glands transiently clearing their infection, but 38% of the treated quarters remained free of *S. aureus* at 14 d. The ratio of cures to responding quarters was .7, a ratio that more closely resembles the therapeutic efficiency of Cefa-Lak®, .84, albeit the overall cure rate is less, 38 versus 77% for the Cefa-Lak®.

Although the ability to potentiate further the activation of milk PMN from infected quarters after cytokine therapy was variable, a decrease in inducible \( \Omega \) was predictive of effective therapy. Inducible \( \Omega \) levels from cured quarters demonstrated a two- to fourfold decrease over relapsed quarters \( (P < .05) \). This would suggest that the activation state of resident mammary gland PMN requires a continuous source of pathogen to maintain the biological activation. Similarly, the activation by cytokines was shown to be transient and reversible. In mastitic glands, activation is maintained through bacterial components and secondary host signals such as cytokines. Exogenous cytokine administration merely mimics normal host reactions to infectious agents, and because phagocytic cell function is significantly depressed in normal milk PMN compared with peripheral blood PMN, overcoming this suppression or maintaining activation with cytokines may be important in the prevention and therapy of mastitis.

If formulations of cytokines can be developed that are equivalent to or better than current therapies, they may totally or partially circumvent prolonged milk withdrawal because of antibiotic residues. Alternatively, cytokines as an adjunct to current mastitis therapy may reduce the amount of antibiotic required. Although previous investigators have proposed an increase in the number of host cells as an approach to prevention (7, 29, 39), we have further shown that the concomitant activation of these cells may be of equal importance in the treatment of bacterial infections. Clearly, this activation can be mimicked by exogenous administration of recombinant cytokines. These observations should be applicable to the therapy and prevention of a wide variety of acute and chronic infectious diseases in both domestic animals and humans.
Figure 4. Correlation of milk polymorphonuclear cells activation and therapeutic efficacy from mastitic glands after intramammary infusion with recombinant bovine interleukin-2 (rbIL-2). A total of 16 cycling *Staphylococcus aureus* mastitis infections were treated with rbIL-2 and monitored for cures. Each symbol represents the phorbol 12-myristate 13-acetate (PMA)-inducible superoxide anion ($O_2^-$) production of individual quarters for 6 consecutive d after the last treatment with rbIL-2. The rate of PMA-induced superoxide induction is expressed as the mean rate of triplicate assays expressed as nanomoles of $O_2^-$/min per $10^7$ milk polymorphonuclear cells. Data are grouped into cured ($n = 10$) quarters (A) or relapsed ($n = 6$) quarters (B). The asterisks indicate that the 136-h grouping was statistically different ($P < .01$) from the 16-h samples by Student's $t$ test.
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