ABSTRACT

Eighteen lactating dairy cows were used to evaluate the physiological response of mammary glands to increasing doses of recombinant bovine interleukin-2. Right front and rear quarters were intramammarily infused with five different doses (0.1 to 100 µg per quarter) of interleukin-2 as either a single or multiple treatment. Left front and rear quarters were intramammarily infused with a saline placebo and served as within-animal controls. Milk secretion samples for compositional analysis were collected from each quarter prior to infusion and at 12, 24, 36, and 48 h following infusion. Animals were slaughtered by exsanguination immediately following the 48-h sampling period, and mammary gland tissue was obtained for morphometric analysis. No changes in milk composition were observed between control quarters and those infused with up to 10 µg of interleukin-2 per quarter, administered as either a single or multiple treatment. Quarters infused with a single 100-µg dose of interleukin-2 or three consecutive doses of 25 and 100 µg of interleukin-2 had significantly lower lactose concentrations; there was a concomitant increase in bovine serum albumin, pH, and SCC compared with preinfusion concentrations or with control quarters. Morphometric analysis of tissue demonstrated an increase in stroma, a decrease in luminal area, and a marked increase in the number of infiltrating leukocytes in those quarters infused with the higher doses of interleukin-2. Results suggest that interleukin-2 can be intramammarily infused at doses as high as 10 µg per quarter without adversely affecting milk quality or normal mammary gland function.

(Key words: interleukin-2, mammary tissue, immunopathology)

Abbreviation key: bIL-2 = recombinant bovine interleukin-2, BSA = bovine serum albumin, IL-2 = interleukin-2, WBC = white blood cell counts.

INTRODUCTION

The term cytokine describes a group of glycoproteins produced by a spectrum of immune and nonimmune cells under diverse circumstances. This heterogeneous group of proteins has the capacity to modulate the proliferation, differentiation, and activation of cells participating in all aspects of immune and inflammatory processes. The immunomodulatory capacity of the cytokine network is complex. The cell-regulating activity of individual cytokines can interact with other cytokines in a synergistic, additive, or antagonistic fashion on multiple cellular targets. Although the in vitro activities of some cytokines indicate tremendous potential to be used as immunotherapeutic agents, it remains to be seen whether these proteins will be beneficial in disease control under in vivo conditions (8).

Interleukin-2 (IL-2) was originally described as T-cell growth factor (8). It is primarily produced by T cell of the helper phenotype and is responsible for clonal expansion of the initial T-cell immune response and establishment of immune memory following mitogenic or antigenic stimulation (19, 20). Interleukin-2 also plays a role in B-cell growth and differentiation, enhancing thymocyte proliferation, activation of natural killer cells, and
inducing cytotoxic T-cell activation (1, 10). Numerous studies with rodents and human lymphocytes suggest that IL-2 can induce or enhance production of a variety of other cytokines, such as B-cell growth factors, colony-stimulating factors, and interferon-γ (5, 10, 12). Studies in mice have shown that IL-2 may protect against lethal challenge with Gram-negative bacteria (4). The diverse functions of IL-2 in orchestrating the immune response suggest that this cytokine may be an important factor in augmenting compromised or suppressed immune systems in domestic food animals.

Most research on the potential therapeutic benefits of IL-2 has been conducted in murine and human systems. Interleukin-2 has especially gained attention as an agent for the treatment of some forms of cancer and immunodeficiency syndromes (9, 17). Although IL-2 therapy can mediate the partial regression of established tumors, there is evidence that infusion of high pharmacologic doses of recombinant IL-2 can cause toxic side effects (7, 17). Very little information is available describing the potential benefits and clinical symptoms of IL-2 therapy in animal health. Beneficial adjuvant effects of recombinant bovine IL-2 (bIL-2) have been demonstrated in cattle vaccinated against bovine herpes virus (16). Systemic administration of higher doses of bIL-2 caused toxic side effects typical of IL-2, which included diarrhea and mild fever during the period of cytokine treatment (16).

It also may be possible to augment bovine mammary gland immune defenses with bIL-2 treatment and to increase resistance to new intramammary infections. Recent studies have shown that intramammary administration of bIL-2 can enhance cellular immune responses in quarters infected with Staphylococcus aureus (14). There also is evidence that IL-2 may enhance the antibacterial capacity of bovine mammary gland lymphocyte populations and increase the resistance of the mammary gland to bacterial infections in a nonspecific manner (21). However, there is no information available describing the optimal intramammary dose of bIL-2 that would enhance local immunity without resulting in toxic side effects, which may decrease the productive efficiency of the cytokine-treated animals. The purpose of this study was to describe the physiologic changes that occur in healthy bovine mammary glands following intramammary infusion of increasing doses of bIL-2.

MATERIALS AND METHODS

Experimental Design

Eighteen late lactation dairy cows from the University of Saskatchewan research herd were used to evaluate the effects of either a single (trial 1) or multiple (trial 2) bIL-2 treatment on local physiological parameters in the mammary gland. Cows had milk from each quarter sampled routinely throughout their lactation for bacteriological analysis and SCC. Animals used in this study had no history of mastitis and had SCC of <250,000/ml before experimental manipulation.

In trial 1, right front and rear quarters of 6 cows were infused intramammarily with four doses of bIL-2 (1, 10, 25, and 100 µg per quarter) administered as a single treatment. Twelve cows were used in trial 2; right front and rear quarters were intramammarily infused with four doses of bIL-2 (1, 10, 25, and 100 µg per quarter) given in three consecutive treatments at 12-h intervals. The bIL-2 was diluted in sterile pyrogen-free saline and administered in 10-ml volumes. The left udder halves of all cows were intramammarily infused with 10 ml of sterile pyrogen-free saline and served as within-animal controls.

Milk samples for bacteriological examination and compositional analyses were collected from each quarter prior to any treatment and at 12, 24, 36, and 48 h following initial infusions to monitor physiological changes in the mammary glands. Venous blood samples were collected at these times in heparinized vacuum tubes (Becton Dickinson, Rutherford, NJ) to determine total and differential white blood cell (WBC) counts. Mammary glands were examined, and rectal temperatures were recorded, at each sampling period to monitor the possible development of local or systemic clinical signs of toxicity. All animals in trial 2 were slaughtered immediately following the 48-h sampling period, and mammary gland parenchyma tissue was obtained from each quarter for morphologic examination.

Recombinant Bovine Interleukin-2

The bIL-2 was expressed in Escherichia coli and purified to homogeneity by SDS-
PAGE. The stock solutions, provided by CIBA-GEIGY Limited (St. Aubin, Switzerland), had a specific biological activity of 3600 units/μg as determined by the ability of this product to induced proliferation of IL-2-dependent bovine cell line. The purified bIL-2 contained less than .01 μg/ml of endotoxin as determined by the Limulus aneocyte lysate test (Whittaker Corp., Walkersville, MD).

### Compositional Analyses

Quarter milk samples were processed and examined for microbial growth (2) immediately following collection. Concentrations of bovine serum albumin (BSA) were quantified in mammary secretion samples by electroimmunodiffusion on cellulose acetate plates (24). Rabbit anti-BSA and BSA standards were purchased from Sigma (St. Louis, MO). Milk SCC were determined using a Fossomatic Cell Counter (Foss Electric Ltd., Hillerod, DK), and concentration of lactose was determined using a Fossomatic Milk Analyzer (Foss Electric Ltd.). Assays were performed on 30-ml milk samples preserved with potassium dichromate at the Provinclal Dairy Laboratory (Regina, SK, Can.).

### Morphometric Analysis

Tissue samples obtained at slaughter were fixed in 2.5% glutaraldehyde in .1 M cacodylate buffer (pH 7 at 37°C) for 2 h. Tissue was then postfixed in .1 M cacodylate-buffered osmium tetroxide (pH 7 at 5°C) for 1.5 h, dehydrated in a graded series of ethanol, and embedded in epoxy resins. Thick sections (.5 to 1 μm) were stained with toluidine blue for light microscopy. Ultrathin sections approximately 60-nm thick were stained with 5% uranyl acetate in 50% methanol for 20 min followed by .4% lead citrate for 10 min.

Quantitative morphometric analysis was used to determine percentage of mammary tissue area composed of epithelium, lumen, and stroma (22). Tissue specimens of mammary parenchyma were examined to quantify numbers and type of infiltrating leukocytes. For each tissue sample, three replications of 1036 contact points were counted per section (3108 contact points per sample) at a magnification of 2200 x.

### Statistical Analysis

Data were analyzed by least squares analysis of variance using the general linear models procedure of SAS (SAS Institute Inc., Cary, NC) to determine the effects of bIL-2 dose and of time after infusion on composition of mammary gland secretion, WBC, rectal temperature, and on morphological parameters. Statistical analysis included effect of cow, treatment, time after treatment, and the interaction of treatment by time. Preplanned comparisons of least squares means from the overall model were made by the pairwise t test. Means were contrasted between treatment groups within a time period and between time periods within a treatment group. No other comparisons were made.

### RESULTS

#### Trial 1: Dose Titration of a Single bIL-2 Treatment

All quarters used in trial 1 were free of mastitis at the start of the study, and all remained uninfected during the trial. Intramammary infusion of a single dose of bIL-2 did not cause any gross abnormalities of milk or induce swelling in infused quarters. No change in total or differential WBC counts were observed in any animal for the duration of the study. Rectal temperatures stayed within the normal range during the entire treatment period (data not shown).

Changes in mammary gland secretion composition following a single intramammary infusion of bIL-2 are summarized in Figures 1 to 4. Pretreatment mean SCC for all quarters in trial 1 were less than 250,000 cells/ml. Mean SCC in placebo-treated quarters and in those treated with .1, 1, and 10 μg of bIL-2 did not differ from pretreatment values throughout the trial. The SCC of quarters treated with 100 μg of bIL-2 increased significantly 12 h following the infusion and remained elevated for the rest of the trial (Figure 1). No changes in milk pH were noted following treatment at any point in the trial, even at the dose of 100 μg of bIL-2 per quarter (Figure 2).

Concentrations of BSA in milk from all quarters in trial 1 were similar prior to any treatment. The BSA profile of placebo-treated
INTRAMAMMARY INTERLEUKIN-2 INFUSION

Figure 1. Changes in milk SCC following a single treatment with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with .1 μg (n = 2), 1 μg (n = 2), 10 μg (n = 4), or 100 μg (n = 4) of bIL-2 or a saline placebo (n = 12) administered at the 0-h treatment period. **Significantly different from placebo (P < .01).

quarters and those infused with .1, 1, and 10 μg of bIL-2 did not change at any time following the treatment. The BSA concentration of milk obtained from quarters infused with 100 μg of bIL-2 increased significantly at 12 and 24 h following the treatment. Thereafter, concentrations of BSA in this high dose group decreased and were similar to pretreatment values (Figure 3).

Milk lactose concentrations for all quarters in trial 1 are shown in Figure 4. Lactose concentrations in milk from placebo-treated quarters and those treated with up to 10 μg of bIL-2 did not change over time compared with pretreatment values. In quarters treated with 100 μg of bIL-2, lactose concentrations decreased significantly by 12 h following infusion. Lactose concentrations remained depressed at 24 h before gradually increasing to baseline values (Figure 4).

Trial 2: Dose Titration of Multiple bIL-2 Treatments

All quarters used in trial 2 were free of mastitis at the start of the study, and all remained uninfected during the trial. Intramammary infusion of three doses of 1, 10, or 25 μg of bIL-2 did not cause any gross abnormalities of milk or induce swelling in the treated quarters. All quarters treated with three doses of 100 μg of bIL-2 showed visible signs of an aseptic inflammation by 24 h following the initial treatment. Treated quarters became swollen, warm, and sensitive to touch. Four of the six quarters treated with the highest dose of bIL-2 had clumps in the milk, as early as 24 h following the initial treatment, that persisted throughout the remainder of the treatment period. No change in total or differential WBC counts were observed in any animal, and rectal temperatures stayed within normal range during the entire treatment period (data not shown).

Changes in mammary gland secretion composition following three consecutive intramammary treatments with recombinant bovine interleukin 2 (bIL-2) are shown in Figure 5. Mammary glands were infused with .1 μg (n = 2), 1 μg (n = 2), 10 μg (n = 4), or 100 μg (n = 4) of bIL-2 or a saline placebo (n = 12) administered at the 0-h treatment period. **Significantly different from placebo (P < .01).

Mammary infusions of bIL-2 are summarized in Figures 5 to 8. Pretreatment mean SCC for all quarters in trial 2 were less than 250,000 cells/ml. Mean SCC in placebo-treated quarters and in those treated with 1 and 10 µg of bIL-2 did not differ from pretreatment values during the treatment period. The SCC of quarters treated with three doses of 25 and 100 µg of bIL-2 increased significantly by 12 h following the initial treatment and remained elevated throughout the rest of the treatment period. There was a greater increase in SCC over pretreatment values at the 12- and 24-h sampling periods in those quarters infused with 100 µg of bIL-2 (P ≤ .001) compared with those treated with 25 µg of bIL-2 (P ≤ .05) (Figure 5). Changes in milk SCC of quarters treated with three doses of 100 µg of bIL-2 followed a trend similar to those treated with only a single dose of 100 µg of bIL-2.

Milk pH in placebo-treated quarters and in those treated with three doses of 1, 10, or 25 µg of bIL-2 did not change at any point following the initial treatment. The pH of mammary secretions from quarters treated with three consecutive doses of 100 µg of bIL-2 increased markedly by 24 h following the initial treatment and remained high for the rest of the treatment period (Figure 6).

Milk BSA concentrations from all quarters in trial 2 are shown in Figure 7. Concentration of BSA in placebo-treated quarters and in those treated with 1, 10, or 25 µg of bIL-2 did not change from pretreatment values during the treatment period. The BSA concentration of milk obtained from quarters treated with three doses of 100 µg of bIL-2 increased significantly by 12 h following the initial infusion. Bovine serum albumin content continued to increase until reaching peak concentrations at 36 h (12 h following the last infusion). By 48 h, BSA concentrations decreased but were.

Figure 4. Changes in lactose concentrations in milk following a single treatment with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with .1 µg (n = 2), 1 µg (n = 2), 10 µg (n = 4), or 100 µg (n = 4) of bIL-2 or a saline placebo (n = 12) administered at the 0-h treatment period. *Significantly different from placebo (P ≤ .05).

Figure 5. Changes in milk SCC following three consecutive treatments with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with 1 µg (n = 4), 10 µg (n = 8), 25 µg (n = 6), or 100 µg (n = 6) bIL-2 or a saline placebo (n = 24) administered at the 0-, 12-, and 24-h treatment periods. *Significantly different from placebo (P ≤ .05). **Significantly different from placebo (P ≤ .01).

Figure 6. Changes in milk pH levels following three consecutive treatments with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with 1 µg (n = 4), 10 µg (n = 8), 25 µg (n = 6), or 100 µg (n = 6) of bIL-2 or a saline placebo (n = 24) administered at the 0-, 12-, and 24-h treatment periods. **Significantly different from placebo (P ≤ .01).
Morphometric analysis of mammary gland parenchymal tissue following treatment with increasing concentrations of bIL-2 is summarized in Table 2. The total number of infiltrating leukocytes was significantly (P ≤ .01) higher in the quarters treated with 100 µg of bIL-2 compared with all other quarters. Macrophages and lymphocytes were the prevalent cell types in tissue obtained from most quarters used in trial 2. Numbers of macrophages, lymphocytes, and plasma cells in the placebo-treated quarters were similar to the quarters intramammary treated with bIL-2. In contrast, significantly more neutrophils were observed in mammary tissue obtained from bIL-2-treated quarters compared with controls. Those quarters treated with the highest doses of the cytokine (25 and 100 µg per dose) had the greatest increases in neutrophil counts. Only those quarters infused with three doses of 100 µg of bIL-2 had significantly more infiltrating eosinophils and fewer mast cells in mammary gland tissues compared with placebo-treated quarters. Prevalence of large numbers of neutrophils and eosinophils within the subepithelial stroma (Figure 10) and alveolar lumen (Figure 11) were often associated with nonactive alveolar epithelium. Neutrophils and eosinophils within the alveolar lumen were observed in various stages of luminal area compared with the placebo-treated group (Figures 9 and 10).

Figure 7. Changes in lactose concentrations in milk following three consecutive treatments with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with 1 µg (n = 4), 10 µg (n = 8), 25 µg (n = 6), or 100 µg (n = 6) of bIL-2 or a saline placebo (n = 24) administered at the 0-, 12-, and 24-h treatment periods. *Significantly different from placebo (P ≤ .05). **Significantly different from placebo (P ≤ .01).

Figure 8. Changes in lactose concentrations in milk following three consecutive treatments with recombinant bovine interleukin 2 (bIL-2). Mammary glands were infused with 1 µg (n = 4), 10 µg (n = 8), 25 µg (n = 6), or 100 µg (n = 6) of bIL-2 or a saline placebo (n = 24) administered at the 0-, 12-, and 24-h treatment periods. *Significantly different from placebo (P ≤ .05).
TABLE 1. Morphometric analysis of bovine mammary gland tissue following intramammary infusions of recombinant bovine interleukin-2 (bIL-2).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Epithelium</th>
<th>Lumen</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SEM</td>
<td>X</td>
</tr>
<tr>
<td>Placebo</td>
<td>37.40</td>
<td>1.83</td>
<td>26.03</td>
</tr>
<tr>
<td>1 µg</td>
<td>42.00</td>
<td>5.13</td>
<td>22.33</td>
</tr>
<tr>
<td>10 µg</td>
<td>40.67</td>
<td>3.65</td>
<td>29.61a</td>
</tr>
<tr>
<td>25 µg</td>
<td>33.38ab</td>
<td>4.51</td>
<td>24.88</td>
</tr>
<tr>
<td>100 µg</td>
<td>41.92</td>
<td>2.87</td>
<td>11.42</td>
</tr>
</tbody>
</table>

Means between treatment groups with different superscripts differ significantly (P ≤ 0.05).

Data are expressed as mean percentage ± SEM of total tissue area measured.

DISCUSSION

Degranulation and were proximal to necrotic or involuting secretory epithelium (Figure 12). Ultrastructural examination of leukocytes migrating towards the lumen revealed the displacement of the basal secretory cell plasma membrane from the underlying basal lamina (Figure 13).

There is evidence to suggest that altered IL-2 production contributes greatly to diminished immune capability, which can lead to the development of bacterial diseases (10) such as mastitis. In fact, recent studies have shown that colostrum samples obtained during the last week of gestation had low IL-2 activity, which correlates with the diminished immune cell function and increased susceptibility to mastitis during this period (23). The availability of large quantities of bIL-2 has made it possible to examine the potential role of this cytokine as an adjunct to mastitis control. Preliminary evidence suggests that therapeutic administration of bIL-2 may heighten both specific and nonspecific defenses of the mammary gland against bacterial infections (14, 21). However, IL-2 is extremely potent, requiring only minute quantities to exert a biological effect. Special care should be taken to select the appropriate dose that will induce the desired response without causing toxic side effects. Results from this study provide information concerning the maximum tolerable dose of bIL-2 that may be used to modulate mammary gland defenses without causing detrimental effects to the host.

TABLE 2. Cytologic analysis of leukocytes infiltrating bovine mammary gland tissue following intramammary infusions of recombinant bovine interleukin-2 (bIL-2).

<table>
<thead>
<tr>
<th>Cell types</th>
<th>0 µg</th>
<th>1 µg</th>
<th>10 µg</th>
<th>25 µg</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SEM</td>
<td>X</td>
<td>SEM</td>
<td>X</td>
</tr>
<tr>
<td>Macrophage</td>
<td>236.00ab</td>
<td>34.43</td>
<td>271.67ab</td>
<td>27.70</td>
<td>217.50b</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>238.25a</td>
<td>28.82</td>
<td>228.33a</td>
<td>32.32</td>
<td>273.60a</td>
</tr>
<tr>
<td>Plasma cell</td>
<td>41.69a</td>
<td>12.48</td>
<td>25.33a</td>
<td>5.34</td>
<td>22.30a</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>10.25c</td>
<td>6.53</td>
<td>86.67ab</td>
<td>18.19</td>
<td>96.70ab</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>.31b</td>
<td>.05</td>
<td>.33b</td>
<td>.03</td>
<td>.50b</td>
</tr>
<tr>
<td>Mast cell</td>
<td>18.75a</td>
<td>4.16</td>
<td>15.67ab</td>
<td>4.27</td>
<td>10.87a</td>
</tr>
<tr>
<td>Total</td>
<td>545.24ab</td>
<td>73.36</td>
<td>627.97b</td>
<td>13.20</td>
<td>621.40b</td>
</tr>
</tbody>
</table>

Means between treatment groups with different superscripts differ significantly (P ≤ 0.05).

Data are expressed as mean number ± SEM of cells infiltrating the total tissue area measured.

Monitoring changes in milk composition can provide an accurate indicator of the mammary gland's functional integrity following bIL-2 treatment. Recombinant bovine IL-2 can be intramammaryly infused as either a single or multiple treatment at doses as high as 10 μg per quarter without altering normal mammary gland secretion composition in the treated quarters. In contrast, intramammary infusion of a single 100-μg dose or three consecutive doses of 25 and 100 μg of bIL-2 led to changes in milk composition that are normally associated with mastitis and reduced milk production (11, 18, 24). Elevated levels of SCC, pH, and BSA in milk at these higher doses reflect disruption of the cellular integrity of parenchyma tissues and increased permeability of the blood-milk barrier (18, 24). Lower lactose concentrations in those quarters treated with 25 and 100 μg most likely indicate impaired synthetic and secretory activity of mammary epithelial cells compared with preinfusion concentrations (13, 18, 24). Because lactose is an osmotic regulator of milk production, disruption of lactose biosynthesis can have a pronounced effect on lactational performance (13). These changes in milk composition accompanied an aseptic inflammatory response that persisted throughout the duration of the trial. Because animals were euthanatized 24 h after the last treatment for tissue collection, it is not known how long the clinical symptoms would have continued or what effect the treatment may have had on subsequent milk production. However, previous studies have shown that, if bacterially induced clinical mastitis persists for extended periods of time, secretory tissue can regress and be replaced.
with scar tissue (18, 22). Intramammary infusion of toxic levels of bIL-2 may cause similar pathological alterations to mammary secretory tissues.

Histological parameters characterizing the functional capacity of mammary gland parenchyma tissue were compared with the composition of milk as a comprehensive indicator of mammary gland function following intramammary bIL-2 treatment. Tissue from quarters treated with three doses of 100 pg of bIL-2 showed less synthetic and secretory ability compared with control quarters, which is consistent with the changes observed in secretion composition. Luminal spaces expand in normal healthy mammary gland tissues because of accumulation of mammary secretion, whereas stromal areas tend to decrease to compensate for the displaced alveolar area. Tissues exposed to the highest doses of bIL-2 exhibited significantly less accumulation of milk product in the alveolar lumen, and stromal areas were larger proportionately to compensate for the reduced luminal area. Similar morphological changes in lactating bovine mammary tissues have been described following natural and experimental B. aureus mastitis (6, 22). These results suggest that therapeutic administration of large doses of bIL-2 can be toxic to the treated glands and possibly cause pathologic damage to the delicate secretory tissues similar to damage from some mastitis-causing organisms.

Tissue specimens of mammary parenchyma were used to estimate the prevalence of infiltrating leukocytes. Elevated leukocyte numbers were observed in tissue from those quarters treated with the highest dose of bIL-2 with significant increases in neutrophil and eosinophil counts. Infiltration of leukocytes, particularly neutrophils, into tissues and milk has been shown to be a major defense mechanism...

Figure 11. Mammary tissue from quarters treated with high dose of interleukin-2 demonstrating extensive infiltration of secretory epithelium (E) and luminal area (L) with neutrophils and eosinophils (arrow heads). x600.

Figure 12. Numerous neutrophils and eosinophils (arrow heads) within the alveolar lumen (L) were observed in various stages of degranulation; E = epithelium. x600.
of the mammary gland against bacterial invasion (15). However, there is considerable evidence that an acute inflammation of the mammary gland can damage the synthetic and secretory potential of mammary epithelium (3, 6, 11, 22). Several previous researchers assessed damage to mammary tissue of lactating cows following neutrophil migration and degranulation in the mammary gland. Extensive leukocyte penetration of mammary parenchyma tissue contributed to the sloughing of epithelium from the underlying basal lamina (3, 6, 22). The release of lysosomal hydrolytic enzymes proximal to delicate secretory cells was also associated with pathological alterations in parenchyma tissue areas (3). In the present study, similar immunopathologic alterations were noted in those quarters infused with three consecutive doses of 100 μg of bIL-2. Neutrophils and eosinophils were observed frequently within the luminal spaces, proximal to secretory epithelium, and lodged between the basal secretory cell cytoplasm and the underlying basal lamina. This association may be responsible for the deleterious effects that high doses of bIL-2 appear to have on mammary tissue structure and function.

Parenteral administration of high pharmacologic doses of recombinant IL-2 in both man and animals cause clinical symptoms usually associated with infection and inflammation (7, 9, 16). Jesmok and Gunther (7) recently reported that continuous intravenous administration of high doses of IL-2 caused changes in blood flow, increased vascular permeability, and increased the margination and emigration of leukocytes. These symptoms of IL-2 toxicity may explain the localized inflammatory response observed in the present study following intramammary infusion of high doses of bIL-2. The mechanisms by which high doses of bIL-2 induce swelling of treated quarters and augment recruitment of neutrophils and eosinophils into mammary tissues and milk are unknown. However, both in vitro and in vivo data suggest that IL-2 may be acting indirectly by causing the release of other inflammatory mediators such as IL-1, tumor necrosis factor, and interferon-γ (7, 9, 12). Although speculative at this point, the localized induction of these secondary mediators may be responsible for the detrimental symptoms of IL-2 therapy.

CONCLUSIONS
Results from this study suggest that the range between therapeutic and toxic doses of bIL-2 may be narrow. Additional information concerning the minimal biologically active dose of bIL-2 required to enhance local immune parameters would be beneficial when considering immunotherapeutic strategies for bovine mastitis.

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REFERENCES


