Relationship of Inflammatory Cytokines, Growth Hormone, and Insulin-Like Growth Factor-I to Reduced Performance During Infectious Disease (43933)

DALE E. SHUSTER,*[,1] MARCUS E. KEHRLI, JR.,* AND CRAIG R. BAUMRUCKER†

Department of Metabolic Disease and Immunology, National Animal Disease Center, U.S. Department of Agriculture—Agricultural Research Service, Ames, Iowa 50010, and Department of Dairy and Animal Science,† Pennsylvania State University, University Park, Pennsylvania 16802*

Abstract. **Production of inflammatory cytokines and concentrations of growth hormone and insulin-like growth factor-I (IGF-I) were studied during experimental *Escherichia coli* mastitis to determine their potential involvement in reduced animal performance during infectious disease. During the first 10 to 14 hr after intramammary infusion of *E. coli*, bacteria multiplied to maximum levels of 10^4–10^9 cfu/ml of milk with no clinical signs of mastitis. A rapid and intense inflammatory response, characterized by udder swelling, increased bovine serum albumin (BSA) and somatic cell count (SCC) in milk of infected glands, and elevated rectal temperature and serum cortisol concentration, began at approximately 12 hr after challenge. Lactational performance was reduced greatly at 24 hr, and the maximal decrease averaged 76% and 63% among infected and uninfected glands, respectively, of challenged cows; three cows became temporarily agalactic in all glands. By 6 days, all cows had nearly or completely eliminated the *E. coli*, and milk production had partially recovered. Milk composition showed an initial decrease in fat percentage followed by an increase thereafter. Protein percentage was increased and lactose content was reduced during most of the mastitic episode. High concentrations of tumor necrosis factor (TNF) and interleukin-1 (IL-1) were detected in milk of infected glands, and their appearance preceded or coincided with development of the mammary inflammation, systemic reaction, and hypogalactia. Serum growth hormone concentration was higher among challenged cows, whereas serum IGF-I concentrations changed little during the mastitic episode. Concentrations of IGF-I in milk whey increased from 5.0 to 12.2 ng/ml among infected glands and from 4.4 to 8.5 ng/ml among contralateral, uninfected glands; IGF binding proteins also increased in the milk of infected glands. These data demonstrate that (i) reduced lactational performance is not caused by reduced concentrations of growth hormone or IGF-I and (ii) inflammatory cytokines are produced at a time consistent with a possible role in the inhibition of milk synthesis.** [P.S.E.B.M. 1995, Vol 210]

Infectious disease among livestock effects large economic losses, estimated at several billion dollars per year in the United States. A primary cost is the reduced productive performance among afflicted animals. Mastitis is one of the most intensively studied livestock diseases and is the most costly disease of the dairy industry, with economic losses of approximately $2 billion in the United States annually (1). Costs associated with mastitis include reduced milk production and milk quality, veterinary costs, culling of afflicted cows due to damaged or chronically infected mammary glands, and death of cows. For this disease, more than 60% of economic losses are due to lost milk production (1). Lack of knowledge about pathophysiological causes of reduced animal performance hampers attempts to minimize adverse effects of infectious disease, including subclinical infections.

We have been studying the effects of mastitis on milk production of lactating dairy cows to gain a better

[1] To whom requests for reprints should be addressed at American Cyanamid Co., Quakerbridge and Clarksville Road, Princeton, NJ 08543-0400.

Received October 3, 1994. [P.S.E.B.M. 1995, Vol 210]
Accepted June 8, 1995.

0037-9727/95/2105-0000$10.50/0

understanding of the effects of infectious disease on animal physiology and performance. Mastitis provides a useful model for these studies because samples of inflammatory fluid can be collected repeatedly and noninvasively from the infection site. In addition, field studies have established that severe episodes of mastitis can readily cause temporary agalactia even in high-producing dairy cows, while subclinical mastitis also has an adverse effect on lactational performance (2, 3).

In our previous research using endotoxin-induced mastitis as an experimental model, we showed that much of the hypogalactia results from a systemic pathophysiological effect of mammary inflammation (4–7). Hormones of the somatotropic axis play an important galactopoietic role in ruminant lactation, and in addition, exogenous somatotropin partially corrects hypogalactia during *Escherichia coli* mastitis in cows (8). Therefore, hypogalactia during mastitis and perhaps other infectious diseases may be due to interference with the somatotropic axis. This hypothesis is supported by several other published observations. Cytokines are produced during bacterial infections and inflammation, and high concentrations of interleukin-1 (IL-1) and IL-6 are found during endotoxin-induced mastitis, while large amounts of tumor necrosis factor (TNF) are produced during *E. coli* mastitis (9, 10). Cytokines and endotoxic reactions induce a number of effects on the hypothalamus, anterior pituitary, and liver, so interference with the somatotropic axis is possible at many levels. Research in cattle has demonstrated suppressed plasma somatotropin concentrations following intravenous administration of endotoxin or recombinant bovine TNF (11). Subsequent work by these researchers showed a direct inhibitory effect of TNF on somatotropin release from the anterior pituitary *in vitro* and the presence of TNF receptors in the pituitary (12). Together, these observations suggest that mastitic hypogalactia may result from interruption of normal galactopoietic signals to the mammary gland, perhaps by cytokines.

The objective of the present study was to expand upon these observations and gain a better understanding of pathophysiological causes of mastitic hypogalactia by characterizing changes in lactational performance, concentrations of somatotropic hormones, production of cytokines, and measures of inflammation during experimental *E. coli* mastitis.

Materials and Methods

Cows. Holstein cows, between 1 month postpartum and 1 month before dry off, were clinically healthy, and all experimental mammary glands were free of bacterial infection prior to challenge. The experiment was conducted with nine cows challenged with *E. coli* and four unchallenged, control cows in five separate trials. Within each challenged cow, one randomly selected gland was inoculated, while the contralateral gland was used as the within-cow control gland. The remaining two glands were not sampled. One randomly selected gland was sampled in each unchallenged, control cow. An intramammary infection did not develop in one challenged cow, so all data from this cow were excluded from the results.

Cows were milked with a quarter milking machine twice daily throughout the experiment. A sample of bulk milk from individual glands was taken after milking for compositional analysis. Sterile foremilk samples (<50 ml) for bacteriological analysis were taken prior to each milking as well as at 2-hr intervals during the first 18 hr after challenge and then at 6-hr intervals for the next 18 hr. A portion of this milk was also used for somatic cell count (SCC) analysis. Another portion of foremilk was ultracentrifuged (48,000*g*, 40 min), and the whey was harvested and stored frozen (−20°C) for subsequent bovine serum albumin (BSA), cytokine, insulin-like growth factor-I (IGF-I), and IGF-binding protein analysis. Coccygeal blood samples were collected at the same times as the foremilk. Blood was allowed to clot at room temperature for 30 min and then refrigerated until serum was harvested by centrifugation for cortisol, cytokine, growth hormone, and IGF-I analyses. Blood and foremilk samples were collected more frequently early after challenge than at other times so that many of the parameters, which change rapidly during this time, could be followed more closely.

Treatments. *E. coli* MacDonald strain 487, a natural mastitis isolate, was prepared for intramammary inoculation as described by Erskine *et al.* (13). Within 2 days of an experiment, a fresh colony of *E. coli* that had grown for 24 hr on blood agar was used to inoculate 10 ml of Todd-Hewlett broth. This broth was cultured for 6 hr at 37°C and then refrigerated before dilution-plating to determine bacterial concentration. Approximately 2 hr before infusion, the broth culture was diluted in pyrogen-free phosphate buffered saline to give 15 colony forming units (cfu)/ml. Challenged glands were infused with 2 ml (30 cfu) of diluted *E. coli* following the morning milking. Control glands in both challenged cows were left untreated.

Assays. Milk samples for SCC or compositional analyses were preserved with bronopol and analyzed electronically at a collaborating Dairy Herd Improvement laboratory. Samples with high cell numbers were diluted 1:10 in phosphate buffered saline before analysis. Concentration of BSA in whey samples was measured by enzyme immunoassay (14). Serum cortisol was measured with a radioimmunoassay kit as described by the manufacturer (Endocrine Sciences, Tarzana, CA). IL-1 and TNF concentration in whey and serum samples were measured as described (9).

Serum growth hormone was assayed as described (15). IGF-I was assayed after samples were acid/ethanol extracted to remove interference from IGF-binding proteins (16). Radioimmunoassay was then performed using polyclonal antibody as described (17). Western ligand blots of IGF-binding proteins were performed on milk as described (18).

Statistical Analysis. Statistical comparisons between treatments were made by comparing area of response curves and values of maximal response for each parameter. Between cow treatment comparisons were made using Student's *t* test. Within cow treatment comparisons (i.e., infected glands versus contralateral, uninfected glands of the same cows) were made using paired *t* test.

Results

Bacterial Growth and Inflammatory Response. Following intramammary inoculation, *E. coli* grew rapidly in milk with roughly a 10-fold increase every 2 hr (Fig. 1). Bacteria grew uncontrolled for the first 10–14 hr after challenge, when no clinical signs were apparent and no response was evident in any of the inflammatory parameters. Peak bacterial concentration averaged 5.9 ± 0.7 log10 cfu/ml (Table I). After the onset of the mastitic response at roughly 12 hr (see below), bacterial concentration decreased. Within individual glands, changes in bacterial concentration were not always characterized by an initial maximum followed by a steady decrease; in most glands, the initial decline was followed by a partial relapse. However, *E. coli* were absent or present only in low numbers in all challenged glands by the end of the experiment, 6 days after challenge.

Udder swelling became apparent 10–14 hr after challenge and was the first clinical sign of mastitis. Concentrations of BSA in milk whey samples from infected glands increased shortly after udder swelling began (Fig. 2). Concentrations were somewhat elevated at 12 hr in two infected glands, but by 14 hr most infected glands exhibited high concentrations of BSA. Peak BSA concentration and area of the response curve were significantly higher among infected glands compared to either contralateral, uninfected glands of the same cows or glands of control cows (Table I and II). Concentrations of BSA increased from a basal level of 0.2 mg/ml to 0.5 mg/ml among contralateral, uninfected glands of challenged cows, and peak BSA concentration among these glands was significantly higher than among control glands (Table I). Leukocyte numbers in milk, as reflected by SCC, increased shortly after challenge (Fig. 2). However, this initial increase occurred in all glands regardless of infection status and probably resulted from normal variations in SCC between milkings. Leukocyte accumulation in milk in response to the intramammary infection did not appear until 16 hr after challenge when SCC of infected glands was first elevated relative to contralateral, uninfected and control glands. The peak SCC and the area under the response curve were significantly higher among infected glands compared with uninfected and control glands (Table I and II). The SCC in uninfected glands of challenged cows were not significantly elevated compared with control glands. The increase in SCC among uninfected and control glands after 24 hr compared with SCC before challenge is unexplained. This increase does not seem to be caused by spontaneous intramammary infection as other indicators of mastitis (e.g., BSA concentration) remained normal.

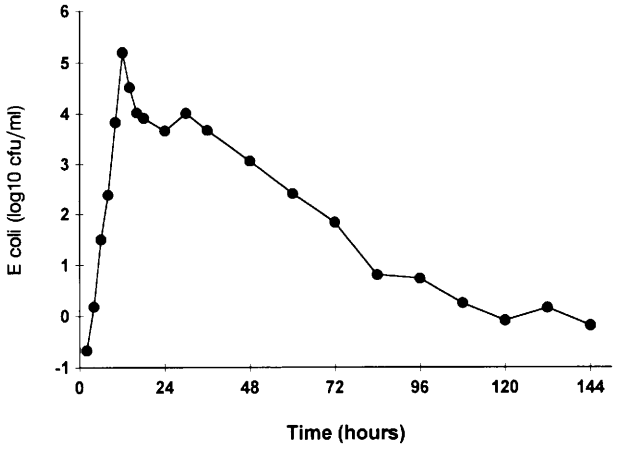

Figure 1. Bacterial numbers in foremilk samples during *E. coli* mastitis. One gland of each of eight cows was inoculated with *E. coli* immediately after milking (time = 0). Data are the geometric means.

A systemic reaction occurred in association with the mastitic episode. Rectal temperatures increased 2°C commencing 12–14 hr after challenge with a maximum at 16 hr (Fig. 3). Similarly, serum cortisol concentrations were markedly elevated with an average concentration of 94 ng/ml at 16 hr post-challenge (Fig. 3). Maximum values and areas of the response curves for temperature and serum cortisol among challenged cows were significantly increased compared to control cows (Table I and II). Other systemic clinical signs that were commonly observed in challenged cows at this time were lethargy and dyspnea. None of the cows became completely recumbent, and systemic signs had almost completely resolved by 24–30 hr.

Lactational Performance. Milk production initially averaged 12 kg/cow and 3 kg/gland at each milking. At 12 hr after intramammary inoculation, milk production was not significantly affected even though bacterial concentration exceeded 10^5 cfu/ml within infected glands (Fig. 4). However, milk production by challenged cows decreased to low levels between 24 and 60 hr after challenge with average minimums of only 24% and 37% of prechallenge production in in-

Table I. Maximum Responses within Glands and Cows Following Intramammary *Escherichia coli* Challenge

	Infected	Uninfected	Control
E. coli (log10 cfu/ml)	5.9 ± 0.7	NA	NA
BSA (mg/ml)	10.9 ± 1.6[a]	0.6 ± 0.2[b]	0.2 ± 0.1[c]
SCC (log10 cells/ml)	7.0 ± 0.1[a]	6.1 ± 0.2[b]	6.2 ± 0.4[b]
Temperature (°C)	41.0 ± 0.3[a]	NA	39.2 ± 0.2[b]
Cortisol (ng/ml)	110 ± 21[a]	NA	37 ± 13[b]
Milk production (%)	24 ± 11[a]	37 ± 13[a]	84 ± 4[b]
Fat at 24 hr (%)	2.2 ± 0.4[a]	3.5 ± 0.4[b]	3.1 ± 0.4[b]
Fat after 24 hr (%)	5.6 ± 0.7[a]	5.9 ± 0.6[a]	3.9 ± 0.3[b]
Protein (%)	5.0 ± 0.5[a]	4.4 ± 0.3[a]	3.4 ± 0.2[b]
Lactose (%)	2.0 ± 0.6[a]	3.5 ± 0.4[b]	4.6 ± 0.1[b]
TNF (U/ml)	5.09 ± 2.85[a]	0.65 ± 0.36[a]	1.48 ± 1.15[a]
IL-1 (log10 ng/ml)	1.23 ± 0.29[a]	0.05 ± 0.09[b]	0.18 ± 0.15[b]
Growth hormone (ng/ml)	12.3 ± 2.0[a]	NA	5.4 ± 1.6[b]
Serum IGF-I (ng/ml)	120 ± 5[a]	NA	128 ± 8[a]
Whey IGF-I (ng/ml)	13.8 ± 1.6[a]	10.0 ± 1.2[b]	5.1 ± 1.3[c]

Note. Data are the means ± SEM for the maximum response to intramammary challenge with *E. coli*. Infected, challenged cows or the challenged gland of these cows as appropriate for the parameter; Uninfected, contralateral, uninfected gland of challenged cows; Control, unchallenged cows or glands of unchallenged cows; NA, not applicable. Values within the same row with the same superscript do not differ ($P > 0.05$).

fected and uninfected glands, respectively (Table I). The maximum decrease and the area of the response curve for both infected and uninfected glands were significant compared with controls (Table I and II), and the decline as measured by area of the response curve was significantly greater among infected glands than among uninfected glands. Inhibition of milk production was related to intensity of the infection. Three cows became agalactic, and their bacterial counts were persistently above 10^6 cfu/ml, whereas all other cows peaked between 10^4 and 10^6 cfu/ml. At the end of the experimental period, milk production was 66% and 90% of normal in infected and contralateral, uninfected glands, respectively. Three of four cows that had cleared the infection by the end of the experiment were producing milk at or above 90% of previous yield. The most severely affected cows were producing milk at less than 60% of normal from infected glands and only 80% of normal from uninfected glands at the end of the experiment, even though bacterial numbers were less than 100 cfu/ml at this time.

Like milk yield, milk composition was normal at 12 hr after challenge, but was markedly altered by 24 hr in both infected and uninfected glands of challenged cows. Fat percentage of milk declined significantly among infected glands at 24 hr (Fig. 4 and Table I and II). This decline was followed by an increase in fat percentage of milk from both infected and uninfected glands. Protein content of milk increased in infected and uninfected glands of challenged cows beginning 24 hr after challenge (Fig. 5). Peak protein concentration was significantly greater for both infected and uninfected glands compared with controls (Table I), and area of the response curve was greater for infected glands than for uninfected or control glands (Table II). In contrast, lactose content of milk from infected glands decreased after challenge (Fig. 5). The magnitude of this decrease and the area of the response

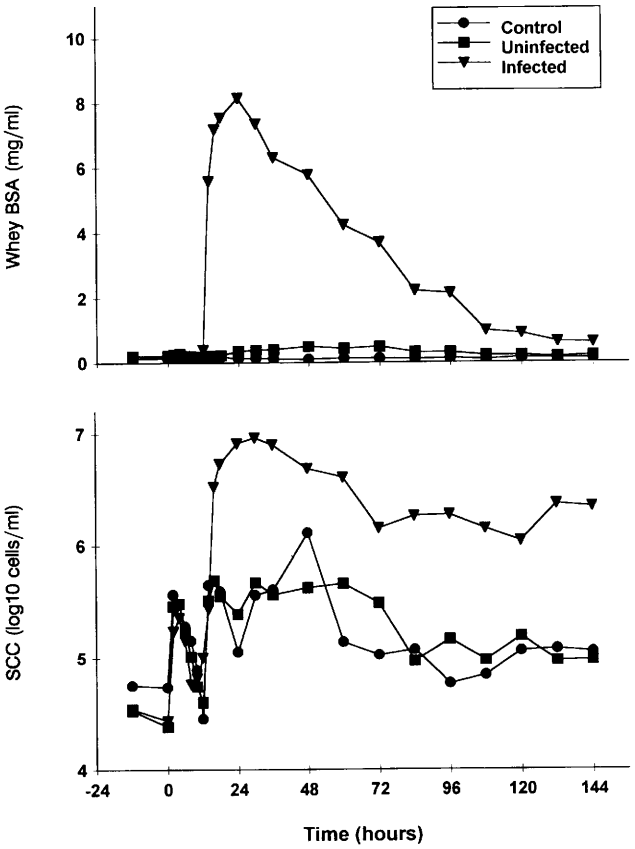

Figure 2. Concentration of BSA and SCC in foremilk samples during *E. coli* mastitis. Infected glands were inoculated with *E. coli* immediately after milking (time = 0). Data are means from the infected and contralateral, uninfected gland of each of eight challenged cows and one gland of each of four unchallenged, control cows.

Table II. Area of the Curve for Responses within Glands and Cows Following Intramammary *Escherichia coli* Challenge

	Infected	Uninfected	Control
E. coli (log10 cfu · hr/ml)	5.9 ± 0.7	NA	NA
BSA (mg · hr/ml)	394 ± 113[a]	14.5 ± 11.3[b]	−1.9 ± 1.5[b]
SCC (log10 cells · hr/ml)	175 ± 38[a]	86 ± 28[b]	50 ± 25[b]
Temperature (°C · hr)	12.9 ± 3.5[a]	NA	−4.4 ± 3.8[b]
Cortisol (ng · hr/ml)	958 ± 354[a]	NA	231 ± 159[b]
Milk production (% · hr)	−4670 ± 900[a]	−3000 ± 880[b]	−310 ± 420[c]
Fat at 12–24 hr (% · hr)	−8.0 ± 4.6[a]	0.1 ± 3.6[b]	0.5 ± 2.5[a,b]
Fat at 36–84 hr (% · hr)	24.3 ± 26.7[a,b]	49.1 ± 14.0[a]	8.3 ± 2.7[b]
Protein (% · hr)	59.8 ± 22.8[a]	17.2 ± 15.6[b]	5.6 ± 3.3[b]
Lactose (% · hr)	−144.2 ± 30.9[a]	−43.2 ± 18.8[b]	2.0 ± 3.4[b]
TNF (U · hr/ml)	29.1 ± 13.9[a]	4.1 ± 2.7[b]	5.9 ± 6.7[a,b]
IL-1 (log10 ng · hr/ml)	19.9 ± 6.7[a]	−3.6 ± 2.1[b]	2.2 ± 3.1[b]
Growth hormone (ng · hr/ml)	183 ± 92[a]	NA	−12 ± 39[a]
Serum IGF-I (ng · hr/ml)	120 ± 390[a]	NA	1270 ± 560[a]
Whey IGF-I (ng · hr/ml)	531 ± 99[a]	252 ± 64[b]	−9 ± 51[c]

Note. Data are the means ± SEM for the area of the response curve following intramammary challenge with *E. coli*. Infected, challenged cows or the challenged gland of these cows as appropriate for the parameter; Uninfected, contralateral, uninfected gland of challenged cows; Control, unchallenged cows or glands of unchallenged cows; NA, not applicable. Values within the same row with the same superscript do not differ ($P > 0.05$).

curve were significant compared with uninfected and control glands (Table I and II). Lactose concentration tended to be lower among uninfected glands compared with controls, but this difference was not significant.

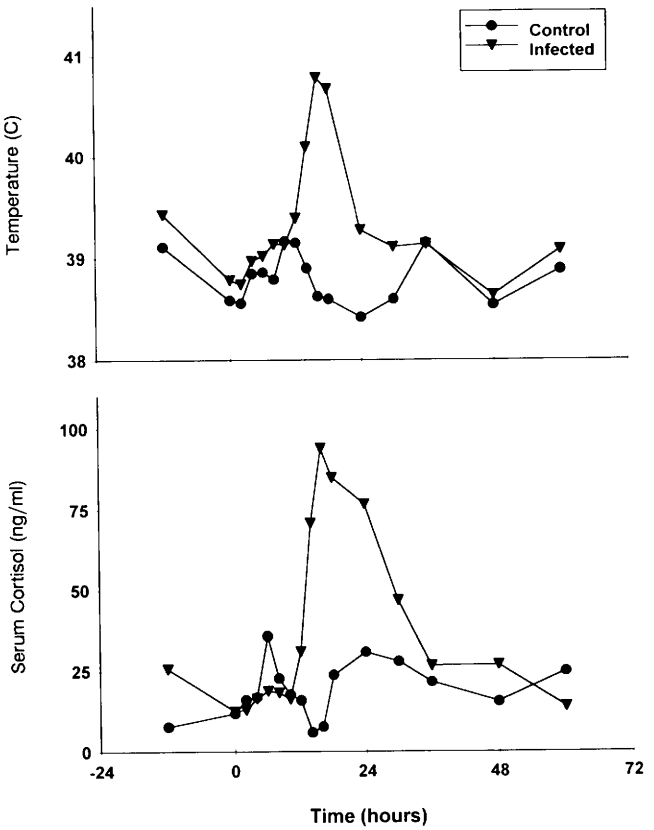

Figure 3. Rectal temperature and serum cortisol concentration of cows during *E. coli* mastitis. Infected cows were inoculated in one gland with *E. coli* immediately after milking (time = 0). Data are means from eight infected cows and four unchallenged, control cows.

Inflammatory Cytokines. Coincident with or preceding development of mammary inflammation, systemic reaction, and hypogalactia, elevated concen-

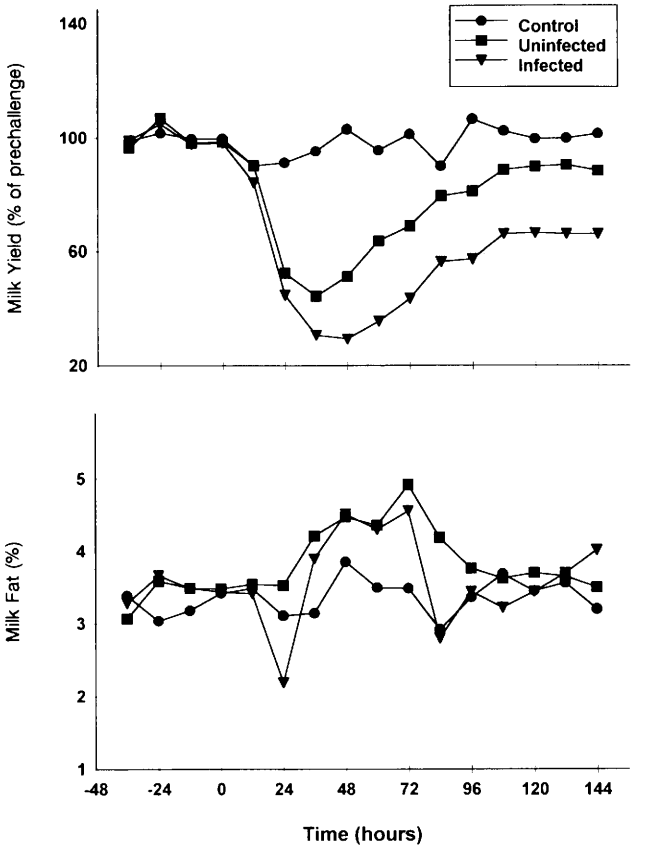

Figure 4. Milk yield and fat content of bulk milk from individual glands during *E. coli* mastitis. Infected glands were inoculated with *E. coli* immediately after milking (time = 0). Data are means from the infected and contralateral uninfected gland of each of eight challenged cows and one gland of each of four unchallenged, control cows.

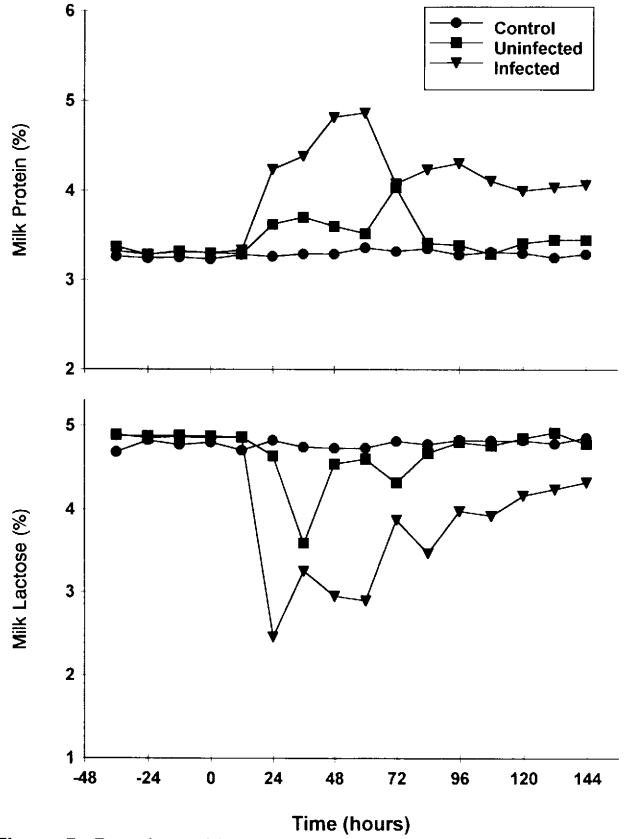

Figure 5. Protein and lactose content of bulk milk from individual glands during *E. coli* mastitis. Infected glands were inoculated with *E. coli* immediately after milking (time = 0). Data are means from the infected and contralateral, uninfected gland of each challenged cow and one gland of each unchallenged, control cow. Protein data are from eight challenged and four control cows, and lactose data are from six challenged and three control cows.

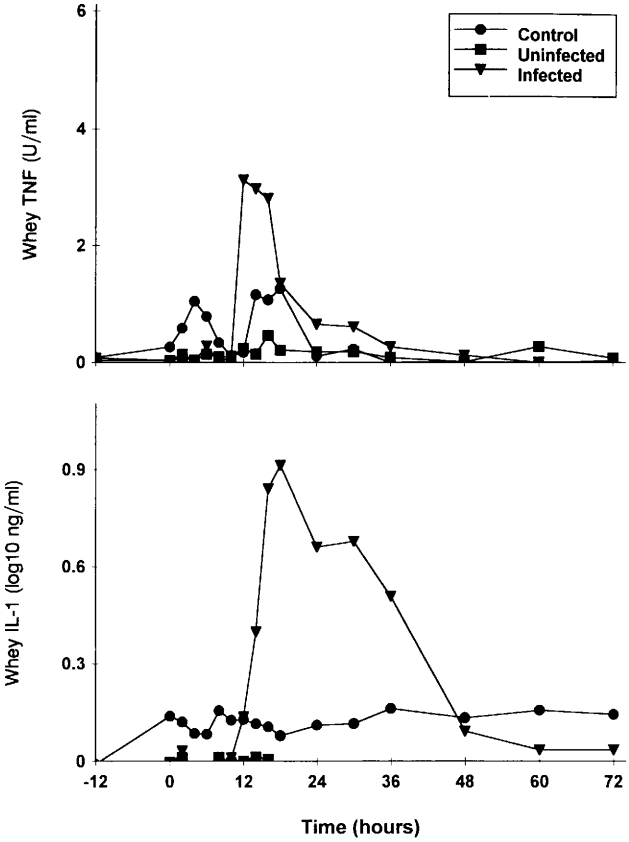

Figure 6. Tumor necrosis factor activity and interleukin-1 concentrations of foremilk wheys at various times relative to intramammary inoculation with *E. coli* at time 0. Data are means from the infected and contralateral, uninfected gland of each of eight challenged cows and one gland of each of four unchallenged, control cows.

trations of TNF and IL-1 were detectable in milk whey from infected glands (Fig. 6). In contrast to changes in milk production, which occurred in both infected and uninfected glands of challenged cows, cytokine responses were restricted to infected glands. The first cytokine to exhibit high concentrations in milk was TNF. Maximal concentrations of TNF were present in infected glands at 12–16 hr after challenge, and thus, coincided with onset of mammary inflammation. Because of the variability of the TNF response among infected glands and the unexplainable TNF-like activity in milk of some control cows at various times during the experiment, the only significant difference was the area of the TNF response curves between infected and contralateral, uninfected glands (Table I and II). Increases in IL-1 activity among infected glands began slightly after increases in TNF, and maximal concentrations were reached later (Fig. 6). The area of the IL-1 response curve and the maximal IL-1 concentration in infected glands were significant compared with uninfected and control glands (Table I and II). Concentrations of each cytokine were related to intensity of the infection; three of eight infected glands with the lowest concentration of bacteria had little or no detectable cytokine response, whereas glands with the highest cytokine responses also had the most severe infections. Serum samples were also analyzed for TNF and IL-1. Little or no activity was detectable in any of the samples, and no increase was observed in response to intramammary infection (data not shown).

Galactopoietic Hormones. Serum growth hormone concentrations increased in challenged cows with maximal concentrations at 30–48 hr after challenge (Fig. 7), coinciding with the time when milk production was minimal. Maximum growth hormone concentration was significantly greater among infected cows compared with control cows (Table I), though areas of the response curves were not different (Table II). In contrast, serum IGF-1 concentrations seemed unaffected during the mastitic episode (Fig. 7), and no difference was found between the two groups of cows (Table I and II).

Concentrations of IGF-1 in milk wheys increased in both infected and uninfected glands of challenged cows, with a significantly greater increase among infected glands (Fig. 8 and Table I and II). The time course of these changes generally paralleled increases

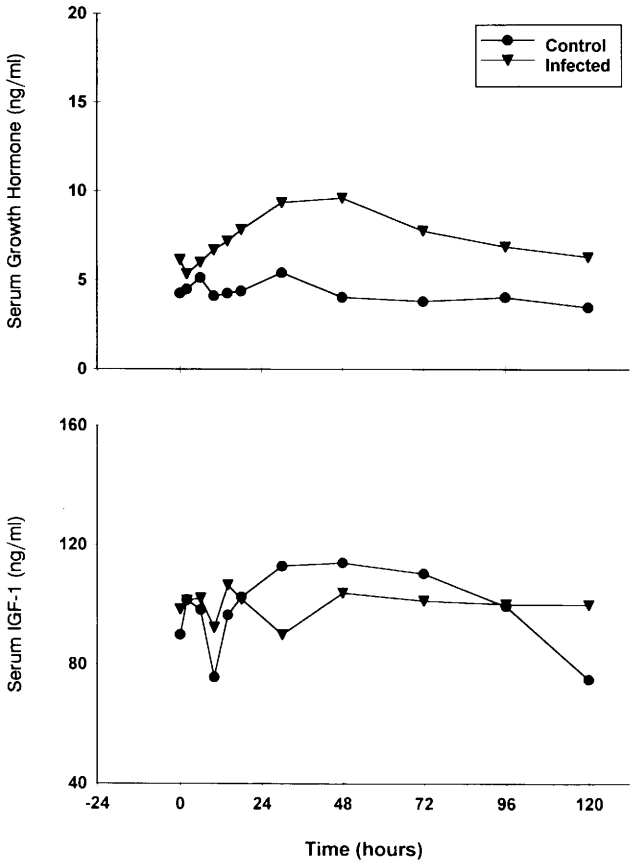

Figure 7. Serum growth hormone and IGF-I concentration of cows during *E. coli* mastitis. Infected cows were inoculated in one gland with *E. coli* immediately after milking (time = 0). Data are means from eight infected cows and four unchallenged, control cows.

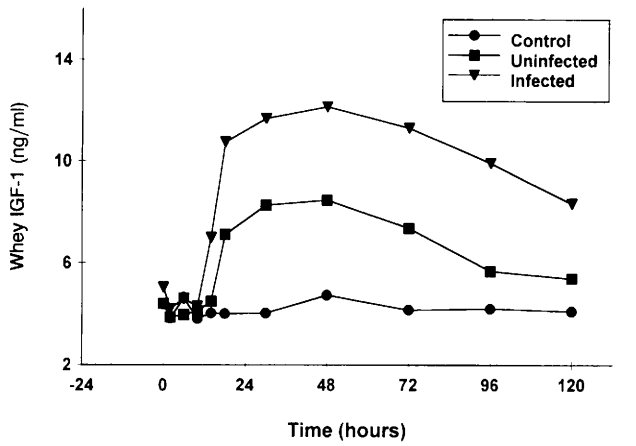

Figure 8. Concentration of IGF-I in whey from foremilk samples during *E. coli* mastitis. Infected glands were inoculated with *E. coli* immediately after milking (time = 0). Data are means from the infected and contralateral, uninfected gland of each of eight challenged cows and one gland of each of four unchallenged, control cows.

in BSA concentrations and decreases in milk production, but the relative magnitude of the IGF-1 increase among uninfected glands was much greater than the increase in BSA. To follow up on these observations, ligand blot analyses were performed on some of these wheys to investigate possible changes in IGF-binding protein levels. Figure 9 shows a representative blot of the pronounced increase in binding protein that occurs in wheys from infected glands at 18 hr and, unlike BSA concentrations, remains elevated at 96 hr. The uninfected glands within the same animals showed no apparent increase and were similar to blots conducted on whey from control cows (data not shown).

Discussion

These results corroborate previous studies of endotoxin-induced mastitis or experimental *E. coli* mastitis (4–7, 9, 13, 19, 20). Together, these studies demonstrate that inflammatory responses are limited primarily to infected glands. Udder swelling is one of the first clinical signs of mastitis, and this response is associated with breakdown of the blood: milk permeability barrier as indicated by a coincident increase in BSA concentrations in milk. Leukocyte recruitment, systemic reaction, and hypogalactia seem to develop somewhat later. In contrast to the localized nature of mammary inflammation, reductions in milk production, though greatest in inflamed glands, are very marked in uninflamed glands as well. Thus, a significant component of the hypogalactic effect of mastitis is apparently mediated through a systemic pathophysiologic effect. Lohuis *et al.* (20) have shown that the severity of systemic reaction is highly correlated to the degree of suppression of milk production. The normal level of milk yield at the first milking after challenge, when bacterial concentration is maximal, supports the hypothesis that milk production is inhibited as a consequence of inflammatory and systemic reactions, which developed later, rather than decreasing as a direct effect of the infection. The collective data show that hypogalactia during mild mastitic episodes is temporary, whether induced by low doses of endotoxin or *E. coli* infections that do not achieve high bacterial numbers.

Changes in milk composition are also consistent across these studies. An initial decline in fat percentage of milk is followed by elevations shortly thereafter. As described previously, these changes are attributable to earlier and less prolonged inhibition of milk fat synthesis relative to synthesis of other milk components (5). Protein content of milk is increased during mastitis, while lactose content is reduced. Some of these changes are explained by mixing of milk and blood components as serum proteins diffuse into milk (e.g., BSA) and lactose escapes from the mammary gland into blood, from which it is excreted into urine (7). Reduced synthesis of these milk components also occurs as their yields decrease markedly even in uninfected glands where permeability changes are small.

Study of galactopoietic hormones provided little

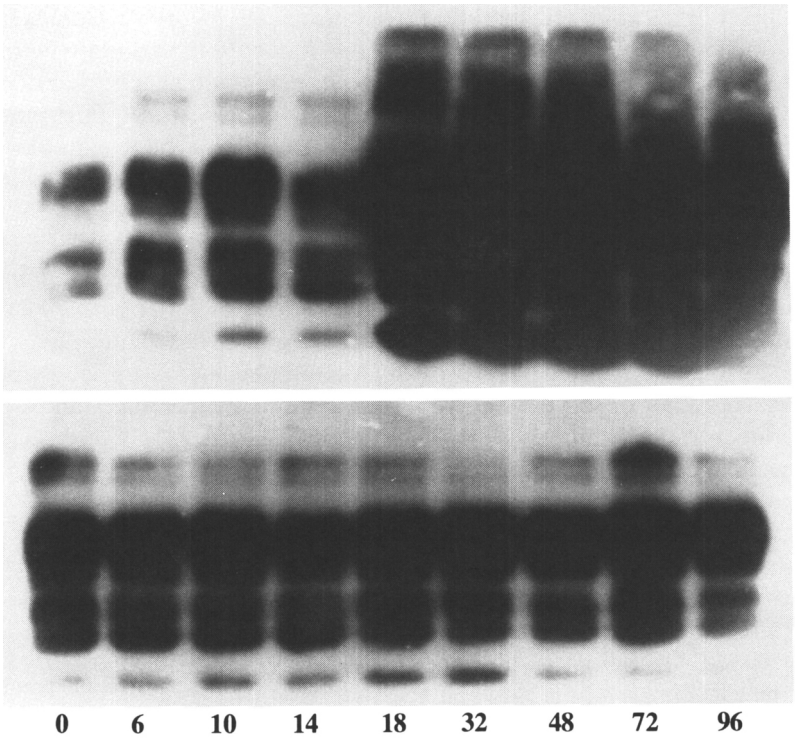

Figure 9. Representative ligand blot of IGF-binding proteins (IGFBP). Wheys from the infected (top) and contralateral, uninfected (bottom) gland were collected at the indicated times after *E. coli* challenge. Analysis of the whey indicated the presence of three major IGFBP bands. By molecular weight and sequence on the blot, IGFBP-3 (first major band) and IGFBP-2 (second major band) are clearly observed. The molecular weight of the remaining ligand blot band suggests that this is IGFBP-4. The minor bands may be the result of protease activity of other IGFBP. This blot is representative of results from four infected glands, three contralateral, uninfected glands and two control glands that were analyzed.

explanation for mastitic hypogalactia. Rather than decreasing, serum growth hormone concentrations actually increased, a result consistent with an earlier observation (21). Despite this increase in serum growth hormone, serum IGF-I concentrations were unchanged. Previous research has demonstrated that growth hormone and IGF-I concentrations remained unchanged in serum during endotoxin-induced mastitis (22), this difference is probably due to the greater severity of *E. coli* mastitis. Concentrations of IGF-I increased in milk whey of both infected and uninfected glands. This result agrees with an earlier observation that IGF-I increases in milk of endotoxin-infused glands (22). This increase can be partially explained by permeability changes, which would allow passive diffusion of IGF-I into milk from serum because of a concentration gradient that is nearly 25-fold higher in serum. Furthermore, this concept is supported by the lack of capacity of normal mammary epithelial cells to synthesize IGF-I (23). A concentrating effect of reduced milk volume could also contribute to the increase in whey IGF-I concentration (e.g., the 50% decrease in milk yield among uninfected glands could completely account for the 2-fold increase in IGF-I concentrations). This concentrating effect may be especially important in uninfected glands where permeability changes were slight. Permeability changes were likely part of the cause of the increased IGF-binding protein in milk of the infected gland as well. However, this hypothesis does not explain our observation that IGF-binding proteins were obviously elevated well after BSA levels had decreased toward normal levels. Another explanation may be increased synthesis of IGF-binding proteins, since mammary epithelial cells can synthesize and secrete IGF-binding proteins (24), and this synthesis is under regulatory control (18). Growth hormone concentrations have also been shown to increase in milk of *E. coli*-infected glands (25).

The significance of increases in galactopoietic hormone concentrations is unclear, but these increases probably would not contribute to hypogalactia. Their changes in milk seem to be an effect of mammary inflammation and hypogalactia rather than a cause. Vandeputte-Van Messom *et al.* (21) likened the increased serum growth hormone concentration during *E. coli* mastitis to that which occurs during endotoxemia and suggested that activation of the sympathetic nervous system may be the effector. No decrease in concentration of either growth hormone or IGF-I was

observed, so it seems unlikely that inhibited secretion of these hormones is a contributor to mastitic hypogalactia. However, growth hormone is normally secreted in a pulsatile manner (26). The present experiment has not ruled out the possibility that growth hormone pulses may have been interrupted, which might contribute to reduced lactational performance.

In this study, TNF and IL-1 were produced before or coincident with onset of systemic reactions and hypogalactia, consistent with our earlier demonstration of IL-1 production during endotoxin-induced mastitis (9). Sordillo and Peel (10) also demonstrated TNF production during *E. coli* mastitis and observed a correlation between TNF concentration and severity of mastitis; serum TNF was also detected in severely afflicted cows. In separate experiments, we also observed TNF in serum of cows with more severe *E. coli* mastitis (unpublished observations). Failure to detect cytokines in serum during the experiment reported here seems more likely a result of rapid clearance and inadequate assay sensitivity, than the actual absence of the cytokines in serum.

Several studies have demonstrated that inflammatory cytokines can elicit systemic reactions characteristic of coliform mastitis (27–29). Thus, cytokines may directly or indirectly mediate the systemic reaction and hypogalactia during mastitis. IL-1 and TNF are known to directly inhibit anabolic metabolism and stimulate catabolic reactions in many tissues, and these effects are thought to cause at least some of the decrease in growth performance during infectious disease (see comprehensive review by Klasing and Johnstone [30]). Thus, these cytokines may also directly alter metabolic activity of mammary tissue to reduce milk synthesis. In support of this hypothesis, Rejman *et al.* (31) demonstrated that recombinant bovine IL-1β inhibited proliferation of the bovine mammary epithelial cell line, MAC-T, *in vitro*. A direct hypogalactic effect of cytokines could explain the greater inhibition of milk synthesis among infected glands, compared with uninfected glands of the same cow. Cytokines are produced locally within mastitic glands and are present in high concentrations, whereas nonmastitic glands would only be exposed to the low concentration of cytokines absorbed into the circulation.

This experiment demonstrates that IL-1 and TNF are produced before or coincident with development of mammary inflammation, systemic reaction, and hypogalactia, and probably mediate these responses either directly or indirectly. Reduced lactational performance is not caused by inhibited secretion of hormones of the somatotropic axis, because serum growth hormone and whey IGF-I concentrations actually increased during *E. coli* mastitis in spite of marked hypogalactia.

Note added to proof: Cohick recently demonstrated that incubation of inflammatory cytokines with the bovine mammary epithelial cell line, MAC-T, increases synthesis of IGF-binding proteins (Cohick WS. Regulation of insulin-like growth factor binding protein synthesis in bovine mammary epithelial cells. J Dairy Sci **78** (suppl. 1):261, 1995). This observation and our finding that synthesis of IGF-binding proteins is increased in the infected mammary gland, where concentrations of inflammatory cytokines are high, suggests a causal association between local cytokine production and increased levels of IGF-binding proteins in the milk of mastitic glands.

The authors acknowledge the excellent technical assistance provided by Dan Valenciano, Scott Bernick, Ron Welper, Wendy Buffington, and Norm Tjelmeland. Dr. Mark Stevens provided very helpful technical support with the IL-1 assay. We thank J. L. Peters and C. Anne Gibson for their valuable contribution of performing the growth hormone and IGF-I assays, and IGF-binding protein ligand blots.

This work was supported in part by a grant from the Biotechnology Research and Development Corporation.

1. Blosser TH. Economic losses from and the national research program on mastitis in the United States. J Dairy Sci **62**:119–127, 1979.
2. Eberhart RJ, Hutchinson LJ, Spencer SB. Relationships of bulk tank somatic cell counts to prevalence of intramammary infection and to indices of herd production. J Food Prot **45**:1125–1128, 1982.
3. Raubertas RF, Shook GE. Relationship between lactation measures of somatic cell concentration and milk yield. J Dairy Sci **65**:419–425, 1982.
4. Shuster DE, Harmon RJ. Lactating cows become partially refractory to frequent intramammary endotoxin infusions: Recovery of milk yield despite a persistently high somatic cell count. Res Vet Sci **51**:272–277, 1991.
5. Shuster DE, Harmon RJ, Jackson JA, Hemken RW. Endotoxin mastitis in cows milked four times daily. J Dairy Sci **74**:1527–1538, 1991.
6. Shuster DE, Harmon RJ, Jackson JA, Hemken RW. Reduced lactational performance following intravenous endotoxin administration to dairy cows. J Dairy Sci **74**:3407–3411, 1991.
7. Shuster DE, Harmon RJ, Jackson JA, Hemken RW. Suppression of milk production during endotoxin-induced mastitis. J Dairy Sci **74**:3763–3774, 1991.
8. Vandeputte-Van Messom G, Burvenich C. Effects of somatotropin on changes in milk production and composition during coliform mastitis in periparturient cows. J Dairy Sci **76**:3727–3741, 1993.
9. Shuster DE, Kehrli ME Jr., Stevens MG. Cytokine production during endotoxin-induced mastitis in lactating dairy cows. Am J Vet Res **54**:80–85, 1993.
10. Sordillo LM, Peel JE. Effect of interferon-γ on the production of tumor necrosis factor during acute *Escherichia coli* mastitis. J Dairy Sci **75**:2119–2125, 1992.
11. Kenison DC, Elsasser TH, Fayer R. Tumor necrosis factor as a potential mediator of acute metabolic and hormonal responses to endotoxemia in calves. Am J Vet Res **52**:1320–1326, 1991.
12. Elsasser TH, Caperna TJ, Fayer R. Tumor necrosis factor-α

affects growth hormone secretion by a direct pituitary interaction. Proc Soc Exp Biol Med **198**:547–554, 1991.
13. Erskine RJ, Eberhart RJ, Grasso PJ, Scholz RW. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient and selenium-supplemented diets. Am J Vet Res **50**:2093–2100, 1989.
14. Shuster DE, Harmon RJ. Enzyme immunoassays of bovine lactoferrin and serum albumin in acid precipitated and ultracentrifugal wheys. J Dairy Sci **73**:3104–3111, 1990.
15. McAndrews JM, Stroud CM, MacDonald RD, Hymer WC, Deaver DR. Age-related changes in the secretion of growth hormone *in vivo* and *in vitro* in infantile and prepubertal Holstein bull calves. J Endocrinol **139**:307–315, 1993.
16. Bruce LA, Atkinson T, Hutchinson JSM, Shakespear RA, MacRae JC. The measurement of insulin-like growth factor 1 in sheep plasma. J Endocrinol **128**:R1–R4, 1991.
17. Etherton TD, Wiggins JP, Evock CM, Chung CS, Rebhun JF, Walton PE, Steele NC. Stimulation of pig growth performance by porcine growth hormone: Determination of the dose-response relationship. J Animal Sci **64**:433–443, 1987.
18. Skaar TC, Baumrucker CR. Regulation of insulin-like growth factor binding protein secretion by a murine mammary epithelial cell line. Exp Cell Res **209**:183–188, 1993.
19. Heyneman R, Burvenich C, Vercauteren R. Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced *Escherichia coli* mastitis in cows. J Dairy Sci **73**:985–994, 1990.
20. Lohuis JACM, Schukken YH, Verheijden JHM, Brand A, van Miert ASJPAM. Effect of severity of systemic signs during the acute phase of experimentally induced *Escherichia coli* mastitis on milk production losses. J Dairy Sci **73**:333–341, 1990.
21. Vandeputte-Van Messom G, Burvenich C, Massart-Leen AM, Fabry J. Endocrinological changes in newly calved cows during experimentally induced *E. coli* mastitis. In: Proc 7th Int Conf Prod Dis Farm Anim, Cornell University, Ithaca, NY, p346, 1989.
22. Bruckmaier RM, Schallibaum M, Blum JW. *Escherichia coli* endotoxin-induced mastitis in dairy cows: Changes and importance of insulin-like growth factor-I and oxytocin. Milchwissenschaft **48**:374–378, 1993.
23. Yee D, Paik S, Lebovic GS, Marcus RR, Favoni RE, Cullen KJ, Lippman ME, Rosen N. Analysis of insulin-like growth factor I gene expression in malignancy: Evidence for a paracrine role in human breast cancer. Mol Endocrinol **3**:509–517, 1989.
24. McGrath MF, Collier RJ, Clemmons DR, Busby WH, Sweeny CA, Krivi GG. The direct in vitro effect of insulin-like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. Endocrinology **129**:671–678, 1991.
25. Massart-Leen AM, Burvenich C, Vandeputte-Van Messom G, Roets E. Effect of acute mastitis and bovine somatotropin (BST) treatment on growth hormone and serum albumin in milk and blood of periparturient cows. Arch Int Phys Bioch **97**: P26–P27, 1989.
26. Gluckman PD, Breier BH, Davis SR. Physiology of the somatotropic axis with particular reference to the ruminant. J Dairy Sci **70**:442–466, 1987.
27. Goff JP, Naito Y, Kehrli ME Jr., Hayes P, Daley M. Physiologic effects of administration of interleukin-1β in cows. Am J Vet Res **53**:1983–1987, 1992.
28. van Miert ASJPAM, van Duin CTM, Wensing T. Fever and acute phase response induced in dwarf goats by endotoxin and bovine and human recombinant tumour necrosis factor alpha. J Vet Pharmacol Ther **15**:332–342, 1992.
29. van Miert ASJPAM, Kaya F, van Duin CTM. Changes in food intake and forestomach motility of dwarf goats by recombinant bovine cytokines (IL-1β, IL-2) and IFN-γ. Phys Behav **52**:859–864, 1992.
30. Klasing KC, Johnstone BJ. Monokines in growth and development. Poultry Sci **70**:1781–1789, 1991.
31. Rejman JJ, Turner JD, Oliver SP. Influence of recombinant bovine cytokines on proliferation of a bovine mammary epithelial cell line. Cell Biol Int **17**:619–621, 1993.