

Effect of Bovine Somatotropin on Neutrophil Functions and Clinical Symptoms During *Streptococcus uberis* Mastitis

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ABSTRACT

The effect of recombinant bovine somatotropin (bST) on the chemiluminescence, diapedesis, and expression of adhesion receptors (CD11a, CD11b, CD18) of isolated polymorphonuclear leukocytes was studied. The plasma concentrations of insulin-like growth factor-I (IGF-I), bST, cortisol, and α -lactalbumin were also monitored. In addition, general and local clinical symptoms and the differentiation of circulating leukocytes were also studied during experimentally induced *Streptococcus uberis* mastitis in cows. Ten cows were infected with 500 cfu of *S. uberis* O140J in both left quarters. Five cows were subcutaneously treated with 500 mg of recombinant bST 7 d before and after infection, and 5 control cows received the excipient. General (fever, tachycardia, inappetance, and depression) and local symptoms (swelling, pain, firmness, and flecks in milk) were more acute, severe, and longer-lasting in control cows. Treatment with bST had no effect on chemiluminescence and diapedesis of circulating polymorphonuclear leukocytes and no effect on the expression of adhesion receptors. Recombinant bST induced significantly higher IGF-I and bST concentrations in plasma. The leukopenia observed after infection was less pronounced in the bST-treated cows, and the number of circulating band neutrophils and metamyelocytes was significantly lower in the treated group. The concentration of cortisol did not differ between both groups, but the blood concentration of α -lactalbumin significantly increased in both groups from 6 d after infection. These results showed that treatment with recombinant bST improves animal welfare by protecting the cows from severe local and general clinical symptoms during subsequent *S. uberis* mastitis, but that it has no effect on

chemiluminescence, diapedesis, and the expression of adhesion receptors of circulating polymorphonuclear leukocytes.

(**Key words:** *Streptococcus uberis* mastitis, recombinant bovine somatotropin, diapedesis, chemiluminescence)

Abbreviation key: AUC = area under the curve, CL = chemiluminescence, DPBS = Dulbecco's PBS solution, HR = heart rate, MFI = mean fluorescence intensity, PMA = phorbol 12-myristate 13-acetate, PMN = polymorphonuclear leukocytes, RT = rectal temperature, ST = somatotropin, TNF- α = tumor necrosis factor alpha.

INTRODUCTION

Recombinant bST has been shown to protect cows from excessive milk production losses and compositional changes during a subsequent, experimentally induced *Escherichia coli* and *Streptococcus uberis* mastitis (25, 49). Recombinant bST also prevented severe local and general clinical symptoms in cows suffering from *E. coli* mastitis, especially in severe responders. Indeed, cows that had been identified as severe responders and previously treated with bST reacted similarly to moderate responders treated with the placebo. Prolactin, somatotropin (ST), and IGF-I are thought to be involved in several immune functions (13, 28, 30), and actions of bST can either be direct or indirectly mediated through IGF-I. The plasma and milk concentrations of IGF-I increase after bST administration (5, 8, 38, 51, 56). Massart-Leën et al. (35) reported an increased number of circulating leukocytes, band neutrophils, and an enhanced phorbol 12-myristate 13-acetate (PMA) induced respiratory burst activity in cows treated with bST after calving. The generation of reactive oxygen species, chemotaxis, random migration, phagocytosis of IgG-opsonized microorganisms, and the PMA-induced oxidative burst can be stimulated or primed by IGF-I and ST (4, 10, 11, 19, 20, 29, 52, 53), and

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the expression of complement receptors can be up-regulated. Increased chemotaxis and random migration (53), increased numbers of circulating neutrophils (7), and increased proliferation of granulocyte and monocyte precursors (36, 37, 44) have also been observed following ST and IGF-I elevations in vivo. Elvinger et al. (13) reported little or no effect on phagocytosis and killing of *E. coli* by circulating bovine polymorphonuclear leukocytes (PMN) or on cytochrome *c* reduction in vitro as well as in vivo. However, Heyneman et al. (22) observed an increased oxidative burst after in vivo administration of bST in healthy cows.

Thus, there is ample evidence that bST and IGF-I can modulate immune response of PMN. The purpose of this study was to investigate whether previous treatment with bST could reduce the severity of a subsequent experimentally induced *S. uberis* mastitis and whether the activity of circulating PMN could be improved in cows previously treated with bST.

MATERIALS AND METHODS

Cows

Ten East Flemish Red Pied cows that were in their first lactation, clinically healthy, and free of mastitis were selected. At the start of the experiment, the cows were between 6 and 15 wk postpartum. Before the study began, the mammary glands of the cows were examined clinically, and quarter milk samples were examined bacteriologically. The SCC was determined. Only those cows with an SCC lower than 2×10^5 /ml that were negative for major mastitis pathogens, such as staphylococci and Gram-negative bacteria, were selected for this study. Mean SCC before infection was $47 \times 10^3 \pm 6 \times 10^3$ /ml. During the experiment, the cows were housed in individual tie stalls. Cows were transferred to these stalls 1 wk before the start of the experiment to allow acclimation. Cows were fed a daily ration of approximately 8 kg of concentrate and had ad libitum access to water and hay. Cows were fed twice daily at 0630 and 1630 h, which did not overlap with sampling or treatment. Cows were milked twice daily at 0700 and 1700 h with a device that measures individual mammary gland quarter milk production. Mean milk production before the first treatment with bST or the excipient (d -7) was 16.8 ± 0.2 kg/d.

Inoculation Procedure

A phagocytosis-resistant *S. uberis* strain O140J (James Leigh, Compton, UK) (32, 33) was main-

tained in a lyophilization medium at -20°C . For experimental use, the bacteria were subcultured in Todd Hewitt broth (Lab M, Bury, UK) at 37°C for 18 h. After washing, the organisms were resuspended and diluted in pyrogen-free PBS. On d 0, 1 h after the morning milking, the cows were inoculated in the left front and left rear quarters with a suspension containing approximately 500 cfu of *S. uberis* O140J in a total volume of 20 ml of sterile pyrogen-free saline solution per quarter. The bacterial suspension was inoculated into the teat cistern using a sterile teat cannula. Before inoculation, the teat ends were disinfected with 70% ethanol containing 0.5% chlorhexidine. After the inoculation, each quarter was massaged for 30 s to distribute the bacterial suspension in the gland.

Experimental Design

All cows were experimentally infected with 500 cfu of *S. uberis* at d 0 of the experiment. Bovine ST treated-cows ($n = 5$) were injected subcutaneously into the ischioanal fossa with 500 mg of recombinant bST (Posilac[®]; Monsanto Co., St. Louis, MO) according to label instructions 7 d before infection (d -7) and 7 d after infection (d +7). Similarly, the control group ($n = 5$) was also subcutaneously injected with excipient only on the same days. A mixture (Nafpenzal[®]; Mycofarm Belga, Turnhout, Belgium) of sodium nafcillin (100 mg), sodium penicillin G (300,000 U), and dihydrostreptomycin sulfate (100 mg) was injected once daily into the infected quarters of all cows 48 h after the first appearance of clinical symptoms. This intramammary antibiotic treatment was continued for 3 consecutive d at 24-h intervals. The experiment was divided into four different periods for analyses: an 8-d period before the first bST treatment (d -7), a 7-d period between the first treatment and infection (d 0), a 7-d period after the infection and before the second treatment (d +7), and a 3-wk period after the second treatment.

Clinical Investigation of Cows

Rectal temperature (RT) and heart rate (HR) were recorded after the morning milking on d -8, -4, and -1 before infection. On the day of infection and on d +1, +2, and +3, RT and HR were recorded at 0800, 1400, and 2000 h. On d +4, +5, +6, +7, +8, +9, +14, +21, and +28, RT and HR were recorded once daily at 0800 h. Rumen motility was determined with a stethoscope each time the HR was recorded. The general behavior of the cows and their appetites were also observed each time the HR was measured.

The mammary gland was clinically examined once daily after the morning milking on d -8, -4, -1, +4, +6, +7, +8, +9, +14, +21, and +28. On d 0, +1, +2, and +3, the mammary glands were examined at 0800, 1400, and 2000 h. Firmness, pain, and swelling were monitored by palpation of the mammary gland, and the appearance of the milk was also recorded.

Determination of Blood Parameters

Blood samples were collected once daily after the morning milking on d -8, -4, -1, +4, +6, +7, +8, +9, +14, +21, and +28. On d 0, +1, +2, and +3, milk samples were taken three times daily after the morning milking at 1400 and 2000 h.

Blood cortisol concentration was measured according to the method of Eskola et al. (17) with the DELFIA[®] cortisol kit (Wallac Oy, Turku, Finland). This assay is a solid-phase, time-resolved fluoroimmunoassay based on the competitive reaction between europium-labeled cortisol and sample cortisol for a limited amount of binding sites on cortisol-specific, biotinylated monoclonal antibodies.

Blood serum α -LA was measured essentially as described by Akers et al. (2). Highly purified bovine α -LA (R. M. Akers, Virginia Polytechnic Institute and State University, Blacksburg) was radioiodinated by a chloramine-T procedure as described by Koprowski and Tucker (31). Its specific activity was 104 to 115 μ Ci/ μ g, and approximately 0.2 ng of ¹²⁵I-labeled α -LA was added per tube. Antiserum was also provided by R. M. Akers and was used at a final dilution of 1/25,000 (vol/vol). Serum samples were assayed at final dilutions of 1/20 (vol/vol) and 1/50 (vol/vol) (20 and 10 μ l, respectively). In addition, some samples that contained very high concentrations of α -LA were reassayed at a dilution of 1/100 (vol/vol). Bound α -LA was separated from free by second antibody (goat anti-rabbit IgG; Linco Research Inc., St. Louis, MO) precipitation. Intraassay and interassay coefficients of variation were 9.5 and 12%, respectively.

Concentrations of IGF-I in plasma were measured by radioimmunoassay according to the method previously described by Vicini et al. (51). Serum bST was measured by radioimmunoassay using an antiserum raised in rabbits (A/S batch R608-6/83; Monsanto Co). Intraassay and interassay coefficients of variation were 12 and 6%, respectively.

The number of circulating leukocytes was counted in an electronic cell counter (Coulter Counter ZF; Coulter Electronics Ltd., Luton, UK). Differential leukocyte counts were performed with a light microscope on smears of 1 drop of whole blood stained with eosin-giemsa (Hemacolor[®]; E. Merck, Darmstadt,

Germany), by counting 100 cells. The number of the different cell types in these 100 cells was expressed as a percentage.

Isolation of PMN from Blood

Blood (40 ml) was aseptically collected from the external jugular vein with evacuated tubes containing heparin (6 IU/ml) as an anticoagulant (Laboratoire EGA, Nogent-le Roi, France). The PMN were isolated according to a method previously described (27). Briefly, after the plasma layer was removed, the buffy coat and the top layer of the packed red blood cells were discarded. After a washing with 0.9% NaCl, the remaining red blood cells were lysed by adding 80 ml of double-distilled H₂O and gently mixing for 1 min. After restoration of isotonicity by the addition of 40 ml of 2.7% NaCl and washing, the final cell pellet was resuspended in 1 ml of Dulbecco's PBS solution (DPBS). After isolation, the cells were enumerated using an electronic cell counter (Coulter Counter ZF). The viability of PMN immediately after isolation was determined by trypan blue dye exclusion, and differential cell counts were performed on smears stained with eosin-giemsa (Hemacolor[®]). On average, 70 to 95% of the isolated cells were PMN with a viability of 97%. After counting and differentiating the cells, cell suspensions for the chemiluminescence (CL) assay were adjusted to a concentration of 5×10^6 viable PMN/ml in DPBS and stored on ice until use. For the diapedesis assay, PMN were suspended (10^7 PMN/ml) in Hanks balanced salt solution containing 0.1% BSA.

CL Assay of Blood PMN

Luminol-dependent cellular CL was used to measure the respiratory burst activity of isolated PMN according to the method previously described (26). A stock solution (0.1 M in dimethyl sulfoxide) of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, MO) was stored at -20°C and, immediately before use, was thawed and diluted to a concentration of 1 mM with DPBS. The respiratory burst of the isolated bovine PMN was activated by PMA (Sigma Chemical Co.) or by latex beads (Serva Feinbiochemika and Co. GmbH KG, Heidelberg, Germany) with a diameter of 0.7 to 0.8 μ m. A stock solution of PMA was prepared by dissolving 200 μ g in 1 ml of dimethyl sulfoxide and storing at -20°C. The ratio of PMN to latex beads was about 1:3. The CL assay was performed at 37°C with a microtiterplate luminometer (LB96P; EG&G Berthold GmbH, Bad Wildbad, Germany). The total

volume used in the wells was 200 μ l. The CL response was registered in duplicate for 30 min at 37°C after adding DPBS, 0.3 mM luminol, and 200 ng/ml of PMA or a suspension of 20×10^6 latex beads to the cell suspension (final concentration of 2×10^6 cells/ml). The area under the curve (AUC) was calculated for the registered impulse rates (counts per minute) over the entire 30-min measurement period.

The CL response was expressed as AUC over the 30-min measurement period. Not only the total response of all isolated cells, of which between 70 and 95% were PMN, was used for statistical analysis, but also the response corrected for the real number of neutrophils in each sample of isolated cells. Corrections were made by first expressing the CL response per 1000 PMN in the sample and consequently by correcting for the percentage of eosinophils in the sample by arbitrarily accepting that the CL response of eosinophils is five times higher than that of neutrophils (23). The following formula was used to perform these corrections:

$$\frac{\left[\frac{T}{400,000 \times \text{PMN}} \right]}{\left[\frac{N}{\text{PMN}} \right] + \left[\frac{E \times 5}{\text{PMN}} \right]}$$

In this formula, T = CL response, PMN = percentage of PMN in the isolated cell suspension, N = percentage of neutrophils, and E = percentage of eosinophils.

For statistical analyses, the AUC of the data, expressed as the AUC per 24 h, of the total CL response was calculated over seven time periods, one preinfection period, and six postinfection periods. Similarly, the AUC of the corrected CL data was calculated over seven time periods, one preinfection period, and six postinfection periods. All AUC were logarithmically transformed before analysis.

Cell Culture of Mammary Epithelial Cells and Diapedesis Assay

The system for culture of bovine mammary epithelial cells was developed according to the method previously described by Smits et al. (45, 46). Briefly, membrane inserts with a diameter of 12 mm and a pore size of 12 μ m (Millicell-PCF; Millipore, Bedford, MA) were coated with collagen by immersion in wells of 24-well plates (Nunc, Roskilde, Denmark) containing 1% calf skin collagen type I solution (Sigma Chemical Co.) and incubated for 48 h at 4°C. These inserts were then inverted in 6-well plates (Nunc) containing culture medium as described previously (45, 46). After adding bovine primary secretory epi-

thelial cells (3×10^5 per insert), the inserts were incubated for 7 d at 37°C in an atmosphere containing 5% CO₂ in air. Confluence of the epithelial monolayer was tested by measurement of the transepithelial electrical resistance using an ohmmeter (Millipore). Only monolayers with a resistance of $>1000 \Omega/\text{cm}^2$ were used for further experiments (45, 46).

The diapedesis assay was performed as previously described by Smits et al. (45, 46). Bovine PMN were isolated and suspended in Hanks balanced salt solution containing 0.1% BSA at a concentration of 1×10^7 cells/ml. Epithelial monolayers were rinsed extensively in Hanks balanced salt solution to remove media and residual serum components. The isolated PMN were added to the upper chamber of the inserts (basal side of the secretory cells). Purified bovine C5a-des-Arg (P. Rainard, Laboratoire de Pathologie Infectieuse et d'Immunologie, Institut National de la Recherche Agronomique, Nouzilly, France) was added to the lower chamber of the inserts (50 ng/ml of medium, apical side of the cells). Five hours after incubation at 37°C in an atmosphere with 5% CO₂ in air, the number of PMN undergoing diapedesis was counted in 50 μ l of the apical medium (lower chamber) using a hemocytometer. Isolated blood PMN from each cow were assayed in duplicate, and results were averaged.

Flow Cytometric Analysis of Bovine Adhesion Receptors

Blood samples (6 ml), collected aseptically in 4 ml of Alsever solution (Gibco BRL, Life Technologies Inc., Gaithersburg, MD), were incubated for 15 min at room temperature (20°C) with 50 μ l of monoclonal antibody to bovine CD11a (IL-A99; International Livestock Research Institute, Nairobi, Kenya), bovine CD11b (IL-A15; International Livestock Research Institute), and bovine CD18 (MF14B4; FUNDP, Namur, Belgium) or with RPMI 1640 medium (Gibco BRL). After this incubation, red blood cells were lysed with ice-cold sterile buffer solution (0.02 M Tris-hydroxymethyl-aminomethane and 0.14 M NH₄Cl in H₂O). After centrifugation ($200 \times g$, 10 min, 4°C), the leukocytes were washed twice in RPMI 1640 medium followed by an incubation with a secondary antibody solution (50 μ l of goat anti-mouse IgG conjugated with fluorescein isothiocyanate; Sigma Chemical Co.) for 30 min on ice in the dark. After centrifugation ($200 \times g$, 10 min, 4°C), the cells were washed twice with PBS, and the cell pellet was fixed by addition of 0.1 ml of paraformaldehyde (1%) in PBS and stored at 4°C until analysis.

The expression of the bovine CD11a, CD11b, and CD18 adhesion receptors was estimated by flow cytometry (FACScan; Becton Dickinson Immunocytometry Systems, San Jose, CA) at an excitation wavelength of 488 nm and emission wavelengths of 530 and 560 nm. The PMN were selectively gated for analysis by forward scatter (size) and side scatter (granularity). The mean fluorescence intensity (MFI) of the PMN gated in the forward scatter-side dot plot was used to quantify the expression of adhesion receptors. The fluorescence of PMN incubated with the secondary antibody but without the primary antibodies to CD11a, CD11b, and CD18 was used as the control for nonspecific fluorescence, which was subtracted from the fluorescence of the primary antibody incubated with PMN.

Statistical Analyses

Means, standard deviations, and standard errors of the means were calculated using Statistix® (47). Statistical analysis of all data was performed using an ANOVA (mixed linear model) with the following model: $Y = \mu + T + t + C/T + \text{Int1} + \text{Int2} + e$, where Y = dependent variable, μ = overall mean, T = treatment, t = time, C/T = cow nested within treatment, Int1 = interaction between treatment and time ($T \times t$), and Int2 = interaction between the cows nested within the treatment and time ($C/T \times t$), and e is the experimental error term. Treatment and time were fixed variables, and individual cows were randomized variables, which were nested within the treatment. To study the effects of treatment, the error term was C/T . To study the effects of time, the Int2 term was the error term. After running the ANOVA, general contrasts were calculated. Significant differences were determined at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

General Clinical Symptoms, RT, and HR

Until 12 h postinfection, no marked clinical symptoms were apparent. Loss of appetite, tachypnea, and general depression were only observed in some control cows, but rumen motility was not affected. Rectal temperature of all cows showed multiple peaks during 8 d in the control cows ($40.3 \pm 0.14^\circ\text{C}$) and during 2 to 3 d in the treated cows ($39.4 \pm 0.19^\circ\text{C}$) (Figure 1). One treated cow showed a RT $>40^\circ\text{C}$, but all control cows had RT $>40^\circ\text{C}$, and two control cows had RT $>41^\circ\text{C}$. Rectal temperature reached the first peak after 24 h in the treated group ($P < 0.05$) and the control group ($P < 0.001$) and the second peak after

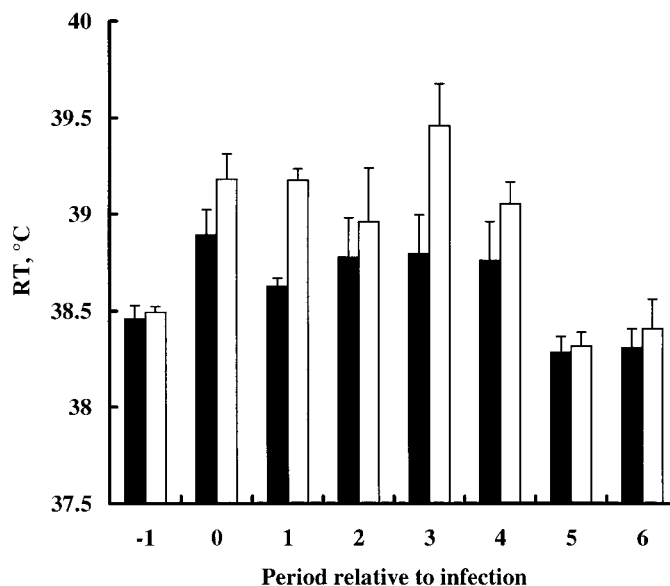


Figure 1. Changes in rectal temperature (RT) of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 0 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = 120 to 156 h after infection, 5 = d +7 to +9, and 6 = d +14 to +28.

84 h in the control group ($P < 0.01$). On d +5, RT started to decrease again ($P < 0.01$), but was still higher ($P < 0.01$) than before infection. From d +7, the RT became normal again in the control cows, and, from 3 d after infection, the RT became normal again in the treated cows. In the treated cows, no changes ($P > 0.05$) in the HR could be observed during the entire experiment (Figure 2). From 24 h after infection, control cows showed tachycardia with HR higher ($P < 0.001$) than before infection. On d +3 HR decreased slightly but remained higher ($P < 0.05$) than those before infection. Tachycardia continued in control cows until about 10 d after infection. The HR of the control cows was higher ($P < 0.001$) than that of the treated cows during the entire postinfection period.

Local Clinical Symptoms

About 6 h after infection, milk leakage was observed in the infected and contralateral quarters of 1 cow in each group. Mastitis symptoms became apparent after 24 h postinfection. The infected quarters were swollen and painful, and firmness was slightly increased. From 30 h postinfection, these symptoms became more prominent, and small flecks appeared in the milk of control cows. Most prominent symptoms

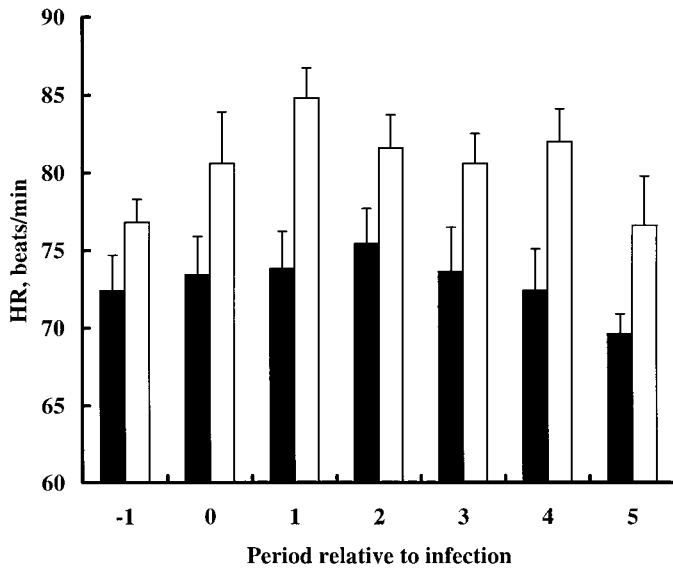


Figure 2. Changes in heart rate (HR) of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 0 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

were observed in 3 control cows and 1 treated cow. On d +3, the firmness, pain, and number of milk flecks increased in these cows, and the milk of the control cow with the most severe symptoms becoming watery and yellow. On d +4, local symptoms and the number of flecks further increased. All control cows and only 2 treated cows showed marked symptoms. On d +6, the glands of all control cows and 3 treated cows were very painful, swollen, and firm, and many flecks were present. The milk of 3 control cows and 1 treated cow became reddish. On d +9, the symptoms began to abate in most cows. Two weeks after infection, the health of the mammary gland of all cows, except 1 control cow, was markedly improved. At d +28, the infected quarters of some cows were still swollen and harder than before infection.

CL

The total CL response after stimulation of leukocytes by PMA slightly decreased ($P < 0.05$) in the control group between 24 and 48 h after infection (Figure 3A). Between 48 and 72 h after infection, CL was normalized, but between 72 and 84 h after infection, the response decreased in both the control ($P < 0.01$) and the treated group ($P < 0.001$). Thereafter, CL response increased ($P < 0.001$) in both groups. From d +9, the total CL response continued to in-

crease ($P < 0.01$) in the treated group and reached levels higher than those observed before infection ($P < 0.001$) in both groups.

The total CL response of leukocytes stimulated by latex increased ($P < 0.01$) in the treated group between 0 and 24 h after infection (Figure 3B). Between 48 and 72 h after infection, CL further increased ($P < 0.05$) in the treated group, but was still comparable to preinfection levels in the control cows.

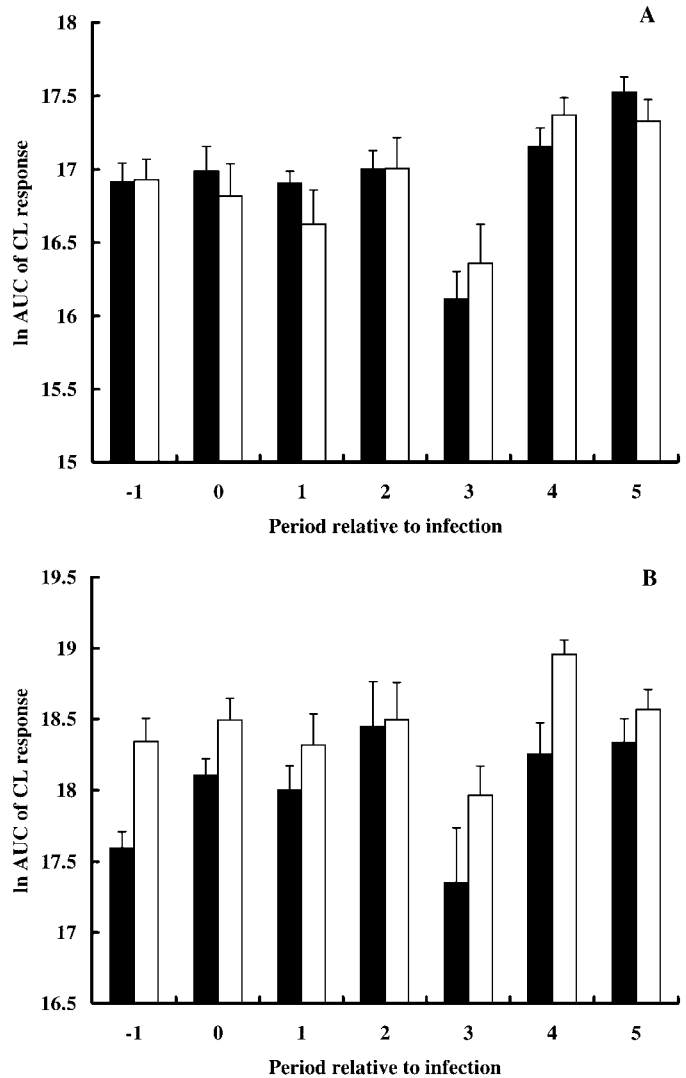


Figure 3. Chemiluminescence (CL) of the total isolated leukocyte population of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis after stimulation by phorbol 12-myristate 13-acetate (A) and latex beads (B). Data are means of the logarithmic transformation of the areas under the curves (AUC) of different periods for five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 0 to 24 h after infection, 1 = 24 to 48 h after infection, 2 = 48 to 72 h after infection, 3 = 72 to 84 h after infection, 4 = 84 to 216 h after infection, and 5 = d +9 to +28.

Between 72 and 84 h after infection, the CL response decreased in the treated ($P < 0.01$) and the control ($P > 0.05$) group toward preinfection values. In the period between d +3 and +9, CL increased ($P < 0.01$) in both groups to values that were higher ($P < 0.01$) than the preinfection values. From d +9, the total CL response after stimulation with latex beads was still higher than the preinfection response in the treated ($P < 0.001$) and control ($P < 0.05$) group.

The corrected CL response (Figure 4A) of the isolated neutrophils after stimulation by PMA did not change ($P > 0.05$) during the first 48 h after infection in either group. Between 48 and 72 h after infection, CL increased ($P < 0.05$) in the control group. From d +4 onward, the CL response started to increase ($P > 0.05$) in both groups toward values that were higher ($P < 0.05$) than the preinfection values in the treated cows. There were no differences between control and treated groups in any of the periods.

The corrected CL response of the isolated neutrophils after stimulation by latex beads (Figure 4B). Between 48 and 72 h after infection, CL increased ($P < 0.05$) in the treated group, but returned ($P < 0.01$) to normal values between 72 and 84 h after infection. Between d +3 and +9, the CL response in the control cows was higher ($P < 0.05$) than the preinfection response, but returned to preinfection values from d +9. From d +3, the CL response in the treated group was higher ($P < 0.01$) than the preinfection CL response.

Diapedesis

At d +21 after infection, the percentage of PMN undergoing diapedesis was higher ($P < 0.05$) in the control cows than in treated cows (Figure 5), but no differences between both groups were observable on any other day. During the first 24 h postinfection, diapedesis decreased ($P < 0.05$) by 83 and 80% in the treated and control groups, respectively. Normal baseline values were reached by the end of the study (d +28).

Expression of Adhesion Receptors

The expression of CD11a on circulating PMN for control and treated cows did not differ ($P > 0.05$) during the entire experiment (Figure 6). Between 24 and 48 h postinfection, MFI for CD11a decreased ($P < 0.01$) by 15% in the control group, which was the lowest MFI between 36 and 48 h postchallenge. From 60 h postinfection, the expression of CD11a started to increase ($P < 0.05$) in the control group, and baseline values were reached between d +4 and +6. In the treated cows, no changes were observed.

The expression of CD11b was higher ($P < 0.05$) in the control cows than in the treated cows between 24 and 48 h after infection (Figure 7). The MFI for CD11b in the control group ($P < 0.05$) increased by 15% to a maximum between 36 and 48 h after challenge and then gradually decreased. The MFI was reduced by 15 and 13% ($P < 0.01$; d +6 and +9) and

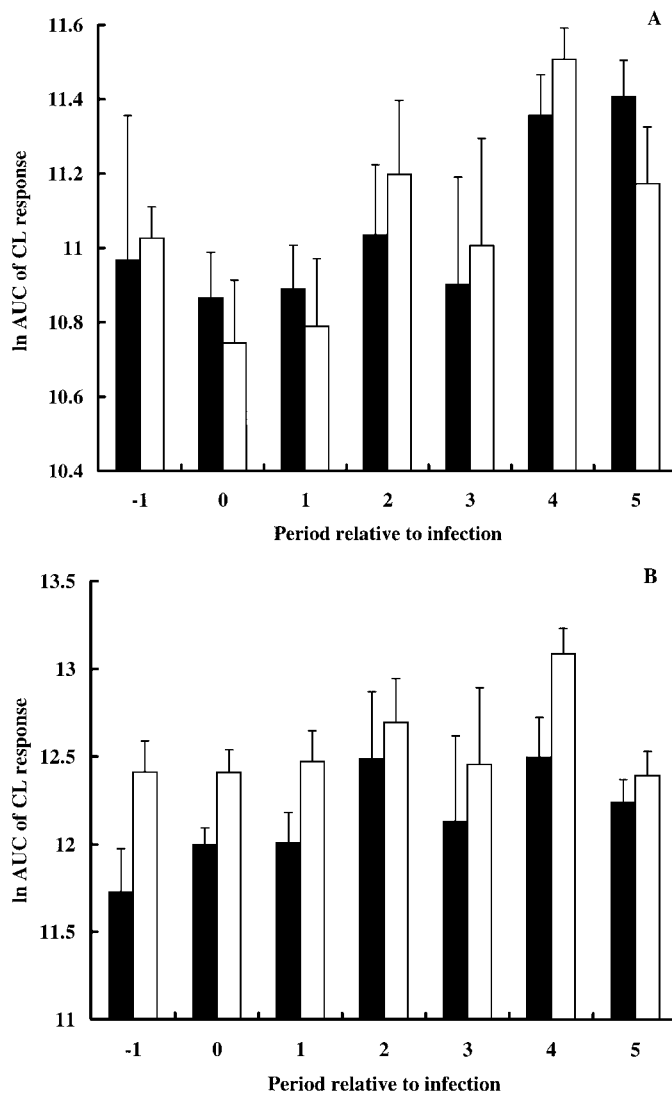


Figure 4. Corrected chemiluminescence (CL) of the isolated neutrophils of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis after stimulation by phorbol 12-myristate 13-acetate (A) and latex beads (B). Data are means of the logarithmic transformation of the areas under the curves (AUC) of different periods for five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 0 to 24 h after infection, 1 = 24 to 48 h after infection, 2 = 48 to 72 h after infection, 3 = 72 to 84 h after infection, 4 = 84 to 216 h after infection, and 5 = d +9 to +28.

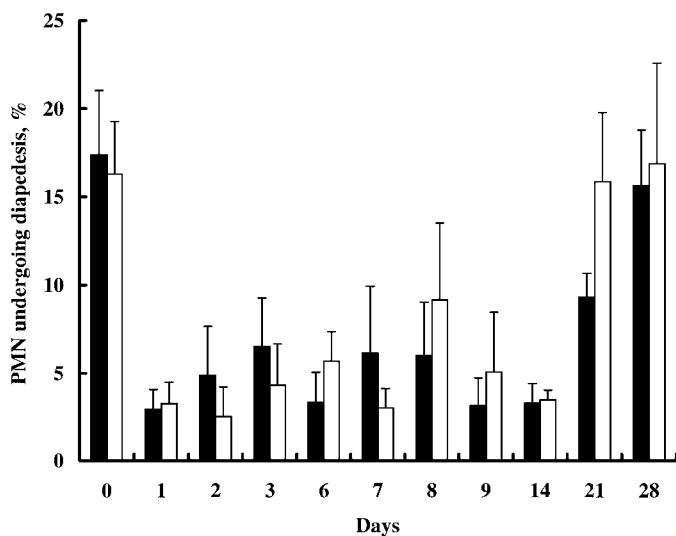


Figure 5. Percentage of polymorphonuclear leukocytes (PMN) undergoing diapedesis isolated from blood of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means.

by 28 and 27% ($P < 0.001$; d +14 to +28) for the control and treated groups, respectively.

Between 60 and 72 h postchallenge (Figure 8), treated cows showed a higher ($P < 0.05$) MFI for CD18 than did control cows because of an increase ($P < 0.01$) of the MFI for CD18 in the treated cows. In the control cows, no changes ($P > 0.05$) in the expression of CD18 on PMN were observed. If the first 72 h postinfection are studied in detail for both groups (data not shown), an increase ($P < 0.001$) was observed at 24 h postinfection, followed by a decrease between 36 and 48 h, and a second increase ($P < 0.01$) at 60 h. Baseline values were reached by d +4.

Blood IGF-I

The concentration of IGF-I in the plasma of the treated cows was higher ($P < 0.001$) from the first treatment until d +14 (Figure 9) compared with the concentration in the control cows. After the first bST treatment, IGF-I increased by approximately 97% ($P < 0.01$) before infection and continued to increase ($P < 0.05$) during the first 24 h after infection. From 24 h postinfection, IGF-I gradually decreased ($P < 0.01$). Between d +6 and +9, concentrations similar to those observed before the first treatment were reached. After the second bST treatment, IGF-I increased by approximately 73% and reached a maximum 1 wk after this treatment (d +14). After this second peak,

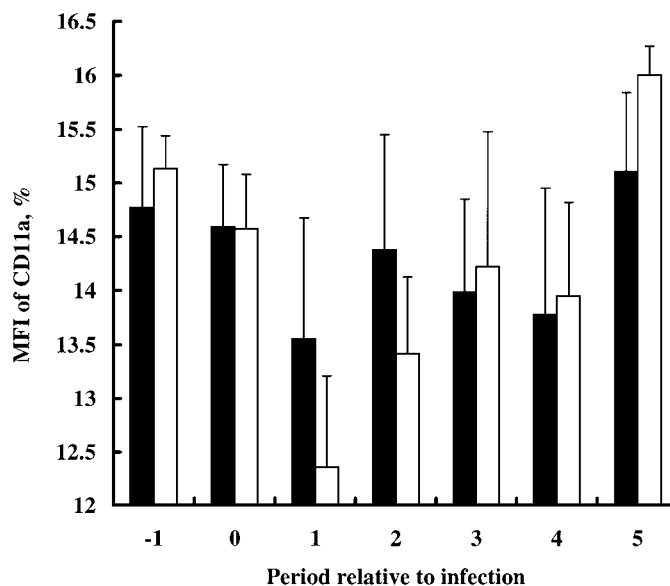


Figure 6. Expression of CD11a on polymorphonuclear leukocytes isolated from blood of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of the mean fluorescence intensities (MFI) of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 12 to 24 h after infection, 1 = 36 to 48 h after infection, 2 = 60 to 72 h after infection, 3 = 84 to 144 h after infection, 4 = d +7 to +9, and 5 = d +14 to +28.

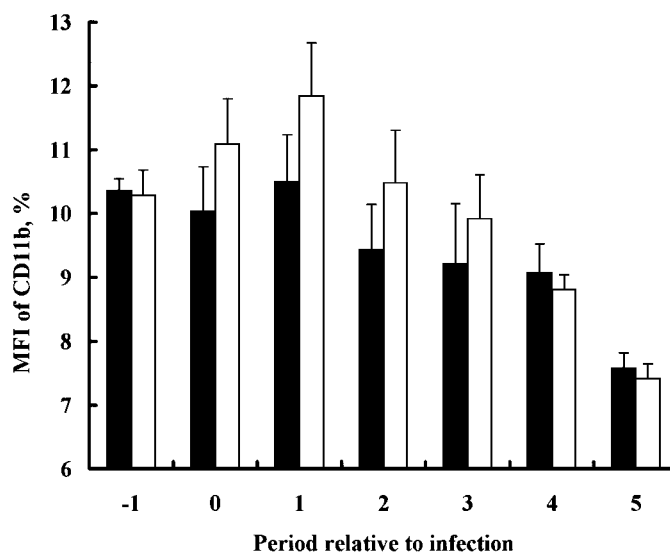


Figure 7. Expression of CD11b on polymorphonuclear leukocytes isolated from blood of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of the mean fluorescence intensities (MFI) of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 12 to 24 h after infection, 1 = 36 to 48 h after infection, 2 = 60 to 72 h after infection, 3 = 84 to 144 h after infection, 4 = d +7 to +9, and 5 = d +14 to +28.

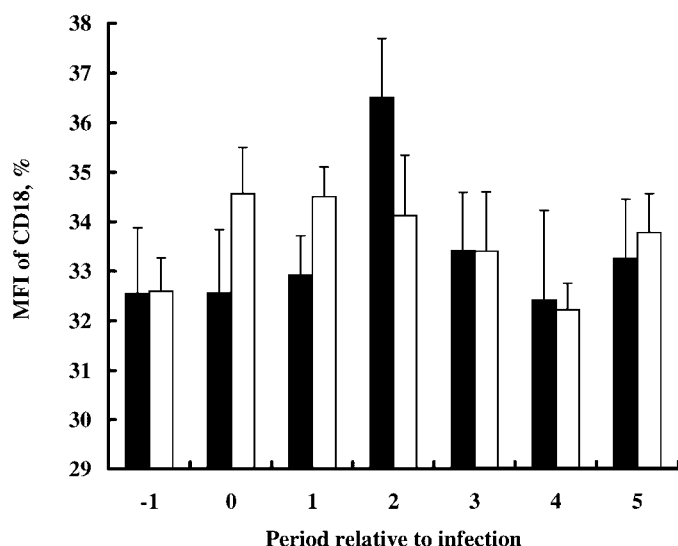


Figure 8. Expression of CD18 on polymorphonuclear leukocytes isolated from blood of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of the mean fluorescence intensities (MFI) of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 12 to 24 h after infection, 1 = 36 to 48 h after infection, 2 = 60 to 72 h after infection, 3 = 84 to 144 h after infection, 4 = d +7 to +9, and 5 = d +14 to +28.

IGF-I decreased ($P < 0.001$) toward concentrations that were similar to those before the first treatment. The IGF-I response after the first and second bST treatment did not differ ($P > 0.05$).

In the control cows, IGF-I decreased ($P < 0.05$) by 40% from d +2. In the most severely diseased cow, a decrease of 86% was observed. The concentration remained lower ($P < 0.05$) until 72 h after infection. From that moment on, IGF-I began to increase again to reach concentrations similar to preinfection concentrations.

Blood bST

A pattern similar to IGF-I was observed (Figure 10). As expected, the concentration of bST was higher ($P < 0.001$) in bST-treated cows than in control cows during the entire experiment. However, prior to injection, treated cows also had higher ($P < 0.01$) plasma bST concentrations than did controls. After the first bST treatment, an increase of 34% in bST concentrations was observed. From 24 h after infection, bST gradually decreased and reached pretreatment values from d +4. After the second treatment, bST increased by 31% ($P < 0.01$) followed by a decrease toward concentrations that were similar to those before the first treatment from d +21 to +28. The concentration

after the first treatment was 23% higher ($P < 0.01$) than the concentration after the second bST treatment. In the control cows, no changes ($P > 0.05$) in the concentration of bST in plasma were observed.

Leukocyte Counts and Differentiation

In the control cows, the number of circulating leukocytes decreased ($P < 0.01$) by 24% between 30 and 48 h after infection (Figure 11). The number of circulating leukocytes reached its lowest value between 48 and 72 h postinfection and was 37% lower than the preinfection number. In the most severely diseased cow, a decrease of 70% was observed. Beginning 72 h after infection, the number of circulating leukocytes started to increase until, between d +14 and +28, normal numbers of circulating leukocytes were present. Between 54 and 72 h after infection, a difference (27%; $P < 0.05$) in the numbers of circulating leukocytes was observed between both groups. The decrease ($P > 0.05$) in the number of circulating leukocytes of the treated cows was less pronounced than that of the control cows.

The number of circulating lymphocytes was similar in both groups during the entire experiment (Figure 12A). From 30 h after infection, the number of lymphocytes decreased by 25% ($P < 0.05$) in the treated

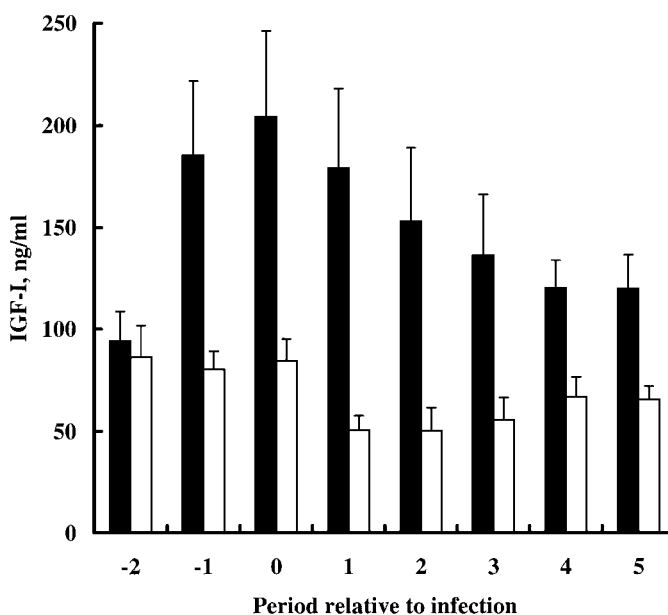


Figure 9. Concentration of IGF-I in plasma of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -2 = day -8, -1 = day -4 to the hour of infection, 0 = 6 to 24 h after infection, 4 = d +6 to +8, 5 = d +9 to +14, and 6 = d +21 to +28.

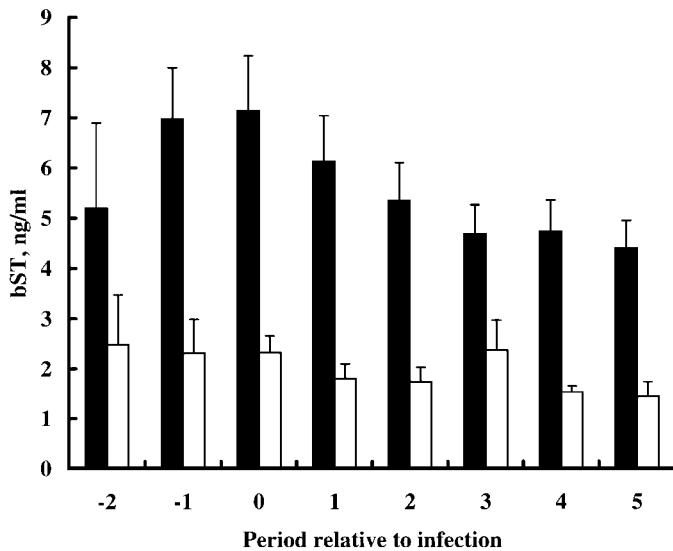


Figure 10. Concentration of bST in plasma of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -2 = day -8, -1 = day -4 to hour of infection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +7, 5 = d +8 to +14, and 6 = d +21 to +28.

cows and by 38% ($P < 0.01$) in the control cows. In the most severely diseased cow, a decrease of 60% was observed. From 72 h, the number of lymphocytes started to increase. The percentage of circulating lymphocytes in the treated group decreased from 63 ± 2.8 to $51.9 \pm 2.7\%$ between 30 and 48 h after infection, but the percentage increased from d 14 to +28 to $64.5 \pm 5.5\%$. In the control group, a decrease from 62.8 ± 1.7 to $54.1 \pm 2.5\%$ was observed between 30 and 48 h after infection followed by an increase to $63.1 \pm 2.8\%$ between d +14 and +28.

Between 48 and 72 h after infection, the number of circulating mature neutrophils was higher (63%; $P < 0.05$) in the treated cows than in the control cows (Figure 12B) but did not change in either group during the entire study. However, in the most severely diseased cow, a decrease of 89% was observed. The percentage of circulating mature neutrophils in the treated cows increased from 33 ± 2.6 to $42.5 \pm 3.3\%$ between 30 and 48 h postinfection followed by a decrease to $31.7 \pm 4.7\%$. In the control cows, an increase from 34.5 ± 1.7 to $40.3 \pm 3.1\%$ was observed during the same period, followed by a decrease to $34.4 \pm 2.6\%$.

On d 0, the number of circulating eosinophils was higher ($P < 0.01$) in the treated cows (Figure 12C). No changes ($P > 0.05$) in the number and the percent-

age of circulating eosinophils could be observed in either of the groups.

The number of circulating monocytes was higher ($P < 0.05$) in the control cows than in the treated cows between 0 and 48 h postinfection (Figure 12D) and increased ($P < 0.01$) in the control cows on d 0. From 54 h, the number of monocytes decreased to values similar to preinfection numbers. The percentage of circulating monocytes showed a similar pattern.

In both groups, band neutrophils appeared in the circulation of all cows at 24 h (Figure 12E). Between 78 and 96 h after infection, the number of circulating band neutrophils was higher ($P < 0.05$) in the control group than in the control group. An increase ($P < 0.05$) occurred in both groups on d 0 followed by a decrease between 54 and 72 h after infection. Between d +6 and +9, an increase was observed in the treated cows but not in the control cows. Between d +14 and +28, the number of band neutrophils was reduced to normal levels again. The percentage of circulating band neutrophils followed the same pattern.

The number of circulating metamyelocytes was higher ($P < 0.05$) in the control cows than in the treated cows between 78 and 96 h postinfection (Figure 12F). Metamyelocytes appeared in the circu-

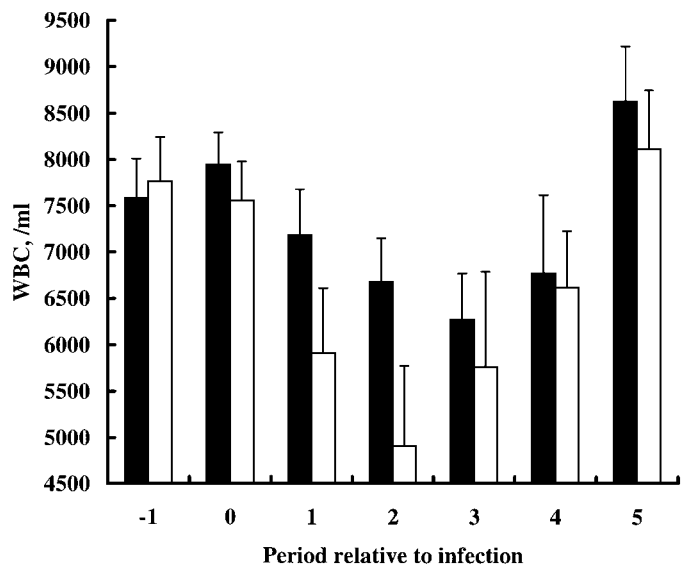


Figure 11. Changes in the number of circulating leukocytes (WBC) of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

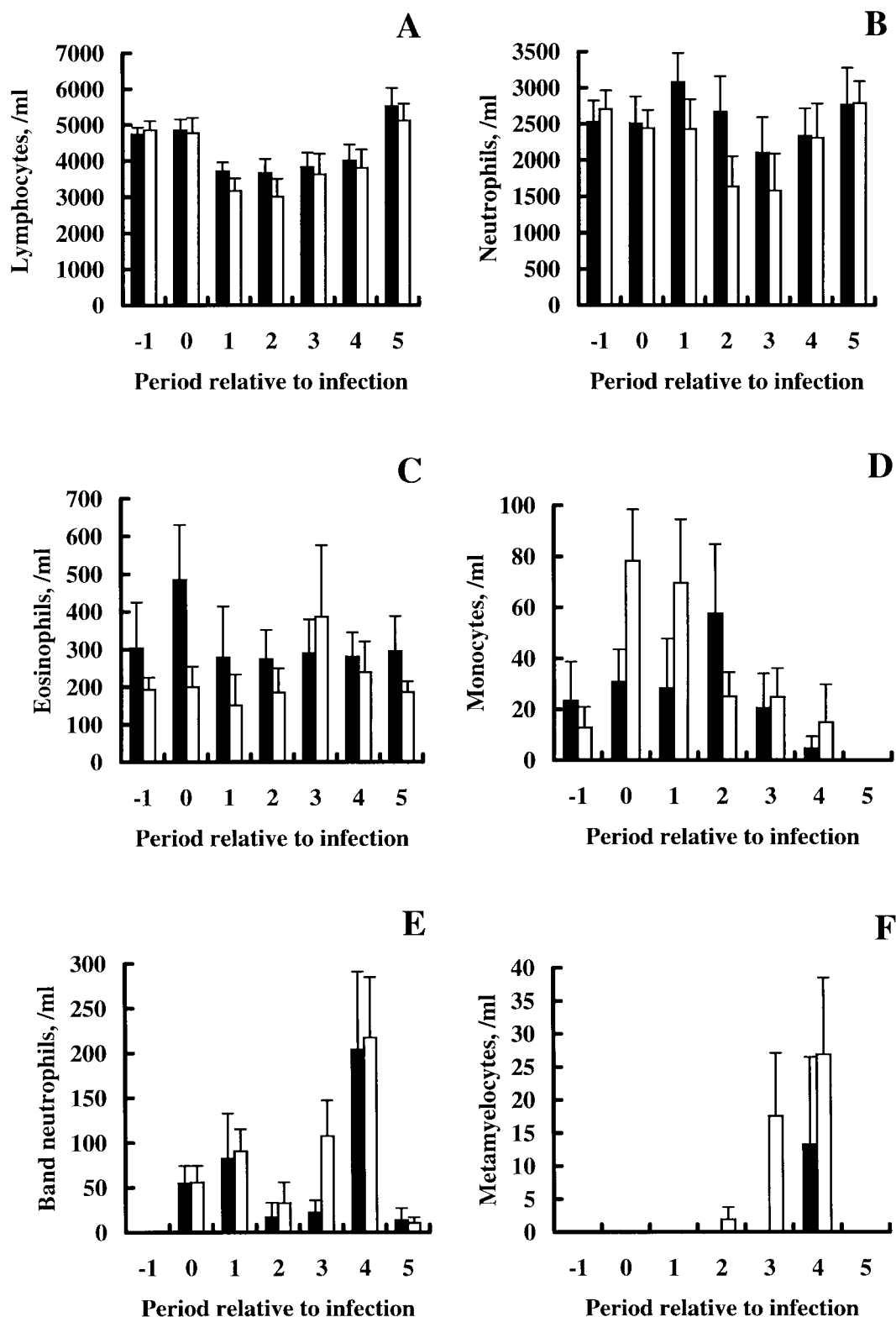


Figure 12. Changes in the number of circulating lymphocytes (A), mature neutrophils (B), eosinophils (C), monocytes (D), band neutrophils (E), and metamyelocytes (F) of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods -1 = preinfection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

lation of 1 treated cow and 5 control cows and disappeared after d +6. The percentage of circulating metamyelocytes followed the same pattern as the absolute number.

The percentage of mature neutrophils in the total neutrophil population differed ($P < 0.01$) between both groups from 72 to 96 h after infection (Figure 13, A and B). The decrease occurred earlier and was more pronounced in the control cows. On d +3, control cows had a higher percentage (2.2 ± 1.35 vs. $11 \pm 5.7\%$; $P < 0.01$) of immature band neutrophils in the circulation than did treated cows. The increase in band neutrophils was more pronounced and longer lasting in the control cows. In the most severely ill cow, approximately 32% of the neutrophil population was band neutrophils. The percentage of metamyelocytes in the entire neutrophil population was elevated in the control cows during the same period in which the percentage of mature neutrophils was very low and the percentage of band neutrophils was elevated. Metamyelocytes were observed from d +2 to +6 in the control cows and on d +6 in only 1 treated cow. In the most severely diseased cow, 7% of the neutrophil population was metamyelocytes versus 2% in the other cows.

Cortisol

No differences between groups were observed. On d 0, plasma cortisol increased in the control cows by approximately 92% ($P < 0.05$) (Figure 14). Between 30 and 72 h postinfection, normal concentrations were observed. On d +3, cortisol increased ($P < 0.05$) in both groups followed by a return to normal preinfection values. When the cortisol concentration is studied in detail during the first 72 h after infection, a 1.6-fold increase is observed at 24 h postinfection in the treated cows, and a 2.4-fold increase is observed in the control cows.

α -LA

In the treated cows, there were no changes ($P > 0.05$) in blood α -LA concentrations during the course of the study because of extreme variability among cows. α -Lactalbumin slightly increased after infection to a maximum around 72 h after infection (Figure 15). The huge increase between d +6 and +28 was due to 1 cow. If the results from this cow were omitted (data not shown), the concentration between d +6 and +28 was only double the concentration before infection.

In the control cows, blood α -LA increased ($P < 0.05$) from 30 to 48 postinfection followed by a return to normal values. However, this increase was due to 1

cow. If the data for this cow were omitted (data not shown), no increase was observed. The concentrations between d +6 and +28 were also higher ($P < 0.05$) than preinfection concentrations and were primarily due to 3 cows. The concentration between d +6 and +9 was higher ($P < 0.01$) than the concentration on d +3.

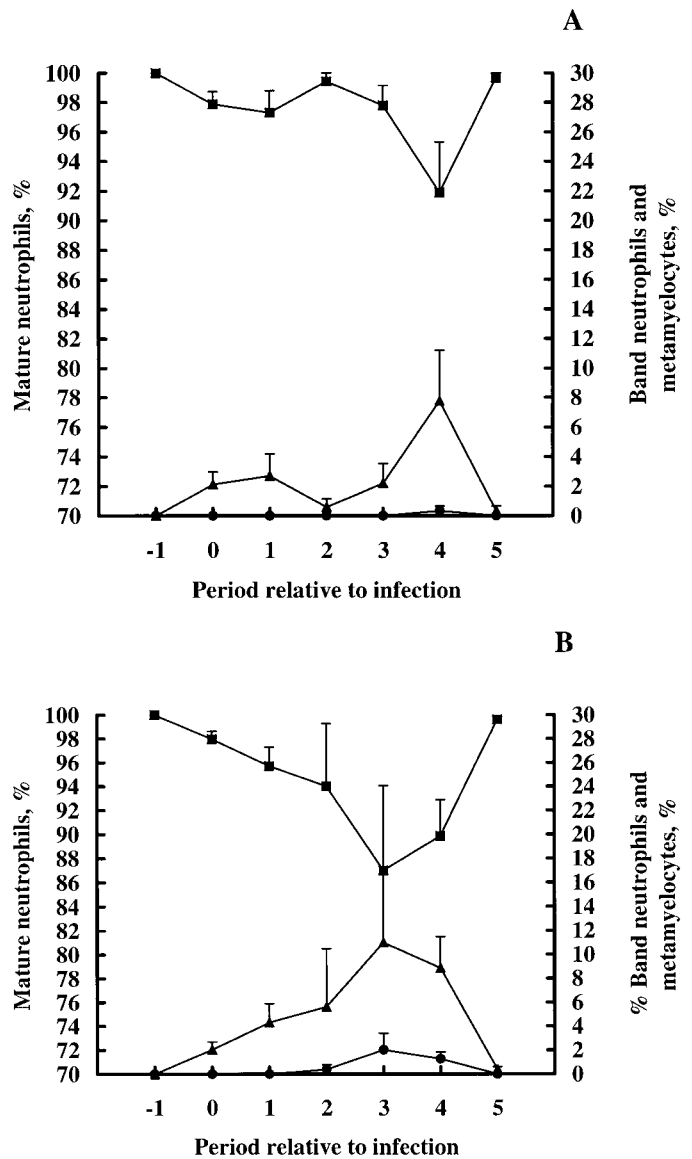


Figure 13. Changes in the percentage of circulating mature neutrophils (■), band neutrophils (▲) and metamyelocytes (●) of the total neutrophilic population in blood of recombinant bST-treated cows (A) and control cows (B) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

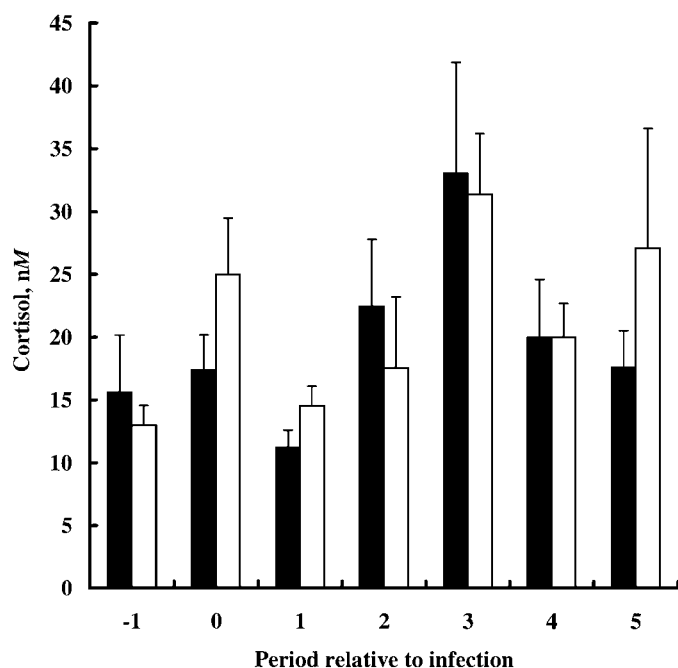


Figure 14. Changes in the concentration of cortisol in the plasma of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

When the first 72 h after infection are studied in detail, a 3- and 8-fold increase was observed in blood α -LA of the treated and control groups, respectively, after 24 h. The concentration of α -LA in the treated group returned to normal values by 36 h postinfection, and, in the control group, the concentration of α -LA returned to normal by 48 h postinfection.

DISCUSSION

Treatment of cows with bST appeared to protect cows from severe clinical symptoms during a subsequent episode of experimental *S. uberis* mastitis. Previously treated cows had a reduced fever, and less tachycardia, and the local symptoms, such as swelling, pain, and firmness of the infected glands, were also less pronounced. Protective effects on the severity of general and local clinical symptoms during mastitis have also been observed in cows suffering from experimentally induced *E. coli* mastitis (49). Cows that were classified as severe responders based on the respiratory burst activity of their circulating neutrophils (23, 50) and on differences in blood and milk components prior to infection reacted as moder-

ate responders when treated with bST. These findings could be ascribed to an enhancement of the rate of diapedesis of neutrophils into the milk, to an increased number of circulating mature and immature neutrophils, and to an enhanced oxidative burst activity of these neutrophils (49).

Indeed, ST primes human and rat monocytes, macrophages, and neutrophils for enhanced production of reactive oxygen species in vitro (10, 20, 52) and in vivo (11, 19). In contrast to those results, Heyneman and Burvenich (21) could not demonstrate any effect on the respiratory burst of bovine neutrophils after administration of bST in vitro. However, in vivo administration of bST induced an enhancement of superoxide production after stimulation with zymosan or PMA in healthy cows and by zymosan only in cows suffering from *E. coli* mastitis (21). The beneficial effects of ST on immune cells might be accomplished either directly or indirectly by the action of IGF-I. Indeed, ST receptors have been identified on bovine thymocytes, and IGF receptors have been detected on peripheral blood mononuclear cells (55). In the present study, administration of recombinantly derived bST increased concentrations of bST in plasma by approximately 34%. This response is consistent with the results of others (8, 38, 51, 56). Somatotropin also may increase concentrations of bST

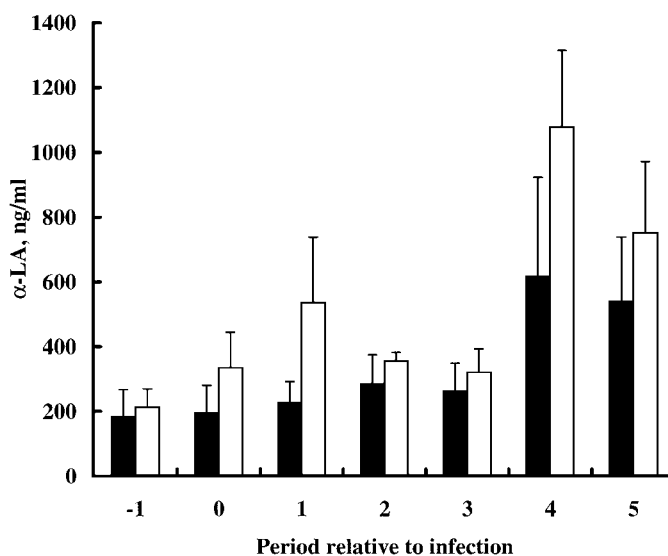


Figure 15. Changes in the concentration of α -LA in the plasma of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: -1 = preinfection, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = 78 to 96 h after infection, 4 = d +6 to +9, and 5 = d +14 to +28.

in milk (41, 56), although Miekle et al. (38) and Eppard et al. (15) could not detect bST in milk. In the present study, plasma concentrations of IGF-I increased after bST administration, which is consistent with the results of other studies (8, 38, 51, 56).

The concentration of IGF-I in milk has also been reported to increase after bST injection (9, 56). According to Prosser et al. (41), this IGF-I originates from the circulation, rather than being locally synthesized *de novo* by mammary epithelial cells (6). Consequently, bST and IGF-I may influence the activity of PMN and macrophages in milk. After infection, plasma IGF-I significantly decreased, especially in the control cows, and plasma bST remained practically unchanged. Gene expression of IGF-I is mainly controlled by ST in hepatic and nonhepatic tissues, but glucocorticosteroids, neuropeptides, estrogens, and cytokines may also regulate IGF-I transcription (1). The interaction of these regulators may be involved in the decreased IGF-I concentration in the control cows. Because of this decreased concentration, the beneficial effects of basal bST, and especially IGF-I, on neutrophils and macrophages might be less pronounced in the control cows, which may explain the more severe illness in these cows. Unlike *E. coli* mastitis, the macrophage is the most important immune cell in the defense against *S. uberis* (24).

Treatment with bST had little effect on the CL response of circulating PMN after stimulation by PMA or latex beads, which is in contrast to the findings of Heyneman and Burvenich (21), Edwards et al. (11), and Fu et al. (19). Total CL response decreased shortly on d +4 followed by an increase to a response that was higher than preinfection responses. The CL response after stimulation of PMN by PMA was more suppressed than the CL response after stimulation by latex. Phorbol 12-myristate 13-acetate directly stimulates the respiratory burst by activating protein kinase C without being internalized. In contrast, latex beads nonspecifically adhere to the phagocyte surface by electrostatic and hydrophobic forces. Consequently, they are internalized within the phagocyte, which then activates the respiratory burst. No opsonization is necessary, and no complement or Fc receptors on the phagocytes are involved. Based on this difference, the results indicate that the respiratory burst is depressed, and phagocytosis is unaffected or possibly stimulated. The phagocytic ability of the PMN was probably less affected or not affected during infection. Indeed, the expression of CD11b or complement receptor 3, which binds the opsonic complement factor 3b, was upregulated after infection in our study, which might indicate that CD11b-mediated

phagocytosis is increased, as perhaps is nonspecific phagocytosis. We also observed that the corrected CL response showed an increasing trend and no decreases, which is in contrast with the total CL response. This decrease coincided with a decrease in the number of circulating WBC and circulating mature neutrophils, but an increase of the number of circulating immature neutrophils that had an impaired activity. This pattern is consistent with a greater depression in the burst activity of eosinophils compared with that of the neutrophils. However, by the end of the trial, CL responses exceeded normal baseline responses. These elevated responses coincided with an increase in the number of circulating mature neutrophils. This increased CL response may be due to a priming effect by endogenous inflammatory mediators and hormones on phagocytes released from bone marrow. Inflammatory mediators could then stimulate the neutrophils released from the bone marrow and prime them for an enhanced production of reactive oxygen species. Similar findings have been reported during acute coliform mastitis (23). Enhanced CL responses were more pronounced in bST-treated cows, which might suggest that, in addition to the influence of inflammatory mediators, the elevated blood concentrations of bST and IGF-I favored the proliferation and generation of PMN that were primed for an increased burst activity. Fu et al. (20) and Bjercknes and Aarskog (4) reported priming of human neutrophils by IGF-I and ST for superoxide secretion after stimulation with PMA. Priming of human neutrophils by interleukin-1, interleukin-8, tumor necrosis factor- α (TNF- α), CD18 cross-linking, and IGF-I has been reported (12, 18, 34, 54). Bovine neutrophils have been shown to be primed by interleukin-1, interleukin-2, and TNF- α (43). Insulin-like growth factor-I also stimulates the production of TNF- α (42) and granulocyte-macrophage colony-stimulating factor (44) by monocytes and macrophages.

In vivo bST treatment did not affect diapedesis of PMN across a monolayer of secretory epithelial cells *in vitro* in the present study. Diapedesis decreased after infection and returned to baseline values at 3 wk after infection in both groups.

No effect of bST was observed on the expression of CD11a on PMN. The down-regulation of CD11a, although of only minor importance, may be partly responsible for the decreased diapedesis because CD11a was shown to be essential for C5a-induced leukocyte adherence in rabbits (3). The expression of CD11b was not affected by bST treatment. In humans, however, upregulation of CD11b on PMN by

IGF-I has been reported (4).

Because the removal of *S. uberis* from the mammary gland is mainly dependent on macrophages rather than neutrophils, the absence of significant effects of bST on neutrophil functions is not detrimental for the outcome of this kind of mastitis, which is in contrast with coliform mastitis. Recombinant bST treatment may affect macrophage and monocyte functions, which were not studied in this experiment.

Treatment with bST protected the cows from excessive leukopenia. The effect may be due to the lower migration of PMN from blood to milk, expressed as a lower SCC in the treated cows (25), or to an increased myelopoiesis induced by ST and IGF-I (37, 39, 40, 44).

Treatment with bST had no effect on the number of circulating lymphocytes. The higher number of circulating mature neutrophils in the treated group may be due to stimulatory effects of IGF-I on bone marrow (7). The percentage of circulating mature neutrophils increased in both groups, especially in the bST-treated cows. This pattern may be partially due to cortisol. Indeed, intravenous injection of hydrocortisone induces a release of young reserve neutrophils from the bone marrow (48). The larger number of circulating immature neutrophils (band neutrophils and metamyelocytes) in the control cows, was probably due to the more severe leukopenia and the higher SCC in these cows. In general, no important effects of bST treatment on the number of mature cells could be observed, which is consistent with the results of other studies (5, 14, 16). Hematocrit significantly decreased in the treated cows (data not shown), which is also consistent with the same studies.

The increase of α -LA in plasma between 30 and 48 h postinfection in the control cows indicated severe damage of the epithelial cell tight junctions of the blood-milk barrier. The changes in milk lactose, BSA, Na^+ , K^+ , and Cl^- are also indicative of this damage (25). In the treated cows, no significant changes were observed, perhaps indicating some protection of the blood-milk barrier by bST. The increase in the bST-treated cows was due to only 1 cow, the most severely diseased cow of that group. This cow was still very ill, milk lactose concentration was still very low, and the SCC, Cl^- , and Na^+ were still very high, although the milk concentration of BSA had already decreased. Differences in molecular mass and in rates of diffusion and secretory rate are responsible for the difference in leakage of BSA and lactose. The elevated blood concentration of α -LA implies that repair of tight junctions was occurring but was not complete. The huge increase of blood α -LA in the control group

was due to 3 cows, which were again, the most severely diseased. The elevated concentration of α -LA indicated that the damaged blood-milk barrier was still incompletely repaired, which was also concluded earlier by Hoeben et al. (25). The concentration of α -LA in serum is the reflection of two processes: first, the integrity of mammary epithelial tight junctions. The more leaky these junctions are, the more α -LA can escape into the circulation. The second process is the rate of secretion of milk, which affects not only the amount of α -LA that can diffuse across the epithelium, but also the intramammary pressure. Increased intramammary pressure increases the flow of α -LA into blood; conversely, decreased intramammary pressure decreases flow of milk components, such as α -LA, into blood. We did not observe a larger increase in blood α -LA during the initial, acute stages of the infection because milk production was dramatically decreased (25) along with intramammary pressure. From d +6, α -LA in serum was elevated because secretory rate (milk production) had almost recovered, but there was still damage to the mammary gland and the epithelial tight junctions. Measuring the concentration of a milk-specific protein such as α -LA in blood will always be a more sensitive measure of mammary damage than measuring blood components in milk, or the ratio of K^+ to Na^+ , or milk lactose concentrations, because the high secretory rate during the chronic phase of the infection tends to mask the effect on milk composition.

In conclusion, the beneficial effects of bST on general and local clinical symptoms during experimentally induced *S. uberis* mastitis are not due to positive effects on the function of neutrophils, as suggested by Vandeputte-Van Messom and Burvenich (49) for *E. coli* mastitis. The neutrophil function of cows previously treated with bST was not improved compared with the control cows. The positive effect of bST is probably due to a protective effect on the blood-milk barrier and possibly to an increased elimination of the infection. As a consequence, fewer inflammatory mediators are released, which positively affects the general and local symptoms.

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