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A Study of the Development of Endotoxin-induced Inflammation in the Bovine Teat

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Persson, K. and C. Hallén Sandgren: A study of the development of endotoxin-induced inflammation in the bovine teat. Acta vet. scand, 1992, 33, 283-295. – Endotoxin-induced local inflammation was studied by frequent samplings in a bovine teat cistern model, which provides a unique possibility for in vivo studies of reactions in the teat without interference from the mammary gland.

A rapid inflammatory response of rather short duration was elicited after endotoxin administration. An initial increase in the concentrations of bovine serum albumin and N-acetyl-ß-D-glucosaminidase, indicating a disturbance in the epithelial integrity, was observed between 1 and 1.5 h post infusion (p.i.). Approximately 0.5 h later, the first influx of leukocytes, mainly neutrophils, appeared. The neutrophils tended to enter the teat cistern in several peaks occurring between 2.5 and 5 h p.i.. The sampling procedure decreased the accumulation of cells by approximately 40%, which was probably due to the removal of inflammatory mediators at an early stage. The parallel use of 2 teats instead of 1 had no major influence on the inflammatory process.

This teat cistern model and the experimental procedure used should be suitable for further studies of the development of local inflammation.

leukocytes; BSA; NAGase.

Introduction

The defence mechanisms in the bovine teat are of the utmost importance in the defence against udder infections (*Nickerson* 1985). However, to date, few investigations of the inflammatory process in the teat tissues have been published (*Frost et al.* 1984, *Nickerson* 1985). Hence, to elucidate this subject, a teat cistern model was developed (*Persson & Åström* 1989). The model provides a unique possibility for frequent sampling of inflammatory exudate in vivo, without any profound influence on the inflammatory process. In the ovine, the non-lactating gland has been used for similar studies (*Colditz & Presson* 1988). Moreover, another experimental approach has been to study the tissue accumulation of leukocytes after intradermal injection of inflammatory substances (*Colditz & Movat* 1984, *Colditz* 1988). However, few such models have been used in the bovine (*Heidel et al.* 1989, *Luthman et al.* 1988).

Endotoxins from Gram-negative bacteria are largely responsible for the morbidity and mortality associated with infections by these microorganisms in various animal species, and have therefore been widely used as inflammatory elicitors. In a recently published study of the endotoxin-induced inflammation in the bovine teat, we found that the cellular response had reached its maximum already at 6 h post infusion (*Pers-*

son 1990). Rapid infiltration of leukocytes has also been observed in other models using endotoxin (*Colditz & Movat* 1984, *Colditz* 1988, *Colditz & Presson* 1988).

Consequently, the aim of this study was to obtain detailed information about the inflammatory response during the first 7 h after infusion of endotoxin into the bovine teat cistern. For this purpose, the alterations in the cell count, the serum protein bovine serum albumin, and the intra-cellular enzyme N-acetyl-ß-D-glucosaminidase were studied.

Materials and Methods

Animals

Six clinically healthy primiparous cows of the Swedish Red and White Breed were used. They were non-pregnant and non-lactating and had no earlier record of udder disease, as measured by clinical symptoms and monthly cell count recordings (<150,000 cells/ml) throughout the previous lactation period. Quarter milk samples at drying off were bacteriologically negative.

In each cow, the teat and udder cisterns were separated from each other by a modification of a surgical procedure described by *Persson & Åström* (1989), to enable studies of reactions in the teat in vivo without interference of the mammary gland secretion. The surgical procedure was performed under general anaesthetic. A linear incision was made through the teat wall along the cranial part of the teat cistern. The inside was exposed, and a horizontal incision (2 mm wide), was made through the epithelial lining as dorsally as possible in the teat cistern. According to the original procedure, the cut through the epithelial lining was made using electrocoagulation (Radioton 704, Siemens) in 9 udder quarters in 3 cows (3 quarters per cow), while the incision was made by cutting a strip of the epithelial lining using a pair of scissors in 15 udder quarters in 6 cows (1 quarter per cow (n=3), or 4 quarters per cow (n=3)). The passage between the teat and the udder cisterns was closed by placing 1 purse string suture of absorbable suture material dorsal to and 1 ventral to the incision. The skin was sutured with single interrupted sutures. Three to 4 weeks post surgery, saline (sterile 0.9% (w/v) NaCl) was infused through the teat canal via a blunt infusion cannula, to determine whether the surgical closure between the teat and udder cisterns was complete. This was the case in all quarters in which the epithelial incision was made with scissors, while a complete closure was found in 6 out of 9 quarters in which the epithelial incision was made with electrocoagulation. No experiments were performed earlier than 6 weeks after surgery.

Endotoxin

The *Salmonella typhimurium* endotoxin (lipopolysaccharide, LPS) was extracted by the hot phenol-water method from batch-grown cultures of formaldehyde-killed *S. typhimurium* SH 4809 and the LPS was further purified and characterised as previously described (*Svenson & Lindberg* 1978, *Lindberg et al.* 1983). The endotoxin was dissolved in sterile 0.9% (w/v) NaCl.

A limulus amoebocyte lysate assay (Kabi, Göteborg, Sweden) was used to measure the presence of detectable concentrations ($>10^{-11}$ g/ml) of endotoxin in teat samples.

Cell Count

The cell count in the teat cistern samples was determined microscopically by counting duplicate samples in a Bürker chamber, after dilution in Türk's solution. Teat cistern samples were centrifuged, smears prepared and stained by the conventional May-Grünewald-Giemsa method. The differential cell counts

were assessed by counting 200 cells in microscope.

Bovine Serum Albumin (BSA)
The BSA content, in mg/ml, was measured by the radial immunodiffusion principle (*Mancini et al.* 1965) using agar plates containing rabbit or porcine anti-BSA serum. BSA standards, 3.0, 1.0, 0.3, 0.1, and 0.03 mg/ml, were present on each agar plate. The diameter of the precipitation zones (x) was measured after 72 h and the BSA values (y) were interpolated using a least squares fit program (log(y)=a+blog(x)).

N-Acetyl-ß-D-Glucosaminidase (NAG)
The NAG value, in units/10 µl, was analysed (*Mattila & Sandholm* 1985) using the Milk NAGase Test (Labsystems, Helsinki, Finland), which essentially is a microplate modification of the fluorogenic method of *Kitchen et al.* (1978). A NAG value of 100 represented a release of 5 picomoles of product (4-methylumbelliferone) per minute at 25°C, as catalysed by 1 µl of milk.

Bacteriological Examinations
Teat cistern samples were examined according to the Scandinavian recommendations given by *Klastrup* (1975).

Blood and Serum Analyses
Total and differential counts of blood leukocytes and measurements of serum cortisol (Coat-A-Count^{R}, Diagnostic Products Corporation, Los Angeles, USA) were performed according to standard procedures as used at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Experimental Procedures
The cows were in mid-cyclus on the day of the experiment. One h before the experiment the teat cisterns were flushed with 10 ml saline (sterile 0.9% (w/v) NaCl) to remove accumulated cells. On each sampling occasion, teat cistern samples were taken by infusing 3.5 ml saline into the teat cistern and immediately stripping it out into a test tube. One flushing of 3.5 ml saline was considered enough as more than 90% of the cells were recovered in the first sample when flushing of the same teat was done twice on the same occasion. Preliminary experiments were performed to select an endotoxin dose which induced a clear inflammatory response in the teat cistern.

Experiment 1 - Endotoxin infusion into 1 teat cistern. The day before the experiment the cows were fitted with a semi-permanent catheter in the jugular vein for blood sample collection.
In 3 cows, 1 teat cistern (A) was infused with 20 µg Salmonella endotoxin in 0.5 ml saline, and 1 teat cistern (B) with 0.5 ml saline as a control. Teat cistern samples were taken just before infusion of endotoxin or saline (0 hour), at 1 h post infusion (p.i.) and then every 30 min up to 7 h p.i.. Blood samples were collected just before infusion of endotoxin and once every h p.i.. The rectal temperature was measured hourly during the experiments. The cell count was determined in the fresh samples while samples for BSA and NAG analyses were frozen (-18°C). Bacteriological examinations of the fresh samples were performed at 0, 1, 2, 3, and 7 h p.i..
The experiment was repeated after six weeks, with infusion of endotoxin into teat cistern B and saline into teat cistern A.

Experiment 2 - Endotoxin infusion into 2 teat cisterns. Minimum 6 weeks after experiment 1, a second series of experi-

ments was performed, in the same cows, for several reasons. Firstly, 2 teat cisterns per cow were infused with endotoxin at the same time, to evaluate the impact of 2 inflammatory foci instead of 1 on the inflammatory response to the endotoxin. Secondly, the origin of NAG, which may be extracellularly released or intracellularly located (*Kaartinen et al.* 1988), was further evaluated by measuring the concentrations of NAG in cell-free samples (FreeNAG). Thirdly, the remaining concentrations of endotoxin in the teat cistern samples after endotoxin infusion were measured.

In 3 cows, 2 teat cisterns in each cow were each infused with 0.5 ml saline containing 20 μg Salmonella endotoxin. The experimental procedure was the same as in experiment 1, but no blood samples were taken. Samples for analysis of cell count, BSA and NAG were treated as in experiment 1. Additionally, samples were frozen for analysis of endotoxin, and cell-free samples for FreeNAG analysis were frozen after centrifugation of the teat cistern samples (200xg, 5 min).

Experiment 3 - Influence of the sampling procedure. As a result of the sampling procedure used in experiments 1 and 2, endotoxin and endogenous inflammatory mediators (including the leukocytes) present in the teat cistern were flushed out on every sampling occasion. To evaluate the effect of this procedure on the inflammatory process, the following experiment was performed.

In 3 cows (not used in experiments 1 and 2), 2 teat cisterns were infused with 20 μg Salmonella endotoxin in 0.5 ml saline each. Samples were taken just before infusion (0 h) in both teats, and a) at 1 and 7 h p.i. in one teat, and b) at 7 h p.i. only in the other teat. The cell count was determined in all the samples, while samples taken at 7 h p.i. were frozen for measurements of endotoxin concentrations.

Statistics

The difference between the value on each sampling occasion and the pre-infusion value was calculated for each teat and parameter. The change over time was evaluated by taking the value for each parameter in each teat on one sampling occasion, minus the value on the previous sampling occasion. In experiment 2, these differences were calculated on the average for 2 teats per cow. The paired t-test on log-transformed data was used for the statistical evaluation, except when comparing experiments not including the same animals (unpaired t-test). Appropriate correlations were calculated using the correlation test in the program.

Results

Throughout the experiments, the general condition of the animals was not affected and the rectal temperature remained normal (38.3-39.0°C). The teats were swollen, sometimes sore, between 2 h and 5 h p.i.. The infusion of endotoxin resulted in a significant influx of leukocytes into the teat cistern. Most of the leukocytes were neutrophils (93.2±3.3%). A small influx of mononuclear cells (5.2±2.3%) and eosinophils (1.6±1.8%) was also seen. No major differences in differential cell count over time post infusion were observed.

Two ways of analysing the material were used. Firstly, the number of cells and the concentrations of BSA, NAG and FreeNAG on each sampling occasion in each cow were compared with the pre-infusion value. Secondly, to illustrate the change over time, the alterations in cell count, BSA, NAG and FreeNAG values, expressed as an increase or

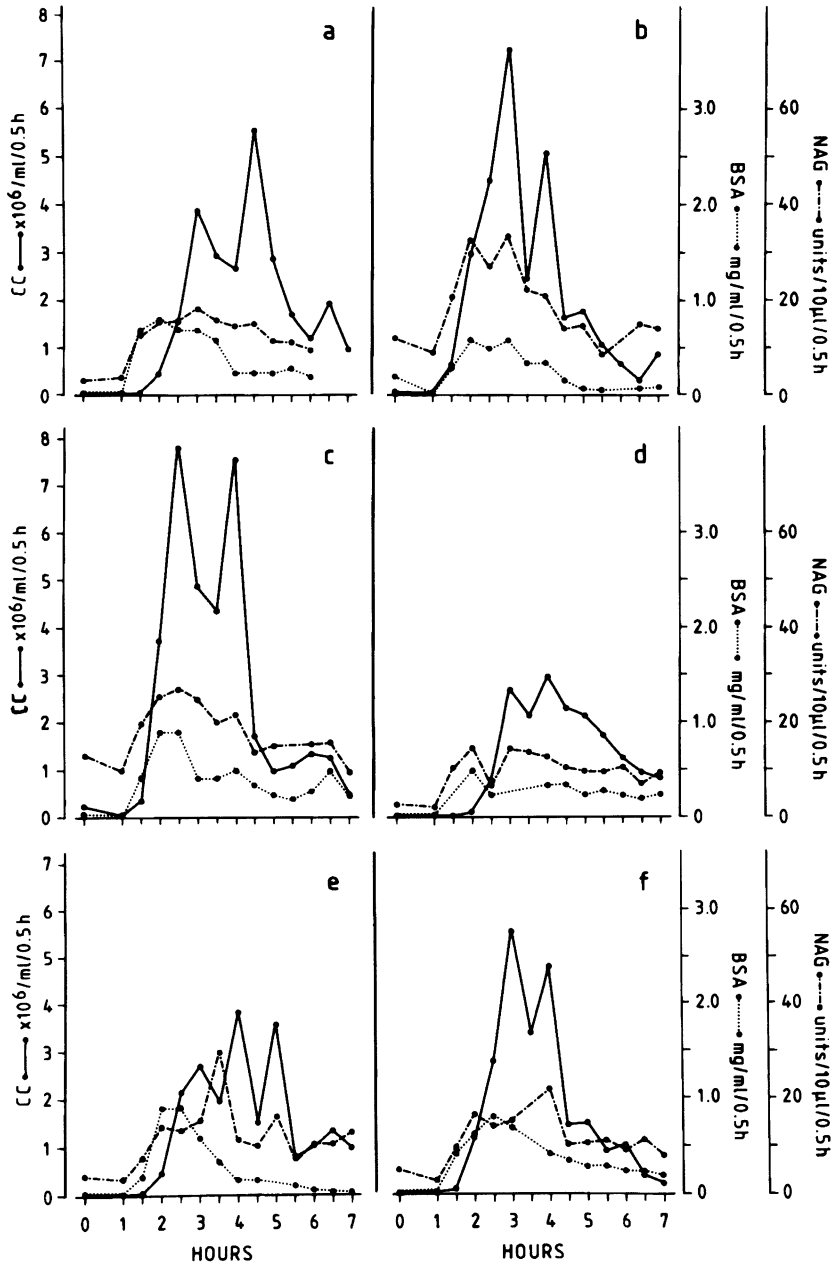

Figure 1. Cell count (CC), and concentrations of bovine serum albumin (BSA), and N-acetyl-ß-D-glucosaminidase (NAG), in teat cistern samples after infusion of 20 µg Salmonella-endotoxin at 0 h into 1 teat cistern per cow, on 2 separate occasions in cow 1 (a,b), cow 2 (c,d), and cow 3 (e,f).

a decrease between 2 successive samplings, were calculated.

Experiment 1 - Endotoxin infusion into 1 teat cistern

The total and differential blood leukocyte counts, and the concentration of serum cortisol were within normal ranges throughout the sampling period. All teat cistern samples were bacteriologically negative.

The results of the teat cistern sample analyses, expressed as the accumulation of cells, BSA, NAG, and FreeNAG per time-unit (0.5 h), are given as individual values in Fig. 1 and as average values (n=6) in Fig. 2.

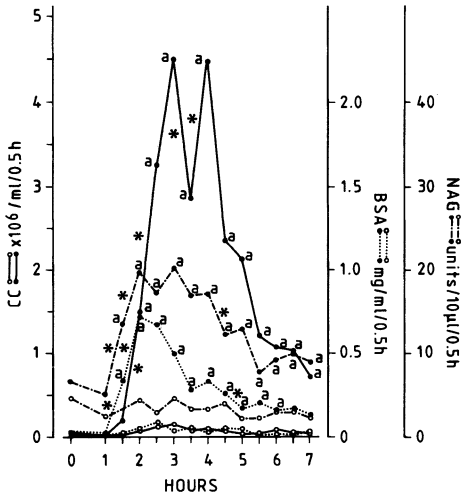

Figure 2. Average cell count (CC), and concentrations of bovine serum albumin (BSA), and N-acetyl-ß-D-glucosaminidase (NAG), in teat cistern samples after infusion of 20 µg Salmonella-endotoxin dissolved in saline into 1 teat cistern (●), and infusion of saline only into another teat cistern (○), at 0 h in 3 cows. The experiment was repeated once.

[a] The value differs from the pre-infusion value at $p<0.05$.

* The change during the time period (0.5h) is significant at $p<0.05$.

Before infusion of endotoxin, the number of cells in the teat cistern samples was 0.06±0.09 x 10^6/ml (Fig. 2). After endotoxin infusion, the influx of neutrophils started to increase between 1.5 and 2 h p.i., and continued between 2 and 2.5 h (Fig. 2). The influx of neutrophils occurred in several peaks as the cell count first decreased and then increased again between 3.5 and 4 h p.i. (Fig. 2). From 2 h p.i. the number of cells on each sampling occasion was significantly higher than before the infusion. The highest cell numbers, 4.5±1.6 x 10^6 cells/ml at both occasions, were recorded at 3 and 4 h p.i. (Fig. 2). In the sampling interval between 2 and 5 h p.i., 81.1% of the total number of cells were recovered (Fig. 2). The cell count then decreased gradually towards the end of the sampling period.

Before infusion, the concentration of BSA was 0.03±0.03 mg/ml (Fig. 2). The leakage of BSA increased significantly already between 1 and 1.5 h, and continued up to 2 h p.i., then decreased gradually (Fig. 2). The BSA concentration on each sampling occasion from 1.5 to 6 h p.i. was significantly higher than the pre-infusion value. The peak concentration of BSA, 0.71±0.16 mg/ml, occurred at 2 h p.i. (Fig. 2).

The NAG concentration was 6.5±4.7 units/10 µl pre-infusion (Fig. 2). A significant increase in NAG release was seen already between 1 and 1.5 h, which continued up to 2 h p.i. (Fig. 2). The NAG concentration on each sampling occasion from 1.5 to 7 h p.i. was significantly higher than pre-infusion. The highest concentrations, 19.7±7.0 and 20.2±7.0 units/10 µl, was detected at 2 and 3 h p.i., respectively (Fig.2).

Moderate to good correlations were found between the cell count and NAG values (0.63), between cell count and BSA (0.48), and between BSA and NAG (0.72).

In the control teat cisterns only minor chang-

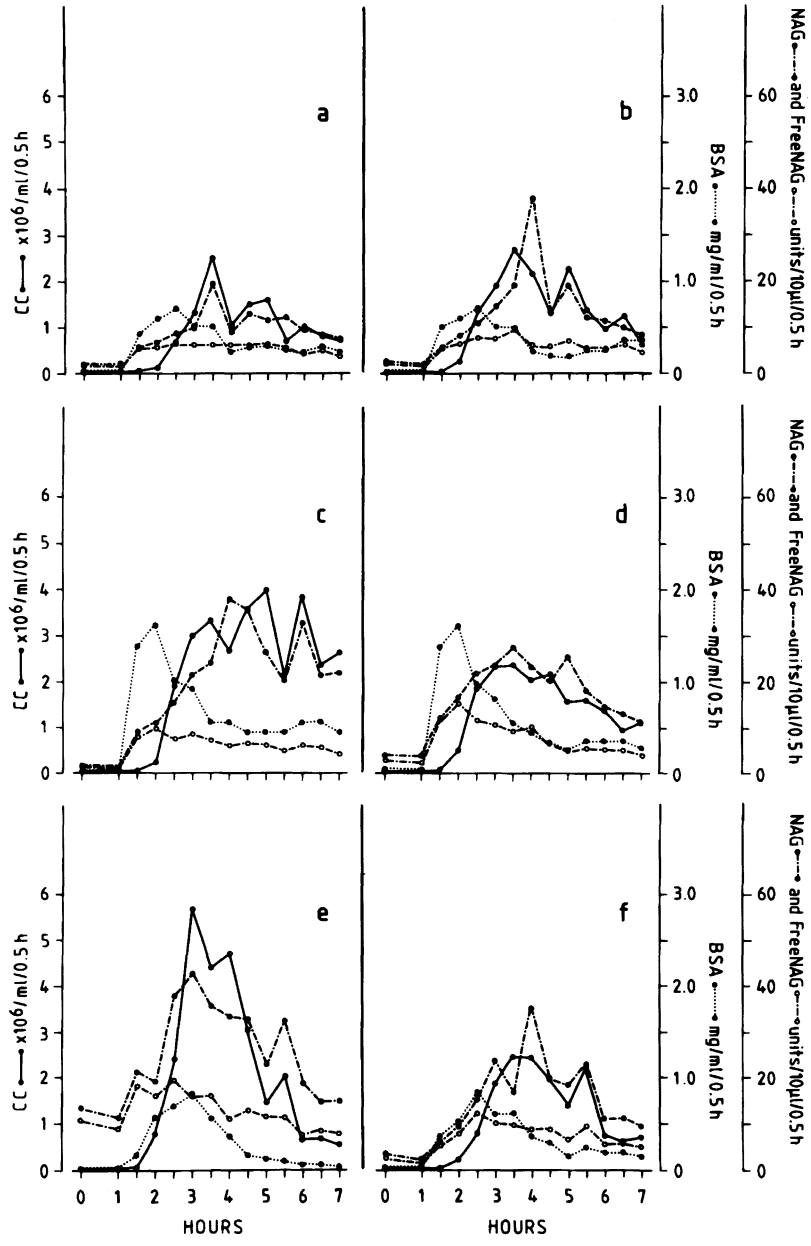

Figure 3. Cell count (CC), and concentrations of bovine serum albumin (BSA), N-acetyl-ß-D-glucosaminidase in samples containing cells (NAG), and N-acetyl-ß-D-glucosaminidase in cell-free samples (FreeNAG), in teat cistern samples after infusion of 20 µg Salmonella-endotoxin each at 0 h into 2 teat cisterns per cow, on 1 occasion, in cow 1 (a,b), cow 2 (c,d), and cow 3 (e,f).

es in the cell count, BSA and NAG values were noted throughout the sampling period (Fig. 2).

Experiment 2 - Endotoxin infusion into 2 teat cisterns

The teat cistern sample results, expressed as the accumulation of cells, BSA, NAG, and FreeNAG per time-unit (0.5 h), in experiment 2 are given as individual values in Fig. 3 and as average values (n=6) in Fig. 4.

Small numbers of cells (0.03±0.01 x 10^6/ml) were found in the pre-infusion teat cistern samples (Fig. 4). After endotoxin infusion, the neutrophil influx started to increase between 1.5 and 2 h, and continued up to 3 h.

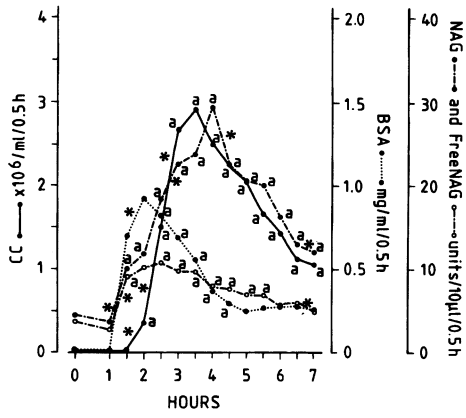

Figure 4. Average cell count (CC), and concentrations of bovine serum albumin (BSA), N-acetyl-ß-D-glucosaminidase in samples containing cells (NAG), and N-acetyl-ß-D-glucosaminidase in cell-free samples (FreeNAG), in teat cistern samples after infusion of 20 µg Salmonella-endotoxin each at 0 h in 2 teat cisterns per cow in 3 cows, on 1 occasion.

[a] The value differs from the pre-infusion value at $p<0.05$.

* The change during the time period (0.5h) is significant at $p<0.05$.

When the values were pooled, only 1 cell peak was seen (Fig. 4), even though more than 1 peak occurred in 5 out of 6 teats (Fig. 3). The number of cells on each sampling occasion from 2 h throughout the experiment, was significantly higher than pre-infusion. The maximum cell number, 2.9±0.4 x 10^6 cells/ml, was observed at 3.5 h p.i. (Fig. 4). In the sampling interval between 2 and 5 h p.i., 71.1% of the total number of cells were recovered (Fig. 4).

The pre-infusion concentration of BSA was 0.02±0.01 mg/ml (Fig. 4). A significant increase in the BSA value was seen between 1.5 and 2 h. The concentrations of BSA on each sampling occasion from 2.5 to 5.5 h p.i. were significantly higher than pre-infusion. The peak concentration of BSA, 0.92±0.0.49 mg/ml, occurred at 2 h p.i. (Fig. 4).

Before infusion, the NAG concentration was 4.4±2.8 units/10 µl (Fig. 4). The NAG release increased already between 1 and 1.5 h (Fig. 4). The NAG concentration was higher than pre-infusion from 1.5 h throughout the experiment. The peak concentration, 29.4±4.4 units/10 µl, was detected at 4 h p.i. (Fig. 4).

The concentration of FreeNAG before infusion was 3.6±2.6 units /10 µl (Fig. 4). The release of FreeNAG was increased significantly already between 1 and 1.5 h p.i.. The concentration of FreeNAG was significantly higher than pre-infusion from 1.5 to 5.5 h p.i.. The peak concentration, 10.7±3.5 units/10 µl, occurred at 2.5 h p.i. (Fig. 4). The FreeNAG concentration was significantly lower than the NAG level on each sampling occasion from 2.5 h p.i..

Moderate to very good correlations were seen between the cell count and NAG (0.91), between the cell count and FreeNAG (0.47), between BSA and FreeNAG (0.87), and between FreeNAG and NAG (0.59), while the correlations between the cell count and

Table 1. Endotoxin concentrations (g/ml) in teat cistern samples after infusion of 20 µg Salmonella-endotoxin, at 0 h, into a teat cistern in 3 cows.

Hours	Cows 1	2	3
0	$<10^{-11}$	$<10^{-11}$	$<10^{-11}$
1	10^{-6}	10^{-6}	10^{-6}
1.5	10^{-8}	10^{-8}	10^{-8}
2	$<10^{-11}$	10^{-10}	10^{-10}
2.5	$<10^{-11}$	$<10^{-11}$	$<10^{-11}$

BSA (0.22) and between BSA and NAG (0.31) were poor.

Detectable concentrations ($>10^{-11}$ g/ml) of endotoxin were found in the teat cistern samples taken at 1, 1.5 and 2 h p.i. (Table 1).

In experiment 1, the increase in the influx of cells between 2 and 2.5 h, and between 3.5 and 4 h p.i. was greater than in experiment 2. A larger total number of cells per teat was collected in experiment 1 (83.4±10.7 x 10^6 cells) than in experiment 2 (63.4±18.2 x 10^6 cells), but the difference was not significant. Regarding the BSA and NAG, no significant differences between the experiments were observed.

Experiment 3 - Influence of the sampling procedure

The total number of cells collected in teats sampled at 7 h only, 120.2±9.1 x 10^6 (n=3), was higher than in teats sampled both 1 and 7 h p.i., 63.9±32.9 x 10^6 (n=3), and also higher than the total numbers of cells collected in experiment 1, 83.4±10.7 x 10^6 (n=6), and in experiment 2, 63.4±18.2 x 10^6 (n=6) (Table 2). No such differences were noted when the teats sampled both 1 and 7 h p.i., were compared with experiments 1 and 2, respectively (Table 2).

Much higher concentrations of endotoxin were detected at 7 h, in the teats sampled at 7 h only (10^{-6} g/ml) than in the teats sampled at both 1 and 7 h (10^{-9} g/ml).

Table 2. Total numbers (× 10^6) of leukocytes collected in a 7 h period after infusion of Salmonella-endotoxin (20 µg/teat) into the teat cistern, following different experimental procedures, in experiments 1, 2, 3a, and 3b (*).

	Experiments 1[1]	2[1]	3a	3b
	91.2	46.2	31.7	119.7
	87.8	78.5	97.4	111.3
	71.2	68.4	62.6	129.5
x±SD	83.4±10.7[a]	63.4±18.2[a]	63.9±32.9[a]	120.2±9.1[b]

* Experimental procedures are given in materials and Methods

[1] Average of 2 teat cisterns/cow

[a,b] Values with different letters differ at $p<0.05$

Discussion

Infusion of endotoxin into the teat cistern induced inflammation, as measured by local clinical symptoms and the inflammatory markers cell count, BSA and NAG, but did not induce any general symptoms, or changes in concentrations of blood leukocytes and serum cortisol. Intramammary infusions of similar doses of endotoxin in the bovine lactating gland, however, were reported to induce general symptoms as well as alterations in blood parameters (*Paape et al.* 1974, *Lohuis et al.* 1988).

The first sign of inflammation in the teat cisterns was an increase in BSA and NAG between 1 and 1.5 h p.i., indicating a disturbance in the epithelial integrity, which occurred approximately 0.5 h prior to the first entry of leukocytes. The highest BSA concentrations were recorded before or during the initial influx of cells, which corresponded to an increase in teat blood flow reported between 1 and 2 h p.i. (*Persson* 1991). After endotoxin infusion into the lac-

tating bovine mammary gland, a rise in BSA prior to a rise in milk somatic cell count was also noted, and was suggested to be due to the accumulation of leukocytes in the tissues (*Frost et al.* 1984).

The initial increase in the concentration of NAG, an intracellular enzyme, paralleled the leakage of BSA. Epithelial cells and leukocytes both contain NAG (*Kitchen et al.* 1978, *Dulin et al.* 1985, *Mattila et al.* 1988), but as few leukocytes were present, the increase in NAG at this early stage indicated epithelial cell damage. Later on, however, when leukocytes were present in the samples, the NAG could have been extracellularly released from epithelial cells and leukocytes in vivo, and/or released from leukocytes disrupted due to freeze-thawing of the sample (*Kaartinen et al.* 1988). The FreeNAG, the NAG measured in cell-free samples, however, only reflects the extracellular release of the enzyme in vivo. This was illustrated by the good to very good correlation between the cell count and NAG, while the correlation between cell count and FreeNAG was only moderate. The FreeNAG was significantly lower than the NAG from 2.5 h p.i., a time when the influx of leukocytes to the teat cistern started to increase, but remained elevated for most of the sampling period.

The influx of leukocytes, mainly neutrophils, into the teat cistern was first seen 1.5 to 2 h p.i., and reached its maximum between 2.5 and 5 h. A similar neutrophil accumulation was seen after endotoxin infusion in the ovine non-lactating gland (*Colditz & Presson* 1988). After intradermal injection of endotoxin in the sheep (*Colditz* 1988) and rabbit (*Colditz & Movat* 1984), the neutrophil influx started earlier and peaked already during the second h p.i.. Although different animal species were used, the response after endotoxin infusion into a body cavity covered with epithelium could be expected to be different from the response after intradermal infusion.

The influx of neutrophils tended to occur in several peaks. The peak pattern was not so prominent when 2 parallel teats were used but could be observed when the individual curves were studied. Only 1 peak was seen after intradermal endotoxin injections (*Colditz & Movat* 1984, *Colditz* 1988) and after endotoxin infusion into the ovine non-lactating gland (*Colditz & Presson* 1988). In the latter experiment, however, samples were only taken every 2 hours. There is no clear-cut interpretation of the neutrophil influx pattern observed. It could be a result, e.g. of the sampling procedure, or of the endotoxin dose which was high compared to the doses used in the sheep and rabbit models. During the first 6 h after i.v. injection of the cytokine tumour necrosis factor (TNF) in rats, 2 peaks of neutrophilia was seen when higher doses of TNF were used; however, not with lower doses (*Ulich et al.* 1987). The appearance of several peaks of neutrophils might also indicate that different mediators, and/or different sources of mediators, were responsible for the recruitment of neutrophils at different stages of the inflammatory process. Leukocytes released into the teat cistern could produce chemotactic substances, identical to or different from the substances responsible for the initial leukocytosis, and thereby induce continued emigration of cells. One important inflammatory mediator, interleukin-1 (IL-1), has been reported to be produced by bovine polymorphonuclear leukocytes and macrophages (*Canning & Neill* 1989, *Politis* 1991). Other factors of importance in the recruitment of leukocytes, such as TNF and leukotriene B_4, are also produced by leukocytes (*Ford-Hutchinson et al.* 1980, *Beutler & Cerami* 1987).

As a result of the sampling procedure used in this paper, endotoxin as well as endogenous inflammatory mediators present in the teat cistern, were flushed out on every sampling occasion. Probably as a result of this, the total numbers of cells collected in experiments 1 and 2 were approximately 40% lower than in teats sampled only at 7 h p.i.. An additional sampling at 1 h p.i. reduced the total number of cells collected during a 7 h period compared to sampling only at 7 h. Additional samplings every 0.5 h up to 7 h, like in experiments 1 and 2, did not, however, reduce the total cellular accumulation more than taking only 1 sample at 1 h p.i. Small doses of endotoxin and short contact times are needed to induce inflammation (*Schultze* 1980, *Colditz & Presson* 1988), and endotoxin was still present at 7 h in teats sampled also at 1 h p.i., while no endotoxin was detected in teat cistern samples after 2 h p.i. in experiments 1 and 2. The early removal of endotoxin as well as the removal of early produced endogenous inflammatory mediators, e.g. IL-1, was probably of importance for the magnitude of the cell accumulation. Flushing samplings every 2 h up to 8 h after endotoxin infusion in non-lactating ovine mammary glands were not reported to affect the accumulation of cells (*Colditz & Presson* 1988), which may indicate that 2 hours are enough for a complete initiation of inflammation. However, in an additional experiment, in which teat cistern samples were taken 2 and 7 h after endotoxin infusion (results not shown), the total number of cells, 52.8±24.3 x 10^6 (n=3), was similar to in teats sampled 1 and 7 h p.i., and lower than in teats sampled at 7 h only.

The mode of action of the endotoxin for the initiation of inflammation is not completely understood, but resident tissue cells are probably the target cells, as a sampling before infusion, and the consequent depletion of the accessible leukocyte population in the teat cistern, still resulted in a considerable inflammatory response, an observation that has also been reported by others from studies in the ovine non-lactating gland (*Colditz & Presson* 1988).

However, a more rapid response was seen in teats in which the pre-infusion samples had a higher cell count (Fig. 1 b,c), which may indicate that the tissues were triggered before the experiment.

Endotoxin may directly alter the cellular function of a number of cells, including macrophages and endothelial cells (*Bradley* 1985), and thereby induce inflammation by initiating the production and/or release of a cascade of inflammatory mediators in the host which, among other things, stimulate the accumulation of neutrophils in the inflammatory lesions. Endotoxin shows chemotaxinigenic properties *in vivo* (*Colditz & Movat* 1984), but was not shown to promote chemotaxis of neutrophils *in vitro* (*Issekutz & Bhimji* 1982). A recent paper by *Zwahlen & Roth* (1990), however, reported a stimulation of bovine neutrophil migration *in vitro*. The purity of the neutrophil preparations could have influenced the results (*Klesius et al.* 1984).

Endotoxin has been shown to induce secretion of the cytokines IL-1 and TNF from monocytes/macrophages and other cells (*Beutler & Cerami* 1987, *Dinarello* 1984), and both substances have been found to be able to induce neutrophil migration in vivo (*Cybulsky et al.* 1988). Substantial evidence suggests that IL-1 and TNF are responsible for many effects previously attributed to endotoxin.

In summary, we conclude that a rapid local inflammatory response of rather short duration was elicited by endotoxin infusion into

the teat cistern. The inflammatory response was somewhat delayed, compared to the response reported after intradermal injections. The influx of leukocytes, mainly neutrophils, into the teat cistern tended to occur in several peaks, maybe indicating the roles for different chemotactic substances and/or different sources of such substances, at different stages in the inflammatory process. The sampling procedure decreased the total accumulation of leukocytes by approximately 40%. This was probably due to the removal of inflammatory mediators at an early stage, suggesting that a longer time period than 1 h was required to elicit a full inflammatory response. The parallel use of 2 teats instead of 1 had no major influence on the inflammatory process. The experimental procedure used should be suitable for further studies of the development of local inflammation.

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Sammanfattning

En studie av utvecklingen av endotoxininducerad inflammation i den bovina spenen.

Bovin endotoxininducerad lokal inflammation i spenen studerades med hjälp av en spencisternmodell. Denna modell ger en unik möjlighet till studier av spenreaktioner in vivo utan påverkan från juverkörteln.

Ett snabbt inflammatoriskt svar med relativt kort duration utlöstes. Ett ökat innehåll av bovint serum albumin och N-acetyl-ß-D-glucosaminidase i spencisternen, vilket indikerade en störning i epitelets integritet, noterades redan 1 till 1.5 t efter infusion. Cirka 0.5 t senare sågs det första inflödet av leukocyter, ffa neutrofiler. Cellinflödet tenderade att ske i flera omgångar mellan 2.5 och 5 t efter infusion.

Provtagningsförfarandet minskade cellansamlingen i spenen med cirka 40%, troligen pga avlägsnande av inflammationsmediatorer under initialskedet. Samtidigt användande av 2 spenar istället för 1 hade ingen större inverkan på inflammationsprocessen.

Denna spencisternmodell bör vara lämplig för vidare studier av lokal bovin inflammation.

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