mRNA expression of immunologically important factors and milk proteins in mammary tissue of dairy cows during LPS induced mastitis

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BACKGROUND

During the early stages of infection, non-specific responses are the predominant defences. A possible approach to study the inflammatory response of the cow's udder is to use endotoxin of *E.coli*, since it elicits mammary gland responses similar to a bacterial infection.

GOAL

The objective was to describe the acute phase time course of mRNA expression of various immunologically important factors (tumor necrosis factor α, insulin-like growth factor-1, cyclooxygenase-2, 5-lipoxygenase, platelet-activating factor acetylhydrolase, lactoferrin, lysozyme, inducible nitric oxide synthase) as well as of milk proteins (αS1-, αS2-, β-, κ-Casein, α-lactalbumin, β-lactoglobulin)

MATERIAL AND METHODS

Five healthy lactating cows were intrammary infused in one quarter with 100 µg *E.coli*-endotoxin (=lipopolysaccharid, LPS) and the contralateral quarter with saline (9 g/l) serving as control.
Mammary biopsy samples of both quarters were taken immediately before and at 3, 6, 9 and 12 h after induction of mastitis and mRNA expression of various factors was quantified via real-time RT-PCR.
Blood samples were taken following the same time course and rectal temperature was measured at 1-h intervals.

Mathematical and statistical analyses

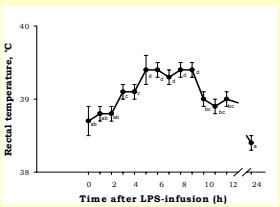
Data are presented as means and SEM.

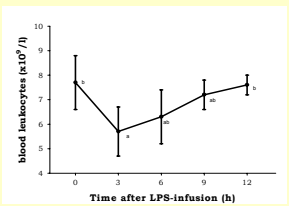
For statistical analysis, the MIXED procedure of the SAS program package (version 8.01) was used. Differences were considered significant if $P < 0.05$.

RESULTS

Temperature increased until 5 h ($P < 0.05$) after LPS administration and decreased to preinfusion level within 24 h after LPS-challenge.
Blood leukocyte concentration decreased ($P < 0.05$) from 0 to 3 h from 7.7±1.1 x 10^9/l to 5.7±1.0 x 10^9/l and thereafter recovered to pretreatment levels until 12 h after LPS-challenge.

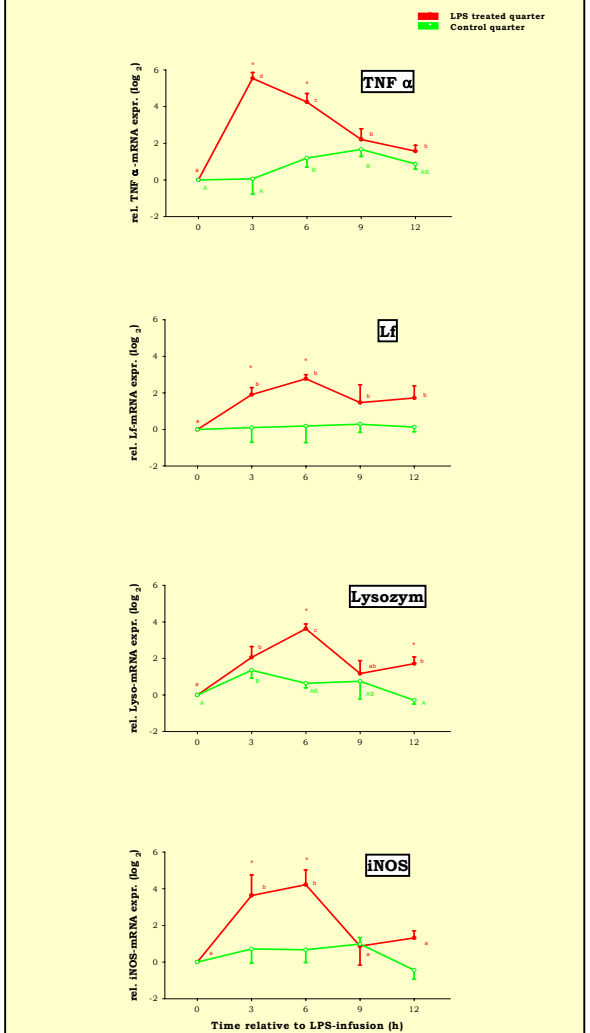
Rectal temperature and blood leukocytes





a,b,c,d: means with uncommon capital letters are significantly different ($P < 0.05$)

mRNA expression in mammary biopsy samples



*, means of LPS and control quarters are significantly ($P < 0.05$) different
A,B, means within-group with uncommon capital letters are significantly ($P < 0.05$) different
a,b,c,d, means within-group with uncommon capital letters are significantly ($P < 0.05$) different

In LPS-challenged quarters tumor necrosis factor α (TNFα) and cyclooxygenase-2 (COX-2) mRNA expression increased to highest values ($P < 0.05$) at 3 h after LPS-challenge.

Lactoferrin (Lf), lysozyme (Lyso), inducible nitric oxide synthase (iNOS) increased ($P < 0.05$) and peaked at 6 h after challenge, while platelet-activating factor acetylhydrolase (PAF-AH) mRNA increased only numerically.

mRNA expression of insulin-like growth factor-1 (IGF-1), 5-lipoxygenase (5-LO) and of αS1-Casein (CN), αS2-CN, β-CN and β-lactoglobulin (b-LG) did not change significantly, whereas mRNA expression of α-lactalbumin (α -LA) decreased ($P < 0.05$) in both quarters and that of κ-CN only in the LPS quarter.

mRNA expression in mammary biopsy samples

Inflammatory factors

Changing of mRNA expression, $\log_2$ (relative to time 0)	Time after endotoxin-infusion: 3 h Control ø±SEM	3 h LPS ø±SEM	6 h Control ø±SEM	6 h LPS ø±SEM	9 h Control ø±SEM	9 h LPS ø±SEM	12 h Control ø±SEM	12 h LPS ø±SEM
COX-2	1.51±1.05	4.45*±0.89	1.23±0.54	3.75*±0.63	2.81±1.62	-0.76*±1.25	-0.83±0.30	0.07±0.43
PAF-AH	0.29±0.82	0.82±0.47	0.2±91.01	1.45±0.41	0.12±0.80	-0.23±0.44	-0.63±0.68	0.50±0.83
IGF-1	0.59±0.96	0.02±0.45	1.42±1.20	0.92±0.81	1.48±1.17	0.34±0.67	0.60±0.52	0.50±0.45
5-LO	-0.54*±0.48	-0.62±0.48	-0.37*±0.33	-1.02*±0.44	-0.66*±0.36	-2.15*±0.59	-1.11*±0.36	-1.26*±0.13

Milk proteins

Changing of mRNA expression, $\log_2$ (relative to time 0)	Time after endotoxin-infusion: 3 h Control ø±SEM	3 h LPS ø±SEM	6 h Control ø±SEM	6 h LPS ø±SEM	9 h Control ø±SEM	9 h LPS ø±SEM	12 h Control ø±SEM	12 h LPS ø±SEM
αS1-CN	-0.58±0.69	-0.33±0.44	-1.20±0.81	0.09±1.00	-1.59±0.70	-0.88±0.25	-0.80±0.35	-0.45±1.08
αS2-CN	-0.61±0.65	-0.50±0.35	-1.40±0.91	-0.27±1.05	-1.63±0.82	-1.46±0.11	-1.07±0.29	-0.97±1.04
β-CN	-0.83±0.74	-0.75±0.34	-1.29±1.02	-0.51±1.25	-1.74±0.81	-1.99±0.48	-1.39±0.26	-1.32±1.17
κ-CN	-0.33±0.36	0.34±0.46	-0.60±0.63	-0.04±0.45	-1.09±0.55	-1.83*±0.60	-0.65±0.24	-0.67±0.64
α-LA	-0.96±0.89	-1.42±0.58	-2.26±1.21	-1.38±1.73	-3.14*±1.44	-2.50*±0.36	-2.14±0.32	-2.13±1.49
b-LG	-0.11±1.09	-0.23±0.39	-1.48±1.32	0.43±1.42	-1.47±1.31	-1.34±0.22	-0.67±0.53	-0.40±1.71

x, means of LPS treated quarters differ significantly ($P < 0.05$) from 0 h
y, means of control quarters differ significantly ($P < 0.05$) from 0 h
*, means of LPS and control quarters are significantly ($P < 0.05$) different

CONCLUSION

mRNA expression of most inflammatory factors changed within hours after LPS challenge, whereas that of most milk proteins remained unchanged.