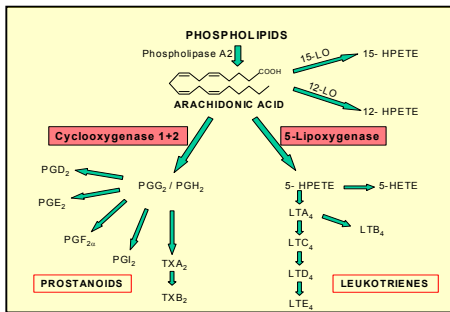


## Introduction

Cytokines and inflammatory mediators, such as prostaglandins and leukotrienes, exhibit potent chemokinetic and chemotactic activity for leukocytes and enhance the bactericidal activity of phagocytes. Moreover, they cause increasing vascular permeability and hyperalgesia during inflammatory disorders [1,2,3].

The goal of this work was to investigate whether leukotrienes and prostaglandins are produced by different fractions of somatic cells in cow milk. Therefore, we have developed a quantitative RT-PCR method to determine the mRNA expression of the key enzymes in leukotriene and prostaglandin biosynthesis, i.e. 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), respectively. The expression of tumor necrosis factor alpha (TNF $\alpha$ ), a cytokine known to be crucial during early inflammatory stages in the mammary gland [3], has been studied concomitantly.

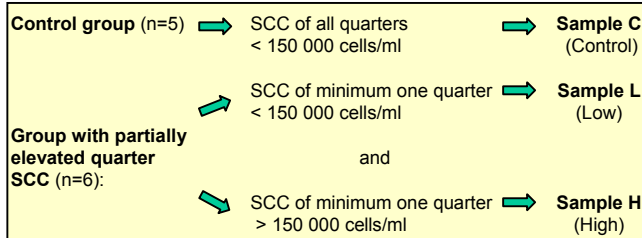


Pathways of leukotriene and prostaglandin biosynthesis

## Material and Methods

### Animals

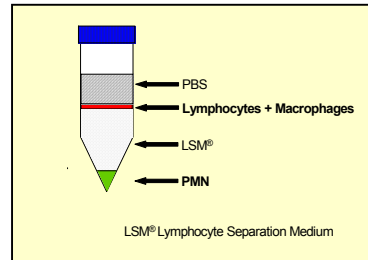
Brown Swiss dairy cows (n=11) with no clinical signs of mammary disease were investigated. Somatic cell counts (SCC) of total quarter milk was measured with a fluoro-opto-electronic method using a Fossomatic cell counter (Foss Electric, Denmark). Based on the SCC results animals were divided in two groups:



### Cell isolation

Total quarter milk from one quarter of control group and from two quarters of cows with partially elevated SCC (one of H and one of L) was collected at one morning milking. The milk was centrifuged and the cell pellet was

washed three times in phosphate buffered saline (PBS), pH 7.4. The cell fractions were separated using a density gradient (Lymphocyte Separation Medium, ICN, Aurora, OH, USA). Lymphocytes and macrophages (Lym+Mac) were distributed within the interphase, polymorphonuclear leukocytes (PMN) were located in the pellet.



Separation of milk cell fractions

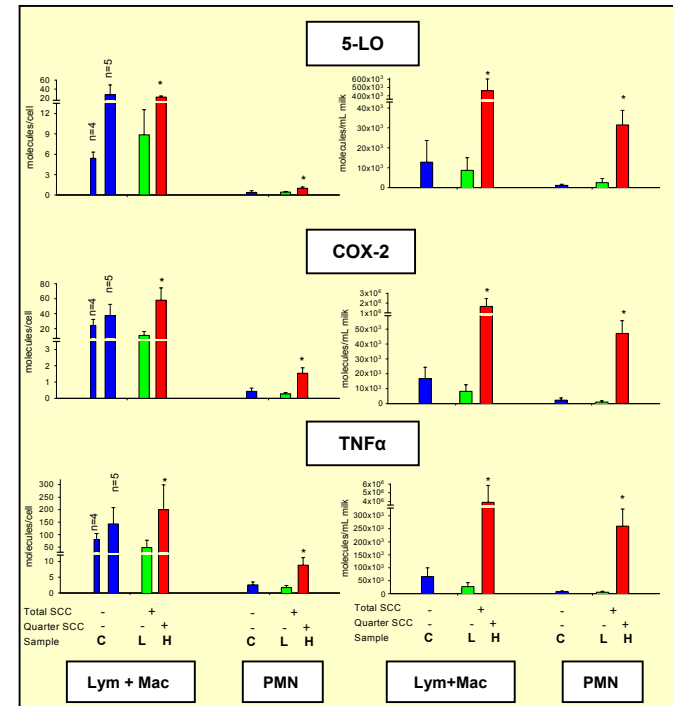
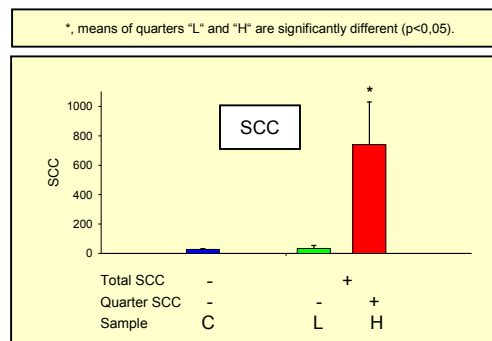
### RNA Isolation and RT-PCR

Total RNA was isolated using TriPure (Roche, Basle, Switzerland) according to the manufacturers instructions. Synthesis of first strand cDNA was performed with MMLV-RT (Promega, Madison, USA) and random hexamer primers. Quantitative analysis of PCR products was carried out in the LightCycler using specific primers and LightCycler DNA Master SYBR Green I (Roche). External DNA standard dilutions were generated from cloned RT-PCR products into pCR 4.0 vector (Invitrogen, Groningen, NL).

### Statistical evaluations

Differences between C and L quarters and differences between Lym+Mac and PMN cell fractions were tested for significance (p<0.05) using Wilcoxon's rank sum test. Differences between L and H quarters (within animal) were tested for significance (p<0.05) by Wilcoxon's signed rank test.

## Results and Discussion



In one control animal gene expression of all factors determined was higher than in the other four animals despite similarly low SCC. The reasons are unclear.

Our results indicate that 5-LO, COX-2 and TNF $\alpha$  are, at least to a certain extent, produced by somatic milk cells, i.e. represent milk borne factors. Expression of each compound studied was locally elevated in quarters with more immunological activity, i.e. higher SCC, indicating that the somatic milk cells themselves are involved in the maintenance of immune response in milk. However, most likely these factors are also synthesized by mammary epithelial cells. Further investigations are in progress.

### References

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- Persson, K., Larsson, I. & Hallén Sandgren, C. Effects of certain inflammatory mediators on bovine neutrophil migration in vivo and in vitro. Veterinary Immunology and Immunopathology 37: 99-112 (1993)
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