# INFLAMMATORY MEDIATORS IN MAMMARY GLAND IMMUNOLOGY: QUANTIFICATION OF KEY FACTORS BY LIGHTCYCLER® REAL-TIME PCR

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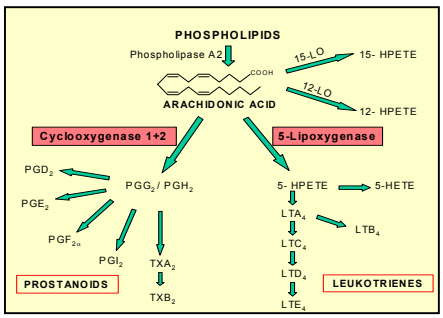
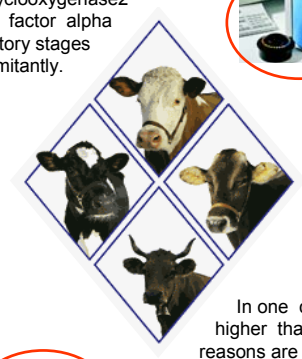
## Introduction

Inflammatory mediators, such as cytokines, 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 cyclooxygenase2 (COX-2), respectively. The expression of tumor necrosis factor alpha (TNFα), 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*

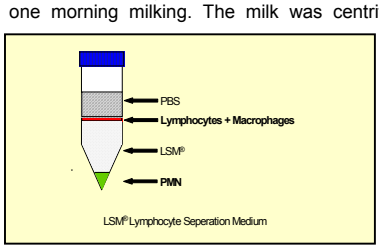
Brown Swiss dairy cows (n=11) with no clinical signs of mammary disease were used. Somatic cell counts (SCC) of total quarter milk was measured with a fluoro-opto-electronic method using a Fossomatic® cell counter (Foss Electric, Denmark). Based on the SCC results animals were devided in two groups:

**Control group** (n=5) → SCC of all quarters < 150 000 cells/ml → **Sample C** (Control)

**Group with partially elevated quarter SCC** (n=6):
- SCC of minimum one quarter < 150 000 cells/ml → **Sample L** (Low)
- and
- SCC of minimum one quarter > 150 000 cells/ml → **Sample H** (High)

*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 PBS (phosphate buffered saline). The cell fractions were separated using a density gradient (LSM®, ICN, Aurora, USA). Macrophages and lymphocytes (Mac+Lym) were distributed within the inter-phase, polymorphonuclear leukocytes (PMN) were located in the pellet.




**Seperation of cell fractions**

*RNA Isolation and RT-PCR*

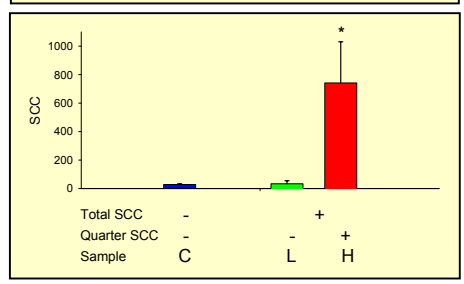
Total RNA was isolated using TriPure® (Roche, Basle, Switzerland) according to the manufacturers instructions. Synthesis of first strand cDNA was performed with MMLV-RT (Promega, Madison, USA) and random hexamer primers. Quantitative analysis of PCR products was carried out in the LightCycler® using 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-TOPO® vector (Invitrogen, Groningen, NL).

*Statistical evaluations*

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

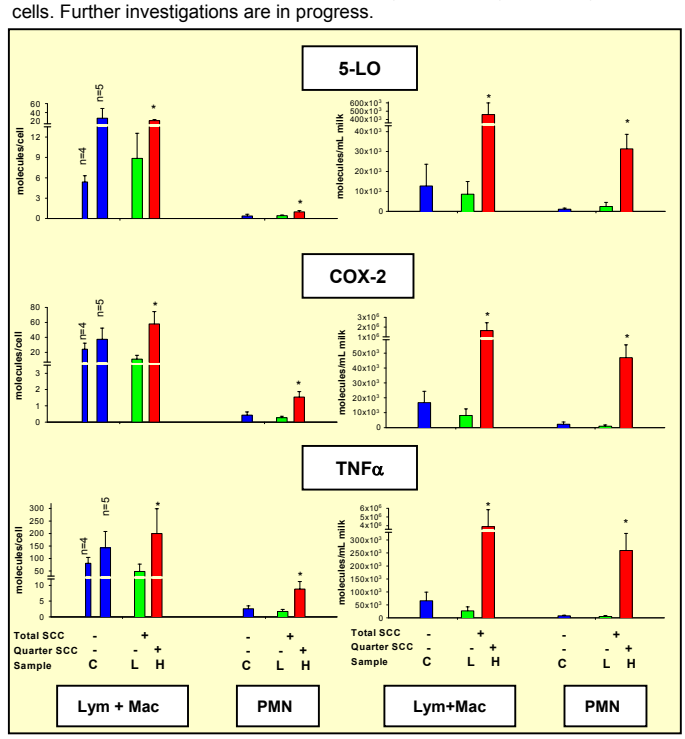
## Results and Discussion

*, means of quarters "L" and "H" are significantly different (p<0,05).




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 TNFα 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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