

## QUANTITATIVE CYTOKINE AND ACUTE PHASE PROTEIN mRNA EXPRESSION IN LIVER AND MAMMARY GLAND FROM DAIRY COWS EXPERIMENTALLY INFECTED WITH *Streptococcus uberis*

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### Introduction

*Streptococcus uberis* cause a significant proportion of clinical and subclinical intramammary infections in lactating dairy cows. In particular, pro-inflammatory cytokines and acute phase proteins are involved in the mammary glands immune response to invading microorganisms. Cytokines stimulate the production of acute phase proteins, primarily produced by the liver. But recently, focus has been on a suggested local production of acute phase proteins in the mammary gland. The objective of this study was therefore to elucidate the gene expression of cytokines and acute phase proteins in the liver and compare that to the gene expression in the mammary gland. Furthermore, we wanted to study in which region of the mammary gland the inflammatory factors were expressed.

### Materials and Methods

Mastitis was induced in the right front quarter of six Holstein-Friesian cows in mid-lactation with no prehistory of *S. uberis* mastitis. The cows were inoculated with approximately 10<sup>7</sup> colony-forming units of *S. uberis* into the teat cistern of the right front quarter. The left front quarter was simultaneously inoculated with sterile milk (control). The six cows were euthanized consecutively at 2-h intervals at 2, 4, 6, 8, 10 and 12 h after inoculation. After euthanasia, liver and mammary tissue was collected and snap frozen in liquid nitrogen. Mammary tissue was collected from the right front mammary gland at four different sections (A: glandular cistern, B: distal, C: central and D: proximal mammary gland). RNA was extracted from liver and mammary tissue using an RNeasy Protect Mini Kit (Qiagen), and concentrations were measured on a GeneQuant *pro* spectrophotometer (Amersham Pharmacia Biotech) and adjusted to 0.2 µg/µl. Specific primers and probes were designed for tumour necrosis factor α (TNF-α), interleukin (IL) 1β (IL-1β), IL-6, IL-10, haptoglobin (Hp), serum amyloid A (SAA) and α1-acid glycoprotein (AGP) mRNA, and the mRNA expression was quantitatively evaluated by real-time RT-PCR (Taqman®).

### Results

TNF-α, IL-1β, IL-6, IL-10, Hp, SAA and AGP mRNAs were found both in the liver and in the mammary gland. Expression levels of liver IL-6, SAA and AGP remained constant, except for cow no 5 (euthanized at 10 h), which had a much higher expression level than other cows. We observed increased expression levels of liver TNF-α, IL-1β, IL-10 and Hp depending on time of euthanasia. TNF-α expression was increased as early as 4h after inoculation (cow 2), IL-10 were highest in cow 4 and 5 (8 and 10h after inoculation) and Hp was highest in cow 6 (12 h after inoculation). Compared to expression profiles in the mammary gland, there were no indications of a relation between liver and mammary gland gene expression for any of the measured genes.

Expression of some cytokines and acute phase proteins appear to change considerably due to time of inoculation, but comparison of average expression levels between liver and mammary tissue showed a higher expression of AGP, Hp, IL-1β and IL-10 in liver tissue (Table 1). The opposite was the case for SAA, IL-6 and TNF-α, which were higher expressed in mammary tissue than in liver tissue (Table 1).

Table 1. Differences in gene expression between liver and mammary tissue for the seven tested genes.

	AGP	Hp	IL-1β	IL-10	SAA	IL-6	TNF-α
Fold difference in gene expression (Liver/mammary tissue ratio)	131072	446	1.6	9.2			
Fold difference in gene expression (Mammary tissue/liver ratio)					4	274	2.3

The acute phase proteins Hp, SAA and AGP were primarily expressed in the glandular cistern and the distal region of the mammary gland. IL-1β and IL-10 had a similar expression pattern, showing the highest expression in the distal part of the gland, while TNF-α primarily was expressed in the central and proximal region of the mammary gland. IL-6 was equally expressed in the mammary gland.

### **Discussion**

TNF- $\alpha$  is one of the fastest responding cytokines. Thus, it was expected to find the early increase in liver expression. Surprisingly, mammary gland TNF- $\alpha$  increased much later, but Pfaffl (2003) have suggested, that “milk cells” are the main source of TNF- $\alpha$  rather than the mammary gland. Although, the contribution of TNF- $\alpha$  mRNA in milk may primarily be from the leukocytes.

Comparison of gene expression between liver and mammary tissue has not been done before. We were surprised to find a higher expression of SAA in the mammary gland than in the liver, since liver is thought to be the main source of SAA.

If the production of acute phase proteins in the mammary gland is regulated in a similar way as in the liver, it is surprising to find the acute phase proteins and the regulatory cytokines expressed in different regions of the mammary gland. Though, IL-6 is thought to be the main stimulator of the acute phase proteins and this cytokine was expressed equally throughout the mammary gland.

Since the presented results are only from one animal at each sample time, data should be verified by further experiments. Furthermore, expression data from the mammary gland should be compared with expression analysis from the control quarter.