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THE IMMUNOSUPPRESSIVE EFFECT OF *STAPHYLOCOCCUS AUREUS* MASTITIS ON IL-8 ACTIVITY

(With One Figure)

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تأثير الثبيط المناعي في ألتهاب الضرع المتسبب بواسطة *Staphylococcus aureus* على نشاط الـ IL-8

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يلعب السايتوكاينز IL-8 الذي له دور مباشر في جذب النيتروفيل دورا مهما في تنظيم الألتهابات. لقد جذب الدور الذي يلعبه IL-8 في التهاب الضرع الذي تسببه *Staphylococcus aureus* إهتماما ملحوظا في السنوات السابقة. ولقد لوحظ اهمية مراقبة مستوى ونشاط الـ IL-8 في الأبقار. لقد تم استخدام تقنية البلمرة المعروفة بـ TaqMan® لمراقبة نشاط انتاج مادة الـ mRNA لـ IL-8 في الأبقار المصابة مخبريا بألتهاب الضرع المتسبب بواسطة *Staphylococcus aureus* في الأوقات صفر 7، 24 ، و 32 ساعة بعد العدوى. لقد لوحظ ازدياد في نشاط الـ mRNA لـ IL-8 في الساعة 24 ولكن لم تبلغ الزيادة مستوا ذو دلالة إحصائية. ان انعدام الدلالة الأحصائية لهذا الأرتفاع متطابقا مع النتائج التي توصلت لها الدراسات مؤخرا. أن عدم الأرتفاع الملحوظ في مستوى نشاط الـ mRNA لـ IL-8قد يكون بسبب التثبيط المناعي المنتج بواسطة الـ *Staphylococcus aureus* من خلال تخفيض مستوى الـ NF-κB المادة الأساسية في انتاج IL-8 .

SUMMARY

IL-8 is a neutrophil chemoattractant that plays important role in the regulation of inflammatory responses. The role of IL-8 in the *Staphylococcus aureus* mastitis has drawn considerable attention in the last years. The level and extent of the IL-8 transcriptional activity was considered vital to be monitored. Bovine TaqMan® real-time PCR was employed to examine the IL-8 mRNA transcriptional activity in cattle experimentally infected with *Staphylococcus aureus* at the time points 0, 7, 24 and 32 hrs post-infection. The level of IL-8 transcriptional

activity increased at 24 hrs post-infection, however non-significantly. The non-significant increase in the IL-8 activity in *Staphylococcus aureus* mastitis was seen in accordance with results that several studies have reported recently. It was postulated that *Staphylococcus aureus* elicits immunosupression by dwonregulating the NF-κB, a major IL-8-inducing factor.

Key words: *bovine, IL-8, mastitis, S. aureus, TGF-β, NF-κB, TaqMan®*

INTRODUCTION

Staphylococcus aureus (S. aureus) mastitis is a contagious disease, characterized by congestion, hardness of udder and formation of milk clots and elevation of somatic cells. The disease starts as acute and becomes chronic at the late stage (Sutra and Poutrel, 1994).

Interleukin-8 (IL-8) is neutrophil-chemotactic chemokine produced by stimulated monocytes, T-lymphocytes, macrophages, neutrophils, endothelial cells and number of tumor cell lines (Matsushima and Oppenheim, 1989). In an explicit approach, Boulanger *et al.,* (2003) revealed an indispensable role for the nuclear factor-κB (NF-κB) genes encoding inflammatory proteins possess κB sites in their promoter, as an important transcriptional regulatory factor in upregulating genes encoding IL-8 and granulocyte-monocyte-colony stimulating factor (GM-CSF). Milk from mastitis-affected cows exhibited intense NF-κB activity with positive correlation with the elevated level of IL-8 and GM-CSF. Accordingly, expression and induction of IL-8 in mastitis appears to be under a tight control of NF-κB factors (Boulanger *et al.,* 2003). Tumor necrosis factor-α (TNF- α) has shown to act as one of the major inducers of the NF-κB expression (Hacker and Karin, 2006).

Distinct differences in the level of IL-8 expression were revealed between *Escherichia. coli* (*E. coli*) and *S. aureus* mastitis (Riollet *et al.,* 2001; Lee *et al.,* 2006; Lahouassa *et al.,* 2007;Yang *et al.,* 2008,). *S. aureus* stimulated weaker IL-8 responses whereas *E. coli* induced copious level of the cytokine. Further studies revealed that the host genetic background and the mammary glands tissue type could play a role in the extent of the *S. aureus*-IL-8 responses (Lahouassa *et al.,* 2007; Tao *et al.,* 2007; Yang *et al.,* 2008). Tissue of mammary glands and milk somatic cells has distinct differences in the level of IL-8 synthesis. Bovine mammary epithelial cells was shown to produce IL-8 in a constitutive manner and its level in immune responses to *S. aureus*

was higher and relentless (Lahouassa *et al.,* 2007; Yang *et al.,* 2008). On the other hand, the pattern of the host gene expression could also influences the lethal effect of the *S. aureus* mastitis (Tao, and Mallard 2007).

The main objectives of this study were to monitor the IL-8 responses at the early stages of the *S. aureus* mastitis and to detect the stage when IL-8 activity will be affected by the mastitis with the aid of the bovine TaqMan® real-time PCR system (Leutenegger, *et al.,* 2000).

MATERIALS and METHODS

Experimental infection, somatic cells extraction and TaqMan® real time PCR

Experiment on the transcriptional activity of IL-8 was carried out on somatic cells from milk samples that were obtained from previous study (Alluwaimi *et al.,* 2003). Isolation and diagnosis, RNA extraction and real-time TaqMan® PCR were as described previously (Alluwaimi, *et al.,* 2003).

Milk samples were collected from infected and non-infected quarters of six Holstein cows at the time points immediately before inoculation, at 7 hr, 24 and 32 hr post-infection (pi). Establishment of the infection in the infected quarters, level of somatic cell counts in the normal and infected quarters and bacterial isolation were described previously (Alluwaimi *et al.,* 2003).

The IL-8 probes and primers

The sequences of the primers and the probes of IL-8 are as follows, forward primer, IL8.250f 5'-CACTGTGAAAAATTCAGAAATCATTGTTA-3', reverse primer, IL8.331r 5'-CTTCACAAATACCTGCACAACCTTC-3'and the probe, IL8.286p 5'-AATGGAAACGAGGTCTGCTTAAACCCCAAG-3' (GeneBank accession no AF232704). Bovine cDNA was assayed for the cytokine profile and for GAPDH as endogenous control in separate wells. The sequence of bovine GAPDH primers and probes are [Forward primer. 463f. 5'-GGCGTGAACCACGAGAAGTATAA-3', Reverse primer, GAPDH. 582r 5'-CCCTCCACGATGCCAAAGT-3' and the probe GAPDH. 489p 5'-ATACCCTCAAGATTGTCAGCAATGCCTCCT-3' (GeneBank accession no AF022183)].

Statistical analysis

Statistical analysis was performed using BMDP statistical software (BMDP Statistical Software, Inc., Statistical Solutions, Limited, Crosse's Green, Cork, Ireland). The data were analyzed with one factor analysis of variance.

RESULTS

The expression of interleukin-8 mRNA was evident at all time points pi. The transcriptional level of IL-8 started to decrease at 7hr pi followed by a sharp increase at 24hr pi and declined gradually at 32hr pi (Fig. 1).

A one-factor analysis of variance with time being within subject (repeated measures) factor revealed non significant linear ($P = 0.60$), quadratic ($P = 0.48$) and marginally significant cubic ($P = 0.051$) time effects. The marginal significance time effect was between 7 and 24 hr pi.

Figure Caption:

Fig. 1: The geometric mean of relative quantification of IL-8 transcriptional activity in somatic cells from quarters of six cows infected with *S. aureus* at different time points data were recorded. The level of IL-8 elevated sharply but non significantly at 24hr post-infection.

DISCUSSION

Immune response to *S. aureus* in bovine mammary gland was a subject of important studies in the last years (Alluwaimi *et al.,* 2003; Takahashi *et al.,* 2005; Lee *et al.,* 2006; Lahouassa *et al.,* 2007; Tao *et al.,* 2007; Yang *et al.,* 2008)**.** Comparison between the *E. coli* and the *S. aureus* mastitis disclosed interesting differences in the nature and extent of the cytokines activity (Riollet *et al.,* 2001; Lee *et al.,* 2006; Lahouassa *et al.,* 2007; Yang *et al.,* 2008). Among these cytokine, IL-8 was studied extensively and its mechanism of expression and regulation in *S. aureus* was addressed in depth (Yang *et al.,* 2008; Lee, *et al.,* 2006). The expression of adhesion molecules CD18/CD11b suffered a distinct set back on neutrophils from mammary gland with *S. aureus* mastitis as well as their cytokines synthesis capability (8). Although the IL-8 transcriptional activity recorded elevation at 24 pi, it failed to indicate significant increase in its level. Similar results were also reported by others (Lee *et al.,* 2006; Yang *et al.,* 2008). It appears that there are no early effects on the IL-8 expression at the *S. aureus* mastitis. Takahashi *et al.* (2005) reported that infusion of mammary glands with recombinant IL-8 caused significant decrease in the milk *S. aureus* count at the early stage of the infection whereas the cytokines failed to provide substantial changes in the infection progress at the late stage of mastitis.

Several mechanisms by which *S. aureus* inflicts lethal suppression on the IL-8 activity were postulated (Bannerman *et al.,* 2006; Hacker and Karin, 2006; Yang *et al.,* 2008). *S. aureus* mastitis was shown to result in a significant elevation of the transforming growth factor-β (TGF- β). TGF- β is well known anti-proinflammatory cytokines (Bannerman, *et al.,* 2006). Hence, the non-significant increase in the IL-8 level in the *S. aureus* mastitis could be influenced by the elevated level of TGF- β. On the other hand, IL-8 activity is under the control of the NF-κB expression (Matsushima and Oppenheim, 1989). TNF-α appears to play a role in regulation of NF-κB (Boulanger *et al.,* 2003). In an conclusive study, Yang *et al.* (2008) indicated that the down regulation of NF-κB could be achieved by either of two important mechanisms. Either by reducing the strength of the trasnducing signal through the toll-like receptor (TLR) or through the down regulation of the TNF-α. Elevated level of TGF-β in the *S. aureus* mastitis renders the strength of the activation signal transduced through the TLR less effective. NF-κB is considered one of the major down stream signalling pathway of TNF-α. Hence, deficiency in the TNF-α synthesis in the *S.*

aureus mastitis causes depression of the NF-κB expression (Yang *et al.* 2008).

In conclusion, *S. aureus* mastitis elicits weak inflammatory responses due to the major changes in the cytokines network, mainly TGF-β and TNF-α that lead to major down regulation in NF-κB. However, IL-8 could be expressed normally at the early stage of the *S. aureus* mastitis but its expression most probably suffers a considerable set back as the disease progresses.

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Figure Caption:
Fig.1. The geometric mean of relative quantification of IL-8 transcriptional activity in somatic cells from quarters of six cows infected with *S. aureus* at different time points data were recorded. The level of IL-8 elevated sharply but non significantly at 24hr post-infection.

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