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Dynamics of Cytokines Associated with IL-17 Producing Cells in Serum and Milk in Mastitis of Experimental Challenging with *Staphylococcus aureus* and *Escherichia coli* in Dairy Goats

X.Q. Jing, Y.Q. Zhao, C.C. Shang, Y.L. Yao, T.T. Tian, J. Li and D.K. Chen
College of Veterinary Medicine, Northwest A&F University,
712100 Yangling, Shaanxi Province, P.R. China

Abstract: Interleukin-17 is a critical pro-inflammatory cytokine in the development of autoimmunity and the immune responses against infection of bacteria, fungus and parasites. In the present study, dynamics of IL-17 and cytokines associated with IL-17 producing in serum and milk in experimental mastitis challenged with *S. aureus* and *E. coli* in dairy goats were monitored using commercial ELISA kits. The results showed that the levels of IL-17 in milk were peaked at 24 or 48 h post challenged with *E. coli* or *S. aureus*, respectively but no detectable peak was found in serum. The levels of TGF-β, IL-6 and IL-1β in milk were elevated in goats challenged with *E. coli* or *S. aureus* but only slight fluctuant were found in serum. These indicated that IL-17 was an important cytokine in the inflammation development of dairy goat mastitis challenged with *E. coli* or *S. aureus* and the local pro-inflammatory cytokines milieu plays an important role in the development of subclinical mastitis whether infected with *E. coli* or *S. aureus*.

Key words: IL-17, mastitis inflammation, dairy goats, cytokines, China, ELISA kits

INTRODUCTION

Mastitis, a highly prevalent disease in dairy goats, cows and ewes is known to affect the production and quality of milk, animal health and even threat human health through consumption of milk (Leitner *et al*., 2008; Halasa *et al*., 2009; Le Marechal *et al*., 2009, 2011). The majority of clinical and subclinical mastitis cases in goat were caused by *Staphylococcus aureus*, *Streptococcus uberis* and *Escherichia coli* (Pisoni *et al*., 2010). Therefore, prevention and control procedures to mastitis of animals mainly depend on antibiotic therapy in dry period in clinic (McDougall *et al*., 2010; Bradley *et al*., 2011). However, considering the shortcomings of residues and resistant of antibiotics (Vaarst *et al*., 2006; Virdis *et al*., 2010), new non-antibiotic therapy strategies are expected to be developed. Recently, several studies showed that the detail understanding the defense mechanism of mammary glands and development of inflammation in mastitis are the key issues to control mastitis (Mazzilli and Zecconi, 2010; Rinaldi *et al*., 2010).

Interleukin-17 (IL-17) is an important pro-inflammatory cytokine which plays a critical role in the development of inflammation in autoimmunity (allergic asthma, rheumatoid arthritis, systemic lupus erythematosus inflammatory bowel disease) (Yen *et al*., 2006; Doreau *et al*., 2009; Shahrara *et al*., 2009; Wang *et al*., 2010) and immune responses against infection of bacterial (*Klebsiella pneumonia*, *S. aureus*, *listeria monocytogenes*) (Aujla *et al*., 2008; Hamada *et al*., 2008; Ishigame *et al*., 2009), fungal (*Candida albicans*) (Huang *et al*., 2004). In these processes, IL-17 could induce and enhance expression of several chemokines (CXCL1, CXCL8) which would indirectly activate and recruit neutrophils into the impaired tissue to trigger effective immune response (Park *et al*., 2005; Hartupee *et al*., 2007; Yu *et al*., 2007; Roussel *et al*., 2010). In IL-17-deficient mice, collagen-induced arthritis was significantly suppressed (Nakae *et al*., 2003) and IL-17RA$^{-/-}$ mice were more susceptible to infected *Porphyromonas gingivalis* and *Candida albicans* than wild mice (Huang *et al*., 2004; Yu *et al*., 2008). Tao and mallard had reported that IL-17 mRNA was up-regulated in *S. aureus* mastitis of cows and IL-17 was more highly expressed in milk somatic cells than blood mononuclear cells but the level of IL-17 protein was not determined in this study (Tao and Mallard, 2007).

In the present study, the mastitis model in Guanzhong dairy goat was established through experimentally challenged with the two main pathogens of

Corresponding Author: D.K. Chen, College of Veterinary Medicine, Northwest A&F University, 1 Xinong Road, The Yangling Agricultural Hi-Tech Industries Demonstration Zone, 712100 Yangling, Shaanxi Province, The People's Republic of China

S. aureus or *E. coli*, respectively and dynamics of the levels of IL-17 and cytokines associated with IL-17 producing in serum and milk were monitored in the development of mastitis.

MATERIALS AND METHODS

Animals: Six healthy Guanzhong dairy goats in mid-lactation were selected that were negative for LMT detection (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu province, China) and bacteriological analysis in milk. The bacteriological analysis was performed according to previous research (Moroni *et al*., 2005).

Preparation of bacteria: The organisms used were *E. coli* and *S. aureus* which were originally isolated from subclinical cases of dairy goat mastitis and had been used to establish mastitis model in dairy goat (Mo *et al*., 2011). Prior to intramammary infection each strain was spread onto trypticase soy agar plates and incubated overnight at 37°C and then a bacterial colony was selected and inoculated with nutrient broth for 18 h at 37°C. After determining the concentration of the stock cultures based on the spread plate colony counts, the stock was diluted in Phosphate Buffered Saline (PBS) to a final concentration of 3×10^3 Colony Forming Unit mL^{-1} (CFU mL^{-1}) of *E. coli* or 3×10^2 CFU mL^{-1} of *S. aureus*.

Intramammary challenge with *E. coli* or *S. aureus*: The dairy goat was infected immediately following the morning milking by hand. Briefly, the right half udder of each goat was infused with 1 mL of prepared *E. coli* (n = 3) or *S. aureus* (n = 3) inoculum and the contralateral udder of each animal was infused with 1 mL of PBS alone. The blood samples from vena jugularis and milk samples were collected pre-infection (time = 0) and post-infection at 4, 8, 24, 48 and 72 h, respectively.

Whey and serum preparation: For the preparation of whey, milk samples were centrifuged at 3,000 rpm for 30 min and the fat layer was removed with a spatula. The middle translucent supernatant was collected and stored at -20°C for further study. For the preparation of serum, vein blood samples were collected, clotted at room temperature for 30 min and centrifuged at 1,500 rpm for 10 min. The supernatant was collected and stored at -20°C.

Enzyme-Linked Immunosorbent Assays (ELISAs): The levels of IL-17, IL-6, IL-1β and TGF-β in whey and serum were determined by commercial ELISA kits (Quantikine M Goat ELISA kit, R and D Systems) according to the manufacturer's instructions.

Statistical analysis: Results were expressed as mean±standard error and statistical significance was analyzed by the Student's t-test (Graphpad Prism Version 5.0 for Windows, Graphpad Software Inc., San Diego, CA, USA) with $p<0.05$ considered significant.

RESULTS AND DISCUSSION

In the present study, two mastitis models of dairy goat challenged with *E. coli* or *S. aureus* were established. The levels of IL-17 in milk were elevated for two models with peaks at 24 or 48 h post challenged with *E. coli* or *S. aureus*, respectively (Fig. 1a). But the level of IL-17 in serum was presented to be slightly fluctuant with no peak detectable from 0-72 h post challenged with *E. coli* or *S. aureus* (Fig. 1b).

Then the levels of cytokines associated with IL-17 producing were studied. The levels of IL-6 in serum and milk were elevated with peaks at 24 and 48 h post challenged with *E. coli* (Fig. 2a, b). But for goats challenged with *S. aureus*, the level of IL-6 was slightly elevated only in milk at 48 h post infection and no detectable increase was found in serum (Fig. 2a, b). Both the levels of IL-1β and TGF-β in serum were presented to be slightly fluctuant from 0-72 h post challenged

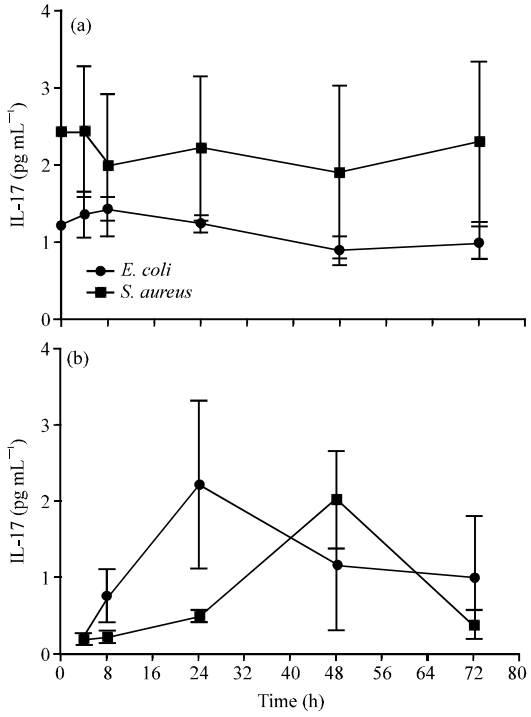

Fig. 1: The level of IL-17 in the blood (a) and milk (b) at different time points post chllenged with *E. coli* or *S. aureus* in dairy goats

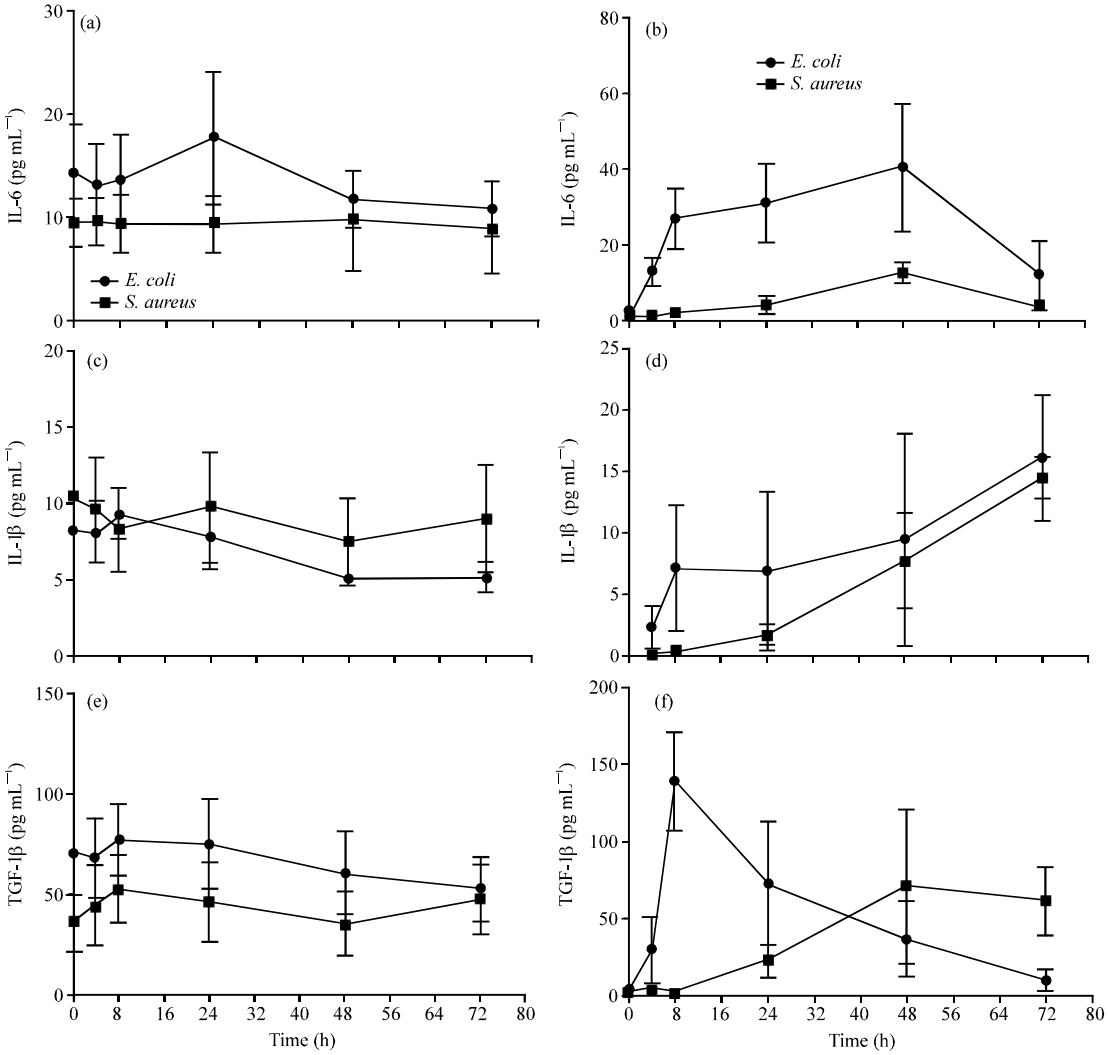

Fig. 2: The levels of IL-6 (a, b), IL-1β (c, d) and TGF-β (e, f) in the blood (a, c, e) and milk (b, d, f) at different time points post chllenged with *E. coli* or *S. aureus* in dairy goats

with *S. aureus* and the levels of these two cytokines were decreased from 8-72 h post challenged with *E. coli* in serum (Fig. 2c, e).

However, the levels of IL-1β were gradually elevated from 8-72 h post challenged with *E. coli* and *S. aureus* in milk (Fig. 2d). The peak of TGF-β level was found in milk at 8 and 48 h post challenged with *E. coli* and *S. aureus*, respectively (Fig. 2f).

IL-17, a key pro-inflammatory cytokine, is produced by Th17 cells and several innate immune cells including γδ T cells, natural killer cells in a cytokine milieu (Roark *et al*., 2008; Cua and Tato, 2010; Hemdan *et al*., 2010) and play pleiotropic biological effects on multiple immune and non-immune cells (Tanaka *et al*., 2009). The present study showed that the levels of IL-17 were elevated in milk of goats challenged with *S. aureus* and *E. coli* which was in accordance with previous studies in mastitis of cows (Riollet *et al*., 2006; Tao and Mallard, 2007).

Therefore, IL-17 would be an important cytokine in development of inflammatory response in mastitis of ruminants.

Local host immune response in mammary gland has been reported *in vivo* and *in vitro* (Beecher *et al*., 2009; Moyes *et al*., 2009, 2010). In the present study, both the changes in levels of IL-17 and IL-17 associated cytokines (TGF-β, IL-6 and IL-1β) in milk were more viable than that in serum. There results further confirmed that mastitis is only a local immune defense against pathogens in mammary gland.

CONCLUSION

IL-17 was an important cytokine in the inflammation development of dairy goat mastitis challenged with *E. coli* or *S. aureus*. However, the levels of IL-17 and cytokines associated with IL-17 producing were highly elevated in milk and slight fluctuant were detected in serum. These results indicated that the local pro-inflammatory cytokines milieu plays an important role in the development of subclinical mastitis whether infected with *E. coli* or *S. aureus* which should be considered in the therapy for mastitis.

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