Kinetics of Interleukin-17 and Interleukin-17 Associated Cytokines in Sera and Milk in Dairy Goat Mastitis Experimentally Induced with Escherichia coli

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Abstract: IL-17 is a crucial mediator of mucosal inflammation for extracellular bacteria clearance. The production of IL-17 was associated with TGF-β and multiple proinflammatory cytokines, especially IL-6 and IL-1β. IL-17 can cooperate additively or synergistically with IL-6 or IL-1β to amplify inflammatory processes. However, the roles of IL-17 and cytokines associated with IL-17 in dairy goat mastitis is poorly understood. In the present study, the concentrations of IL-17, IL-6, IL-1β and TGF-β were assayed in sera and milk of Guanzhong dairy goat mastitis induced with Escherichia coli using the Enzyme-Linked Immunosorbent Assay (ELISA) at several time points before and after challenge. The results showed that the levels of all cytokines varied significantly in sera while raised significantly in milk post intramammary inoculation with E. coli. The results suggest that IL-17 is one of important mediators in mammary gland inflammation for bacterial clearance in dairy goat mastitis and IL-6 and TGF-β play an important role or help for development in IL-17 producing cells in goat.

Key words: Dairy goat, IL-17, mastitis, proinflammatory cytokines, milk, cell

INTRODUCTION

Mastitis, an inflammation of the mammary gland for infection of contagious and environmental bacteria is one of the most important diseases in the dairy animals (Virdis et al., 2010, Zhu et al., 2007). Mastitis in dairy goat has a major effect in reducing both yield and quality of milk, leading to strong economic losses. Mastitis is a complex disease and a classical example of the interaction of microorganisms, host factors and the environment in dairy ruminants (Leitner et al., 2004). There have been reports on immune response against these antigens and clinical changes during clinical infection and experimental mastitis model of cows, sows and mice (Notebaert et al., 2008; Ranaldi et al., 2010; Swanson et al., 2009; Zhu et al., 2008).

Cytokines appear to be an important component of a paracrine or autocrine communication network in the mammary gland inflammation (Ramard and Riolley, 2006). Increased concentrations of proinflammatory cytokines such as Interleukin (IL)-1β, IL-6, Interferon (IFN)-γ and Tumor Necrosis Factor (TNF)-α in milk responses to Intramammary Inoculation (IMI) in cows have been well documented (Notebaert et al., 2008; Osman et al., 2010; Persson et al., 2003). Proinflammatory cytokines recruit the immunocytes including macrophages, monocytes, neutrophils and T lymphocytes which constitute the first line of defense in the host response to the site of inflammation for antigens clearance (Oviedo-Boys et al., 2006). Recently, the advance of the cytokine IL-17 on orchestrating mucous immune function, promoting peripheral tissues for pathogen clearance and mediating inflammation of local bacterial infection and autoimmunity diseases has been proved in multiple studies (Cho et al., 2010; Egan et al., 2008). IL-17 can cooperate either additively or synergistically with IL-6, TNF-α or IL-1β to amplify the inflammatory processes (Dragon et al., 2007; Hartup et al., 2007). However, systematical study on proinflammatory cytokines in dairy goat mastitis has rarely reported.

The objective of this study was to investigate the dynamics of IL-17 and cytokines associated with IL-17 production such as IL-1β, IL-6 and TGF-β in dairy goat mastitis experimentally induced with E. coli.

MATERIALS AND METHODS

Bacteria: E. coli strains were isolated from the milk of the clinical mastitis of Guanzhong dairy goats in Guanzhong area of Shaanxi province, Northwest of The People's
The E. coli strains was identified as serotype O117 by China Institute of Veterinary Drugs Control. The bacterial inoculum was grown overnight in nutrient broth at 37°C. The bacteria were washed once in Pyrogen Free Saline (PFS) and diluted in PFS. The number of Colony-Forming Units (CFU) was determined after serial dilution and plate counting.

**Animals:** Three Guanzhong dairy goats in their second or third lactation were used. All goats were about 45 kg and clinically healthy by diagnosis using the California Mastitis Test (CMT) at the start of the experiment. The goats were in mid-lactation, producing approximately 3 L milk per day each. The udders of the goats were pathogen-free prior to the experiment as determined by bacteriological examination of milk samples 1 week before the start of the experiment. The animal experimental protocols are in accordance with the Animal Care and Use Committee of Northwest A & F University and have been approved by the Animal Ethics Committee of the University.

**Intramammary challenge with E. coli:** In these studies, the two teat on the right side of mammary glands was inoculated with 1.0 mL bacterial suspension containing E. coli (3×10⁶ CFU) and the contrary one with Phosphate Buffer Solution (PBS).

**Clinical observations:** Inoculated goats were examined at 0, 4, 8, 24, 48 and 72 h post IMI for generalized and local reactions. The generalized reactions included awareness of the environment, activity and grooming, weakness and mortality, food and water uptake and rectal temperature. The local reactions such as redness and swelling of the mammary gland were observed as well.

**Histological observation:** The mammary gland tissues (5×5×5 mm³) were collected by aseptic operation from local anaesthetic goats infected with E. coli at 24 h post IMI, respectively. The normal mammary gland tissue collected from the left udder.

The tissues were fixed in 10% formalin and embedded in paraffin. About 8 μm thick sections were stained with Hematoxylin and Eosin (H and E) and slides were assessed for inflammatory cell infiltration and tissue destruction.

**Serum and milk samples preparation:** The blood and milk samples were collected at 0, 4, 8, 24, 48 and 72 h post infection. The peripheral blood obtained from the jugular vein. Serum was separated and serum samples stored at -80°C until use. The 5 mL milk collected in sterile tubes were centrifuged at 3000 rpm for 40 min, discarded the precipitant and fat. The residual stored at -80°C until analysis.

**The cytokines assays by Enzyme-Linked Immunosorbent Assay (ELISA):** The levels of the cytokines IL-1β, IL-6, IL-17 and Transforming Growth Factor (TGF-β) in the sera and milk samples of goats were quantified by Goat ELISA kit (Shengwu biotechnology Ltd., Shanghai, China), according to the manufacturer’s instructions.

**Statistical analysis:** Data were expressed as Means± Standard Error (SEM) and differences were considered significant at p<0.05 by unpaired t-test for independent samples with GraphPad prism 5.0 for Windows (GraphPad Software Inc, San Diego CA, USA).

**RESULTS AND DISCUSSION**

**Clinical observations:** All goats developed acute mastitis with clinical signs post IMI with E. coli. The rectal temperatures of the E. coli group rose from 4 h and returned to normal at 72 h (Fig. 1) and the goats had droopy appearance and poor appetite from 8-48 h post IMI.

The right udder became red, swelling, heat and pain and the milk appeared light yellow compared with normal milk. The activity and appetite of goats, the udder appearance and milk character returned to normal at 72 h.

**Histological observation:** The mammary tissue of the right udder was infiltration by lots of inflammatory cells in interstitial and acinar lumina and the acinar structure.

![Graph showing rectal temperature of goats pre (0 h) or post IMI (4, 8, 24, 48 and 72 h) with E. coli](image)
Cytokines responses in sera: The concentrations of IL-17, IL-6, IL-1β and TGF-β in sera at 4, 8, 24, 48 and 72 h post IMI with E. coli had no significant change compared with 0 h in this study (Fig. 3).

Cytokines responses in milk: The concentration of IL-17 in milk increased markedly from 0.10±0.02 pg mL⁻¹ at 0 h to its maximum 2.82±1.31 pg mL⁻¹ at 24 h post IMI with E. coli (Fig. 4a).

The production of cytokine IL-6 was observed in the milk. The level of IL-6 in milk elevated significantly to its peak (67.59±12.61 pg mL⁻¹) at 24 h compared with 0 h (2.84±1.25 pg mL⁻¹) post IMI with E. coli (p<0.01, Fig. 4b).

Increased concentrations of cytokine TGF-β were recorded in the milk of the experiment. Milk TGF-β rose to 142.00±31.35 pg mL⁻¹ significantly at 8 h compared to the level (6.28±1.86 pg mL⁻¹) at 0 h post IMI (p<0.05, Fig. 4c). The value of IL-1β in milk was 0.96±0.52 pg mL⁻¹ and rose to its maximum 23.28±7.20 pg mL⁻¹ at 72 h significantly post IMI with E. coli (p<0.05, Fig. 4d). Mastitis onset has been associated with many different environmental or infectious bacteria in dairy animals but the most common are staphylococci, streptococci and coliform bacteria (Riekerink et al., 2008). The inflammation of mammary gland is the result of a number of cell and soluble factors that function together to eliminate invading microorganisms but the factors involved in this inflammatory response differ depending on the infectious agent. To investigate the inflammation response of goat mammary gland, experimental mastitis was induced in
Guangzhou dairy goats with *E. coli*. In the present study, elevation of rectal temperature, droopy appearance and poor appetite of goats and the redness, swelling, heat and pain of the right udder (infected) indicated that the clinical mastitis was induced by *E. coli*. Many studies have shown that the gram positive bacteria like *Streptococcus uberis* or *S. aureus* tend to cause less severe clinical, often subclinical inflammations in contrast, the gram negative coliform very often cause heavy, acute inflammations with clinical symptoms (Yang et al., 2008). The inflammatory cells in interstitial and acinar lumina and the acinar structure disintegration in histological sections of mammary gland (Fig. 2) indicated an acute inflammation of mammary gland in Guangzhou dairy goats were induced by *E. coli* in this study.

The results showed that the concentrations of IL-17, IL-1β, IL-6 and TGF-β were all increased in milk of goats post IMI with *E. coli* (Fig. 2) which were in accordance with studies in cattle (Bannerman et al., 2004). However, the levels of these cytokines in sera had not varied significantly compared with 0 h (Fig. 1). The results were similar with other studies (Blum et al., 2000; Zhu, 2007) and indicated there was a discrepancy between systemic and local cytokine responses in goat mastitis.

The IL-17 as a pleiotropic biological effects on multiple immune and non-immune cells in various tissues (Pappu et al., 2010) is a key barrier function regulator of epithelium, a mediator of neutrophil recruitment and plays a critical role in mucosal immunity to many extracellular pathogens (Kolls, 2010). Increasing evidence demonstrated that IL-17 was associated with protective immune responses in the lung against *S. aureus, Bordetella bronchiseptica* and *Klebsiella pneumoniae* (Cho et al., 2010; Ye et al., 2001). In the present study, the increase of milk IL-17 concentration markedly post IMI with *E. coli* were consistent with studies on cattle or sow mastitis (Tao and Mallard, 2007; Zhu, 2007) which suggested IL-17 play an important role on mediating of inflammatory response against *E. coli* in dairy goat mastitis.

IL-17 was mainly produced by CD4⁺ Th17 cells in adaptive immunity (Park et al., 2005). However, more evidences have shown that Nature Killer T (NKT) cells and γδ T cells were another important sources of IL-17 in innate immunity at early stage of infection (Cua and Tato 2010; Roark et al., 2008). As the crucial sentinels of the immune system, γδ T cells and NKT were the majority of T cells and the first line of defense in epithelial tissues including the skin, intestines, lung and reproductive tracts (Komori et al., 2006). In this study, the IL-17 in milk was observed firstly at 4 h post IMI with *E. coli* which time was at very early stage of infections. The increase of IL-17 concentration at the very early stage of infection in the study indicated IL-17 in milk may be produced by γδ or NK T cells because the activation of adaptive Th17 cells was impossible at so early time. However, the specific IL-17-producing cell types in innate immunity of goat mastitis require further studies although the key role of IL-17-producing γδ T cells in response against *E. coli* (Shibata et al., 2007), *Mycobacterium tuberculosis* infection (Lockhart et al., 2006) and arthritis (Roark et al., 2007) in mice had been reported.
IL-6 and IL-1β were important pro-inflammatory cytokines and more evidence displayed they were associated with the development of Th17 in mice or human. Studies had showed that the phenotypic profile of IL-17 producing T cells such as γδ or NK cells was similar to that of Th17 cells (Bird, 2009). In vitro skewing experiments displayed TGF-β and IL-6 mediate the initial Th17 differentiation in mouse (Veldhoen and Stockinger, 2006) while IL-1β and IL-23 are the crucial inducers for Th17 development in human (Acosta-Rodriguez et al., 2007) which demonstrated a fundamental difference in the biology of human and mouse Th17 cells. In the present study, the peak value of IL-6 was observed at 24 h and the peak of TGF-β were at 8 h which were earlier than or coincident with the peak of IL-17 while IL-1β arrived at its peak value at 48 h, later than IL-17. According to the results, the good correlation on the time and concentration between TGF-β, IL-6 and IL-17 indicated that these three cytokines are either coregulated or TGF-β and IL-6 help in differentiation of IL-17 producing cells in goat.

CONCLUSION

In the study, the dynamics of IL-17 in sera and milk were investigated in goat mastitis induced with E. coli experimentally. The results indicated IL-17 is one of important mediators in mammary gland inflammation for bacterial clearance in dairy goat mastitis and IL-6 and TGF-β play an important role or help for development in IL-17 producing cells in goat.

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