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

[1, 2]Xiaoqi Jing, [1]Yansen Han, [1]Duoyao Cao, [1]Shan Mou, [1]Hui Liu,
[1]Jing Yao, [1]Lijuan Zhao, [1]Yanqing Zhao, [1]Chuanchang Shang and [1]Dekun Chen
[1]College of Veterinary Medicine, Northwest A&F University,
712100 Yangling, Shaanxi Province, P.R. China
[2]Life Science College, Yulin University, 719000 Yulin, Shaanxi Province, P.R. China

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 proinflammatary cytokines, especially IL-6 and IL-1β. IL-17 can cooperate additively or synergistically with IL-6 or IL-1β to amplify of 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 slightly 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; Rinaldi *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 (Rainard and Riollet, 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-Boyso *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; Hartupee *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

Corresponding Author: Dekun Chen, College of Veterinary Medicine, Northwest A&F University, 712100 Yangling, Shaanxi Province, P.R. China

Republic of China (PRC). 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* (3×10^3 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 ($5\times5\times5$ mm^3) 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

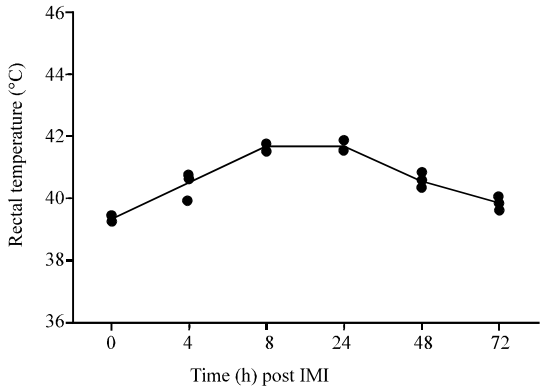

Fig. 1: The rectal temperature of goats pre (0 h) or post IMI (4, 8, 24, 48 and 72 h) with *E. coli*

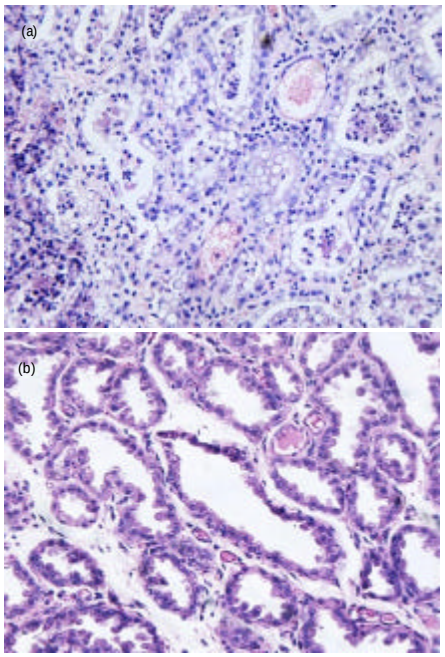

Fig. 2: Histology of goat mammary gland at 24 h post IMI. a) Infiltratration of inflammatory cells in interstitial and acinar lumina and the acinar structure disintegration at 24 h post IMI with *E. coli*; b) Epithelial tight junction, intact acinar structure and no infiltration of inflammatory cells in mammary tissue of uninfected udder (left)

disintegration at 24 h post IMI with *E. coli* (Fig. 2a) compared to the epithelial tight junction and intact acinar structure of the left udder (Fig. 2b).

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).

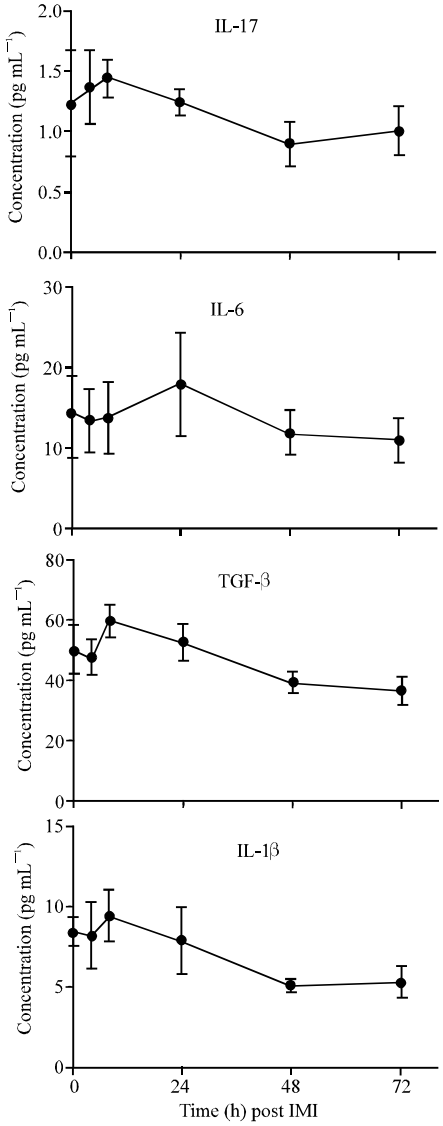

Fig. 3: The level of IL-17, IL-6, TGF-β and IL-1β in sera had no marked change at 4, 8, 24, 48 and 72 h post IMI with *E. coli* compared with 0 h

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

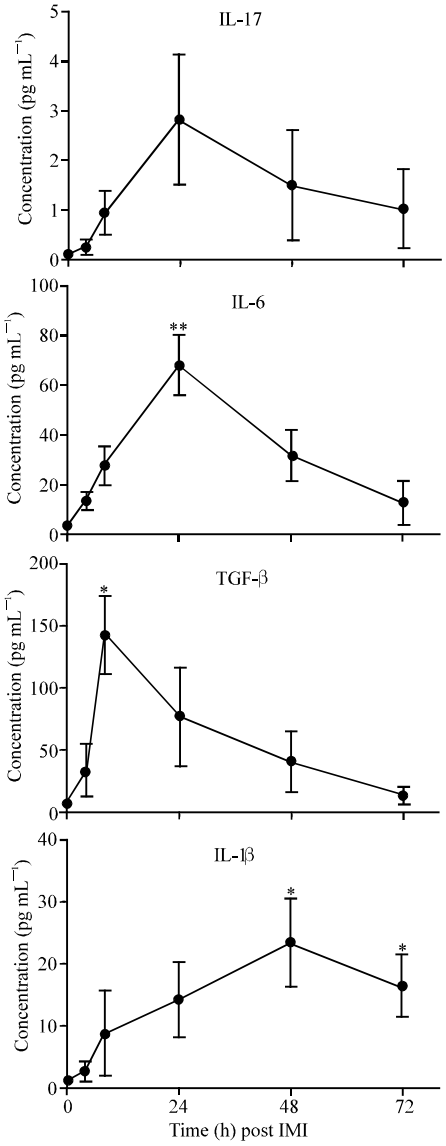

Fig. 4: Concentrations of IL-17, IL-6, TGF-β and IL-1β (mean±SEM, n = 3, respectively) at 0, 4, 8, 24, 48 and 72 h in milk of Guanzhong goats experimental induced with *E. coli*. The concentrations of IL-17 (24 h), IL-6 (24 h) and TGF-β (8 h) in milk increased significantly compared with 0 h post IMI; *Significant difference ($p<0.05$), **Extremely significant difference($p<0.01$)

Guanzhong 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 Guanzhong 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 signifycantly 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 pneumonia* (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 proinflammatory cytokines and more evidence displayed they were associated with the development of Th17 in mouse 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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REFERENCES

Acosta-Rodriguez, E.V., G. Napolitani, A. Lanzavecchia and F. Sallusto, 2007. Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17–producing human T helper cells. Nat. Immunol., 8: 942-949.

Bannerman, D.D., M.J. Paape, J.W. Lee, X. Zhao, J.C. Hope and P. Rainard, 2004. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. Clin. Diagn. Lab. Immunol., 11: 463-472.

Bird, L., 2009. γΔ T cells: Innate source of IL-17. Nat. Rev. Immunol., Vo. 9, 10.1038/nri2645

Blum, J.W., H. Dosogne, D. Hoeben, F. Vangroenweghe, H.M. Hammon, R.M. Bruckmaier and C. Burvenich, 2000. Tumor necrosis factor-alpha and nitrite/nitrate responses during acute mastitis induced by *Escherichia coli* infection and endotoxin in dairy cows. Domest. Anim. Endocrinol., 19: 223-235.

Cho, J.S., E.M. Pietras, N.C. Garcia, R.I. Ramos and D.M. Farzam *et al*., 2010. JIL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. Clin. Invest., 120: 1762-1773.

Cua, D.J. and C.M. Tato, 2010. Innate IL-17-producing cells: The sentinels of the immune system. Nat. Rev. Immunol., 10: 479-489.

Dragon, S., M.S. Rahman, J. Yang, H. Unruh, A.J. Halayko and A.S. Gounni, 2007. IL-17 enhances IL-1beta-mediated CXCL-8 release from human airway smooth muscle cells. Am. J. Physiol. Lung Cell. Mol. Physiol., 292: L1023-L1029.

Egan, P.J., A. van Nieuwenhuijze, I.K. Campbell and I.P. Wicks, 2008. Promotion of the local differentiation of murine Th17 cells by synovial macrophages during acute inflammatory arthritis. Arthritis. Rheum., 58: 3720-3729.

Hartupee, J., C. Liu, M. Novotny, X. Li and T. Hamilton, 2007. IL-17 enhances chemokine gene expression through mRNA stabilization. J. Immunol., 179: 4135-4141.

Kolls, J.K., 2010. Th17 cells in mucosal immunity and tissue inflammation. Semin. Immunopathol., 32: 1-2.

Komori, H.K., T.F. Meehan and W.L. Havran, 2006. Epithelial and mucosal ãä T cells. Curr. Opin. Immunol., 18: 534-538.

Leitner, G., U. Merin, N. Silanikove, E. Ezra and M. Chaffer *et al*., 2004. Effect of subclinical intramammary infection on somatic cell counts, NAGase activity and gross composition of goats milk. J. Dairy Res., 71: 311-315.

Lockhart, E., A.M. Green and J.L. Flynn, 2006. IL-17 production is dominated by γδ T cells rather than cd4 t cells during *Mycobacterium tuberculosis* infection. J. Immunol., 177: 4662-4669.

Notebaert, S., D. Demon, T.V. Berghe, P. Vandenabeele and E. Meyer, 2008. Inflammatory mediators in *Escherichia coli*-induced mastitis in mice. Comp. Immunol. Microbiol. Infect. Dis., 31: 551-565.

Osman, K.M., H.M. Hassan, I.M. Ibrahim and M.M.S. Mikhail, 2010. The impact of staphylococcal mastitis on the level of milk IL-6, lysozyme and nitric oxide. Comp. Immunol. Microbiol. Infect. Dis., 33: 85-93.

Oviedo-Boyso, J., J.J. Valdez-Alarcon, M. Cajero-Juarez, A. Ochoa-Zarzosa, J.E. Lopez-Meza, A. Bravo-Patino and V.M. Baizabal-Aguirre, 2006. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J. Infect., 54: 399-409.

Pappu, R., V. Ramirez-Carrozzi, N. Ota, W. Ouyang and Y. Hu, 2010. The IL-17 family cytokines in immunity and disease. J. Clin. Immunol., 30: 185-195.

Park, H., Z. Li, X.O. Yang, S.H. Chang and R. Nurieva *et al*., 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol., 6: 1133-1141.

Persson, W.K., I.G. Colditz, S. Lun and K. Ostensson, 2003. Cytokines in mammary lymph and milk during endotoxin-induced bovine mastitis. Res. Vet. Sci., 74: 31-36.

Rainard, P. and C. Riollet, 2006. Innate Immunity of the bovine mammary gland. Vet. Res., 37: 369-400.

Riekerink, R.G.M.O., H.W. Barkema, D.F. Kelton and D.T. Scholl, 2008. Incidence rate of clinical mastitis on canadian dairy farms. J. Dairy Sci., 91: 1366-1377.

Rinaldi, M., R.W. Li, D.D. Bannerman, K.M. Daniels and C. Evock-Clover *et al*., 2010. A sentinel function for teat tissues in dairy cows: Dominant innate immune response elements define early response to *E. coli* mastitis. Funct. Integr. Genomics, 10: 21-38.

Roark, C.L., J.D. French, M.A. Taylor, A.M. Bendele, W.K. Born and R.L. O'Brien, 2007. Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing γδ T cells. J. Immunol., 179: 5576-5583.

Roark, C.L., P.L. Simonian, A.P. Fontenot, W.K. Born and R.L. O'Brien, 2008. Γδ T cells: An important source of IL-17. Curr. Opin. Immunol., 20: 353-357.

Shibata, K., H. Yamada, H. Hara, K. Kishihara and Y. Yoshikai, 2007. Resident Vδ1+ γδ T cells control early infiltration of neutrophils after *Escherichia coli* infection via IL-17 production. J. Immunol., 178: 4466-4472.

Swanson, K.M., K. Stelwagen, J. Dobson, H.V. Henderson, S.R. Davis, V.C. Farr and K. Singh, 2009. Transcriptome profiling of *Streptococcus uberis*-induced mastitis reveals fundamental differences between immune gene expression in the mammary gland and in a primary cell culture model. J. Dairy Sci., 92: 117-129.

Tao, W. and B. Mallard, 2007. Differentially expressed genes associated with *Staphylococcus aureus* mastitis of Canadian *Holstein* cows. Vet. Immunol. Immunopathol., 120: 201-211.

Veldhoen, M. and B. Stockinger, 2006. TGFâ1, a Jack of all trades: The link with pro-inflammatory IL-17-producing T cells. Trends Immunol., 27: 358-361.

Virdis, S., C. Scarano, F. Cossu, V. Spanu, C. Spanu and E.P. de Santis, 2010. Antibiotic resistance in *Staphylococcus aureus* and coagulase negative staphylococci isolated from goats with subclinical mastitis. Vet. Med. Int., 10.4061/2010/517060.

Yang, W., H. Zerbe, W. Petzl, R.M. Brunner and J. Gunther *et al*., 2008. Bovine TLR2 and TLR4 properly transduce signals from *Staphylococcus aureus* and *E. coli* but *S. aureus* fails to both activate NF-κB in mammary epithelial cells and to quickly induce TNFα and interleukin-8 (CXCL8) expression in the udder. Mol. Immunol., 45: 1385-1397.

Ye, P., P.B. Garvey, P. Zhang, S. Nelson and G. Bagby *et al*., 2001. Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. Am. J. Respir. Cell Mol. Biol., 25: 335-340.

Zhu, Y., 2007. Early inflammatory response in periparturient sows to experimentally induced *Escherichia coli* mastitis. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Zhu, Y., U. Magnusson, C. Fossum and M. Berg, 2008. *Escherichia coli* inoculation of porcine mammary glands affects local mRNA expression of Toll-like receptors and regulatory cytokines. Vet. Immunol. Immunopathol., 125: 182-189.

Zhu, Y.M., J.F. Miao, Y.S. Zhang, Z. Li, S.X. Zou and Y.E. Deng, 2007. CpG-ODN enhances mammary gland defense during mastitis induced by *Escherichia coli* infection in goats. Vet. Immunol. Immunopathol., 120: 168-176.