

ajava

Asian Journal of Animal and Veterinary Advances



Academic
Journals Inc.

www.academicjournals.com

Pivotal Role of IL-17-producing $\gamma\delta$ T cells in Mouse Chronic Mastitis Experimentally Induced with *Staphylococcus aureus*

^{1,2}Xiaoqi Jing, ¹Duoyao Cao, ¹Hui Liu, ¹Xiaoyu Wang, ¹Xuanduo Zhao and ¹Dekun Chen

¹College of Veterinary Medicine, Northwest A and F University, Yangling 712100, Shaanxi Province, People's Republic of China

²Life Science College, Yulin University, Yulin 719000, Shaanxi Province, People's Republic of China

Corresponding Author: Dekun Chen, College of Veterinary Medicine, Northwest A and F University, Yangling 712100, Shaanxi Province, People's Republic of China

ABSTRACT

Staphylococcus aureus is a contagious, mastitis-causing pathogen that is highly adapted to survive in mammary gland. This study focuses on $\gamma\delta$ T cell populations in mammary gland involved in the immune response against *S. aureus*. The C57BL/6J mice in 10-15th day of lactation were intramammary challenge with *S. aureus* isolated from goat chronic mastitis or with PBS (control). $\gamma\delta$ T1 (interferon (IFN)- γ -producing) cells and $\gamma\delta$ T17 (interleukin (IL)-17-producing) cells in the mammary gland during infection were evaluated by cytometry. The results showed that the percentage of $\gamma\delta$ T1 in $\gamma\delta$ T cells decreased following intramammary injection and significance was observed at 8, 36, 48, 60 and 72 h compared with control. At the same time, expression of IFN- γ mRNA was decreased. However, percentage of $\gamma\delta$ T17 in $\gamma\delta$ T cells was increased significantly at 18, 48 and 60 h post challenge, accompanied by an increase in the expression folds of IL-17, IL-23p19 and CCR6 mRNA following intramammary injection. Overall, these data suggest that $\gamma\delta$ T17 cells, not $\gamma\delta$ T1 cells, in mammary gland tissue were activated rapidly and played an important role in mediating mammary inflammation caused by *S. aureus*.

Key words: Mouse, *Staphylococcus aureus*, mastitis, interleukin-17, $\gamma\delta$ T cell

INTRODUCTION

Staphylococcus aureus is one of the most frequent causes of subclinical mastitis in the ewe, dairy cow and goat in nature (Al-Graibawi *et al.*, 1986; Mork *et al.*, 2007; Tenhagen *et al.*, 2009), accounting for as many as 4-30% of all intramammary infections (Contreras *et al.*, 2007). Although, the subclinical mastitis in ruminants appear chronic and no clinical signs (Yang *et al.*, 2008), such as fever and lethargy, it account for approximately 80% of mastitis related costs due to reduced milk yield and product quality (Shim *et al.*, 2004). The extensive use of antibiotics in the treatment and control of mastitis has implications for human health through an increased risk of antibiotic-resistant bacterial strains and the presence of antibiotics in milk intended for human consumption (Berry and Hillerton, 2002). One of the most practical means for dealing with mastitis in the dairy industry is to enhance the natural host defence mechanisms (Kotwal, 1997).

Immune responses are frequently categorized as “innate” versus “adaptive” on the basis of the time course kinetics and contributing cells and mediators. $\gamma\delta$ T cells are generally regarded as a part

of early induced immune responses, bridge of innate and adaptive immune responses and a first line of host defense controlling neutrophil-mediated innate immune responses (Shibata *et al.*, 2007; Kabelitz, 2011). Recently, it has become clear that $\gamma\delta$ T cells are functionally committed to the effector cells producing IFN- γ ($\gamma\delta$ T1) and IL-17 ($\gamma\delta$ T17) in the thymus and then moved to epithelium (Haas *et al.*, 2009; Jensen and Chien, 2009; Ribot *et al.*, 2009; Korn and Petermann, 2012). The vast majority of $\gamma\delta$ T cells reside in epithelial layers of tissues such as skin, gut, lung, tongue and reproductive tract and rapidly produce cytokines that regulate pathogen clearance, inflammation and tissue homeostasis in response to tissue stress (Witherden and Havran, 2011). However, the mediator role of $\gamma\delta$ T cells in mammary gland infectious diseases had been scarcely reported.

To address this problem, in this study, we investigate the dynamics of $\gamma\delta$ T1 and $\gamma\delta$ T17 cells in mice mammary gland challenged with *S. aureus* during the early infectious stages and also the mRNA profiles of transcription factors and effector cytokines T-bet, ROR γ t, IFN- γ , IL-17 and IL-23 (p19) were analysed. The results of this study provide new insights in the functions of $\gamma\delta$ T cells in immune responses against *S. aureus*.

MATERIALS AND METHODS

Bacteria: *S. aureus* strains were isolated from the milk of the subclinical mastitis of Guanzhong dairy goats in Guanzhong area of Shaanxi province, Northwest of The People's Republic of China (PRC) and were identified as *S. aureus* 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.

Mice: Seven-week-old C57BL/6J mice were purchased from the Laboratory Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China). The animals were group housed under constant temperature (22°C) and humidity, a 12 h light/dark cycle and free access to food and water in the animal facility at the Northwest A and F University. The animals were allowed to acclimatize for one week before the start of each experiment and females were mated. Following parturition, lactating females were selected for further experiments when they were raising at least five pups. The animal experimental protocols are in accordance with the Animal Care and Use Committee of Northwest A and F University and have been approved by the Animal Ethics Committee of the University.

Intramammary challenge with *S. aureus*: Lactating C57BL/6 mice were challenged 7 to 12 days after parturition. The intramammary inoculation technique was based on the method described by Brouillette and Malouin (2005). Mice were anaesthetized using ketamine (150-200 mg/kg mouse) (Fujian gutian Pharmaceutical Co, Ltd., Gutian, Fujian, China). After disinfecting the teats of the fourth and fifth pairs of mammary glands, found from head to tail, the very near end of the teats was cut with small scissors. A 32 G needle with a blunt end was then inserted into the teat canal and a volume of 50 μ L was slowly injected. Challenged mice received 1×10^7 CFU of *S. aureus* in every challenged mammary gland while control mice were inoculated with PBS in all challenged glands. Mice were allowed to recover in their cage.

Clinical observation: Inoculated mice were examined at serial time points post challenge for generalized and local reactions. The generalized reactions included awareness of the environment, activity and grooming, weakness and mortality, food and water uptake. The local reactions such as redness and swelling of the mammary gland were observed as well.

Samples preparation: At serial time points after *S. aureus* challenge mice were sacrificed by CO₂ inhalation. The entire infused mammary glands were removed. One-fifth of each mammary gland (~100 mg) was immediately homogenized in 1 mL of Trizol (TaKaRa), frozen in liquid nitrogen and then stored at -80°C until further RNA extraction was performed. One-fifth of each mammary gland tissues were collected and fixed in 10% formahln. The remainder was cut into pieces to isolate the single cell suspensions of the mammary gland.

Histological observation: The fixed tissues were embedded in paraffin. Eight micrometer-thick sections were stained with hematoxylin and eosin (H and E) and slides were assessed for inflammatory cell infiltration and tissue destruction.

Mammary gland lymphocytes harvest: The protocol for isolation of lymphocytes from mammary gland was based on and modified the method about colonic tissue described by Weigmann *et al.* (2007). Specifically, the tissues were cut into pieces using scissors and the single-cell suspensions were obtained by mincing the tissue through a 100 µm nylon mesh in PBS. Lymphocytes were then separated from the other cells by Ficoll-Hypaque gradient centrifugation.

Primers, RNA isolation, cDNA synthesis and real-time PCR: The primers and the expected PCR product length for IFN-γ, IL-23 p19, IL-17, RORγt, T-bet and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are listed in Table 1. Primers were synthesised by Invitrogen.

The reagents of total RNA isolation, cDNA synthesis and real-time PCR were the products of TaKaRa Biotechnology (Dalian) Co., Ltd. (Dalian, China). Total RNA was isolated from homogenate of mammary gland according to the manual of RNAiso plus. First-strand cDNA was synthesized using PrimeScript Reverse Transcriptase according to the manufacturer's instructions.

Table 1: Real-time PCR primers used in gene expression detection

Gene	GenBank accession No.	Primer sequence (5'→3')	Product size (bp)
IFN-γ	NM_008337	F: GTGGCATAGATGTGGAAGA	173
		R: GTGTGATTCAATGACGCTTAT	
IL-23 p19	NM_031252	F: CTGCTTGACTCTGACATCT	162
		R: CCACTGCTGACTAGAACTC	
IL-17	NM_010552	F: CTCCAGAATGTGAAGGTC	89
		R: GAACGGTTGAGGTAGTCT	
RORγt	AJ132394	F: ATTCAGTATGTGGTGGAGTT	141
		R: GTGGTTGTTGGCATTGTAG	
T-bet	NM_019507	F: GGAGGTGAATGATGGAGAG	200
		R: GGACACTCGTATCAACAGAT	
GAPDH	NM_008084	F: ACCTGCCAAGTATGATGAC	119
		R: GGGAGTTGCTGTTGAAGT	

Relative quantity real-time PCR was performed using SYBR[®] Premix Ex Taq[™] II (TaKaRa) and iQ[™]5 Real-Time PCR Detection Systems (BIO-RAD) according to the manufacturer's instructions. All of gene expression data were normalized against GAPDH. Thermocycler conditions comprised an initial step at 95°C for 5 min which was followed by a 2-step PCR program at 95°C for 15 sec and 55°C for 30 sec for 40 cycles. Data were collected and quantitatively analyzed on an iQ[™]5 optical system software (BIO-RAD) and presented as ddCt.

Intracellular cytokine staining: The following Absorption and corresponding isotype control Absorption have been used for flow cytometry: FITC anti-mouse IL-17A Antibody, PerCP/Cy5.5 anti-mouse IFN- γ Antibody and APC Anti-mouse TCR γ/δ Antibody from Biolegend Inc. (San Diego, CA, USA).

Total mammary gland lymphocytes cells were restimulated with 500 nM PMA (Sigma, St Louis, MO) and 5 ng mL⁻¹ ionomycin (Sigma) for 6 h in RPMI 1640 (Invitrogen, Grand Island, NY) containing 10% FBS, 10 μ g mL⁻¹ BFA (Brefeldin A Sigma), 100 U mL⁻¹ of penicillin G sodium and 100 mg mL⁻¹ of streptomycin sulfate (Shandong Lukang Pharmaceutical Group Co., Ltd.). Cells were incubated with mAbs for extracellular proteins and intracellular cytokines for 30 min at 4°C.

Flow cytometric analysis: Splenocytes were used to determine lymphocyte forward and side scatter properties and as compensation controls. Cells were sorted using a FACSAria Flow Cytometer (BD Biosciences, Mountain View CA, USA) and analyzed by FCS Express[™] 4, Diva (De Novo Software, Thornhill Ontario, Canada).

Statistical analysis: Data are presented as the Mean \pm SEM. Statistical significance was calculated by Student's t test using Prism software (GraphPad Software Inc, San Diego CA, USA) or Microsoft Excel 2010. Differences were considered significant if p<0.05 or p<0.01.

RESULTS

In the present study, mice showed minor indications of illness and no obvious signs of infection. Food and water uptake was not diminished obviously all through the experiments following challenge with 1 \times 10⁷ CFU of *S. aureus*.

The histological sections displayed that mammary alveoli with intact structure was filled with the milk in control group (Fig. 1a). In contrast, inflammatory cells infiltration at the alveoli was seen and the numbers increased gradually in the infected gland in the following series stages (Fig. 1b-j). Lots of cell fragments, possibly due to apoptosis, were observed in alveoli together with inflammatory cells at 60 and 72 h post challenge (Fig. 1i, j).

The $\gamma\delta$ T1 cells and $\gamma\delta$ T17 cells in mammary gland were sorted through cytometry. The results showed that the percentage of $\gamma\delta$ T1 in $\gamma\delta$ T cells had no obviously change at 2 and 12 h and decreased significantly at 8 h (p<0.01), 18, 24, 36 h (p<0.01), 48 h (p<0.01), 60 h (p<0.001) and 72 h (p<0.001) post challenge compared with control (Fig. 2). Besides, the percentage of $\gamma\delta$ T17 in $\gamma\delta$ T cells was increased significantly at 18, 48 and 60 h post challenge (Fig. 3) compared with control. Figure 4 shows representative flow cytometry data.

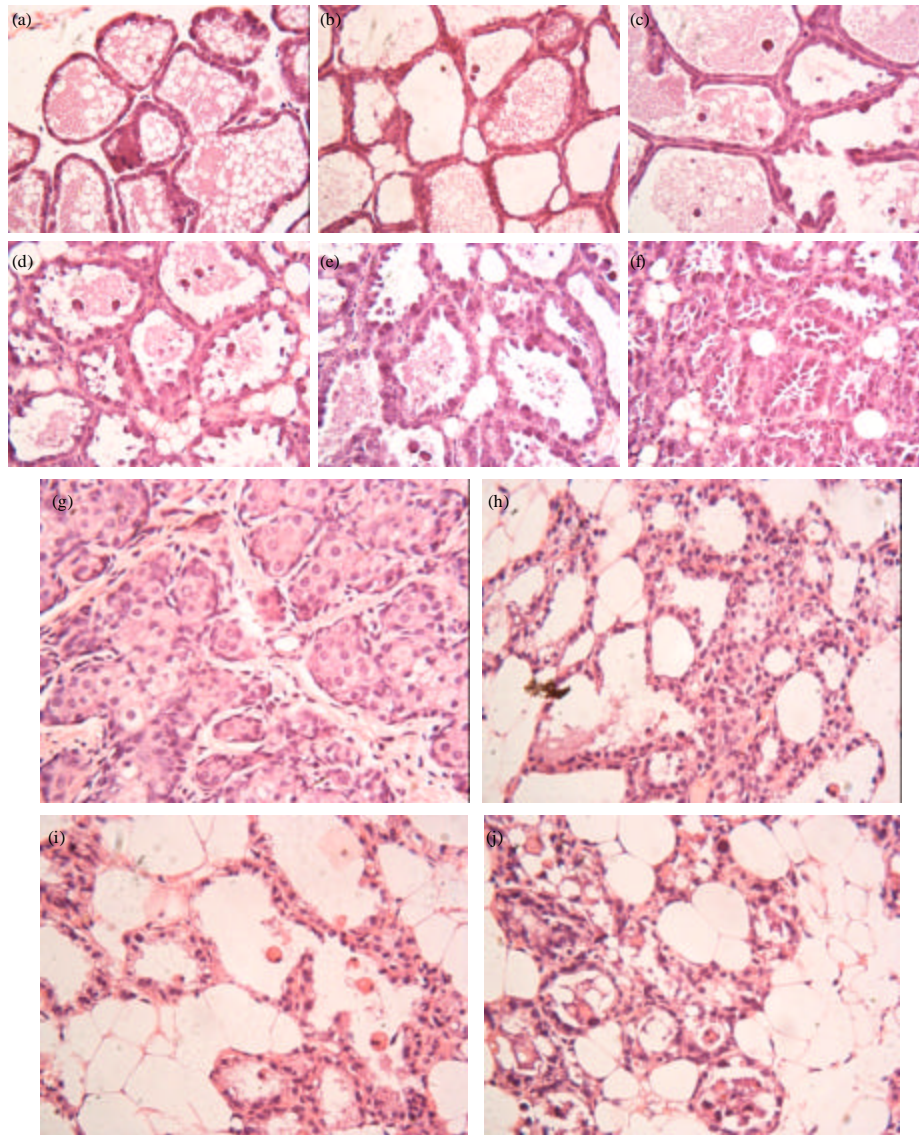


Fig. 1(a-j): Microscopic alterations in the mammary glands of female C57BL/6 mice infected with 10^7 CFU *Staphylococcus aureus* per mammary gland, x400, H and E stained, (a) Control: normal mammary tissue, (b) At 2 h p.i.: Intact structure of mammary gland and a little PMNs in milk of acinar lumina, (c) At 8 h p.i.: A little PMNs in milk of acinar lumina and migrating cell on the acinar wall, (d) At 12 h p.i.: Lots of PMNs in milk of acinar lumina, (e) At 18 h p.i.: Lots of PMNs in milk of acinar lumina and migrating cell on the acinar wall, (f) At 24 h p.i.: Amount of immunocytes in milk of acinar lumina, (g) At 36 h p.i.: intense interstitial inflammatory cell infiltration, (h) At 48 h p.i.: mild to moderate interstitial fibrosis and intense inflammatory cell infiltration, (i) At 60 h p.i.: largely predominate over infiltrating cells, (n). H and E stained. Bar, 10 mm, (J) At 72 h p.i.: Mammary gland tissue degenerated and part of epithelia were substituted by fatty tissue and neutrophil accumulation

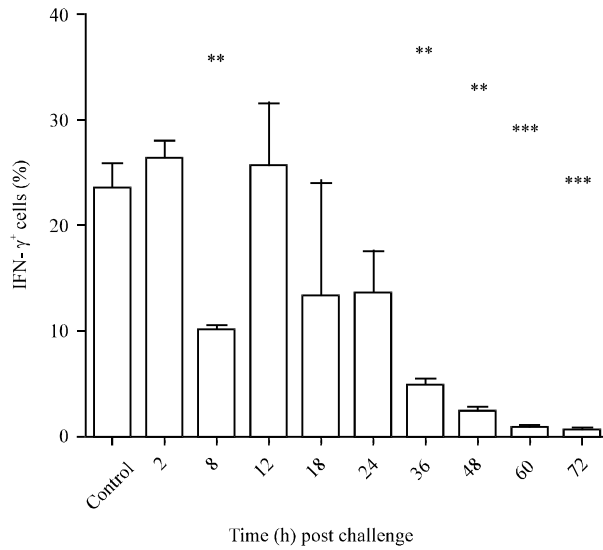


Fig. 2: Dynamics of IFN-γ⁺ cells in mammary gland γδ T-lymphocytes, **p<0.01, ***p<0.001

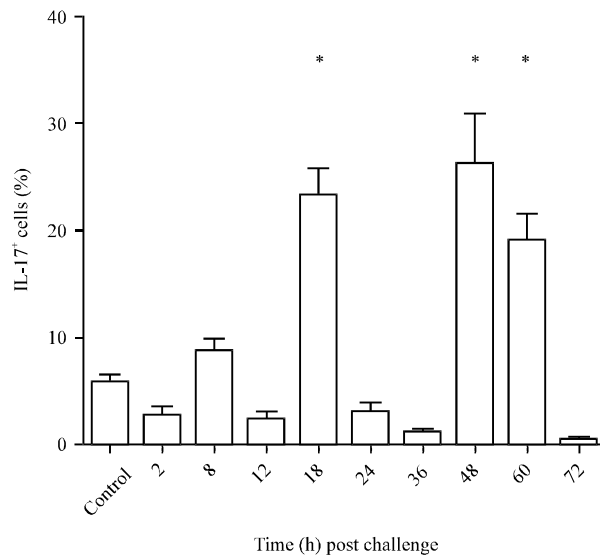


Fig. 3: Dynamics of IL-17⁺ cells in mammary gland γδ T-lymphocytes, **p<0.05

The average folds of induction for IFN-γ were always higher in glands at all time points after challenge. The expression of IFN-γ increased post challenge, peaked at 8 h and decreased gradually afterward (Fig. 5). The temporal profiles of IL-17 and IL-23p19 expression following infection were similar. The expression of IL-17 increased at 2 h, peaked at 12 h and then decreased until 24 h post challenge. Afterward, the folds of IL-17 rose again and up to its second peak at 48 h and then decreased gradually (Fig. 6). The mRNA level of IL-23p19 increased markedly post challenge and peaked at 8 and 60 h, respectively (Fig. 7). The expression folds of CCR6 were always higher in glands challenged all through experiments and the peak folds appeared at 8 and 48 h post challenge (Fig. 8).

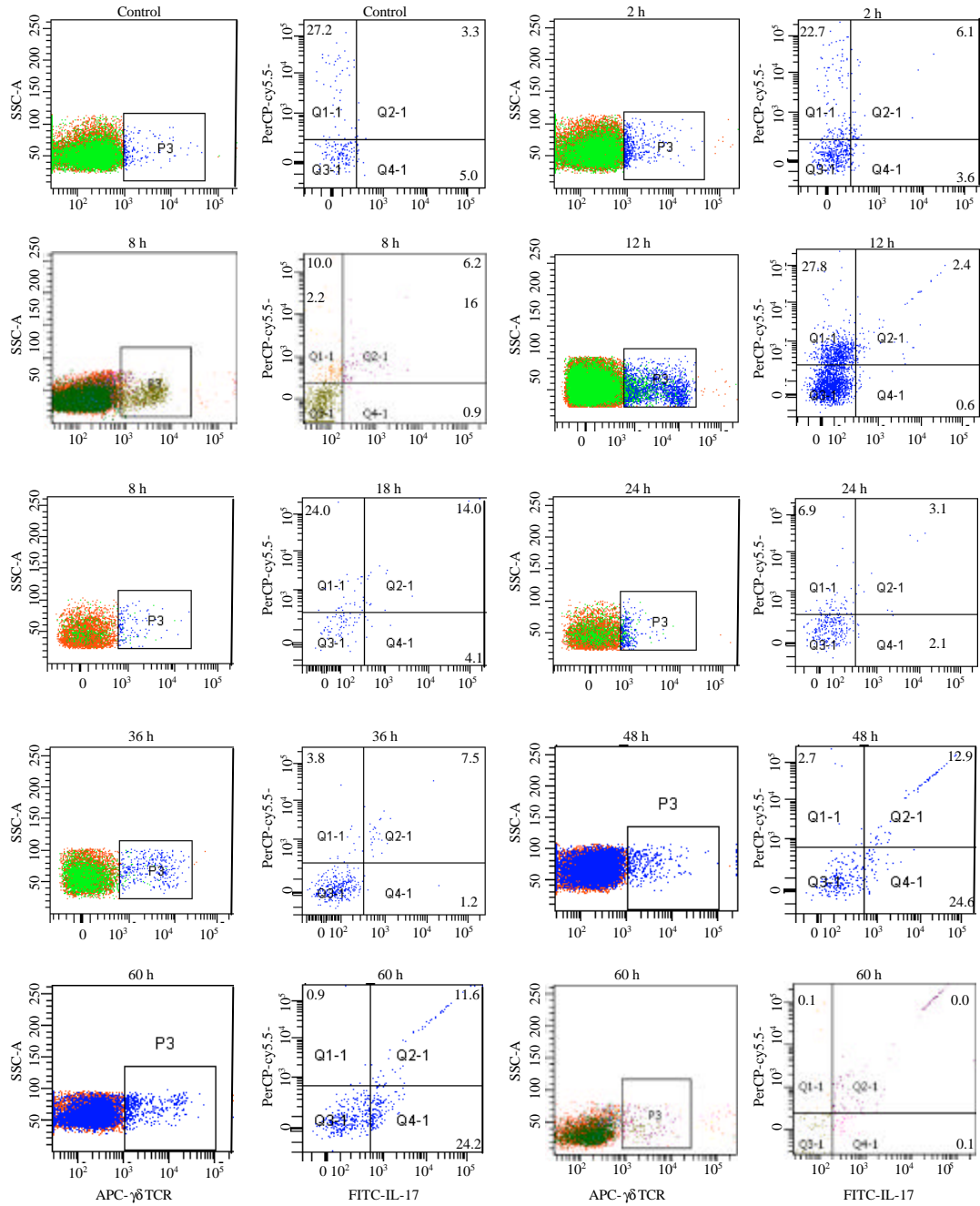


Fig. 4: Representative cytometry plots of IFN- γ^+ and IL-17 $^+\gamma\delta$ T cells in mammary gland challenged with *S. aureus*

DISCUSSION

Although mastitis is one of the main diseases in dairy ruminants, mouse models are increasingly used as an ideal immunological alternative for the study of intramammary infections due to major costs and management problems (Notebaert and Meyer, 2006). Chandler (1970) described a mouse model of infectious mastitis firstly in the 1970s. Since then, this model has been induced with a

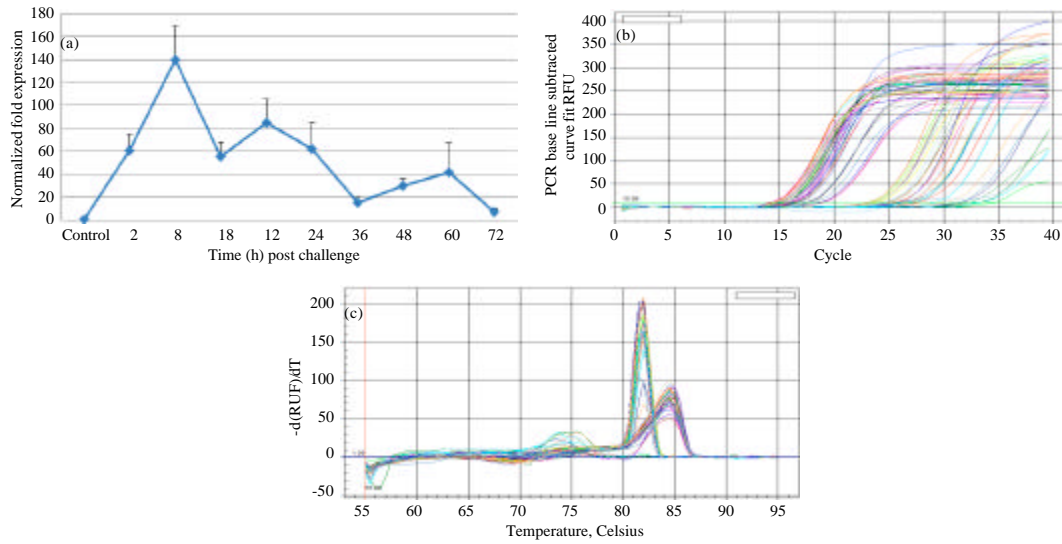


Fig. 5(a-c): (a) Dynamics of IFN- γ mRNA in mammary gland of mice challenged with *S. aureus* and Real-time PCR amplifications (b) Curve and (c) Melt peak chart of IFN- γ expression in mammary gland of mice challenged with *S. aureus*

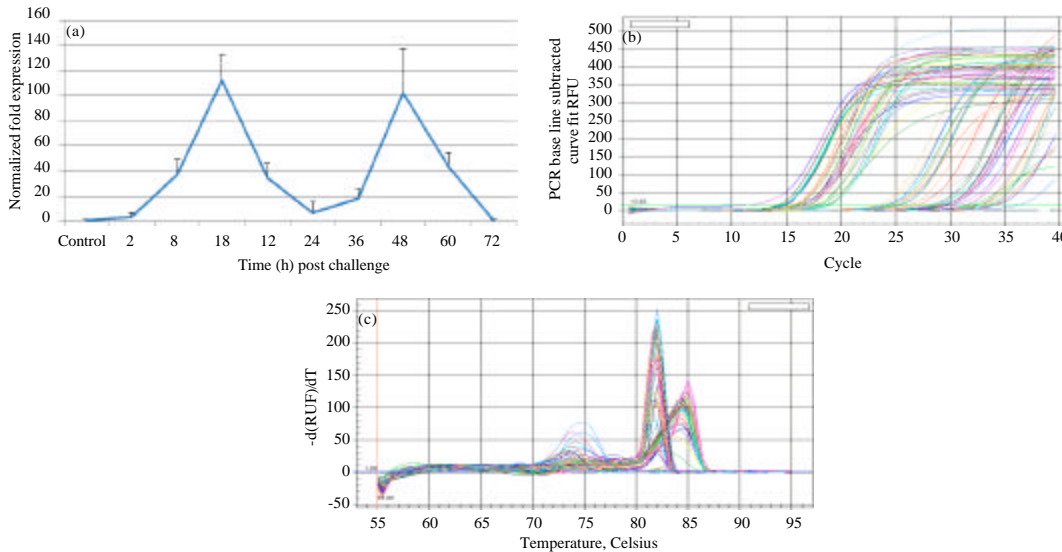


Fig. 6(a-c): (a) Dynamics of IL17 mRNA in mammary gland of mice challenged with *S. aureus* and Real-time PCR amplifications (b) Curve and (c) Melt peak chart of IL-17 expression in mammary gland of mice challenged with *S. aureus*

variety of pathogens to gain knowledge about the pathogenesis of mastitis, the pathogen's mode of action and the control of intramammary infections by new drugs and vaccines (Notebaert *et al.*, 2008). However, extensive studies had focused on the acute, but not chronic mastitis in mice model induced with *E. coli* or *S. aureus* experimentally in the past (Brouillette *et al.*, 2005; Notebaert *et al.*, 2008). In the present studies, we injected 50 μ L *S. aureus* (1×10^7 CFU) isolated from milk of Guanzhong dairy goat that suffered subclinical mastitis into mice mammary. Results

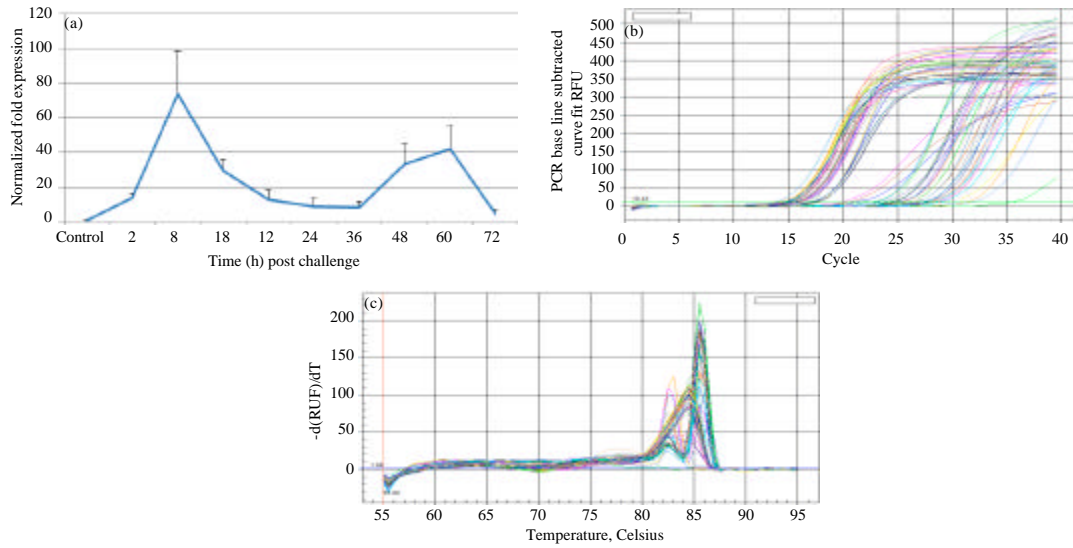


Fig. 7(a-c): (a) Dynamics of L-23 p19 mRNA in mammary gland of mice challenged with *S. aureus* and Real-time PCR amplifications (b) Curve and (c) Melt peak chart of L-23 p19 expression in mammary gland of mice challenged with *S. aureus*

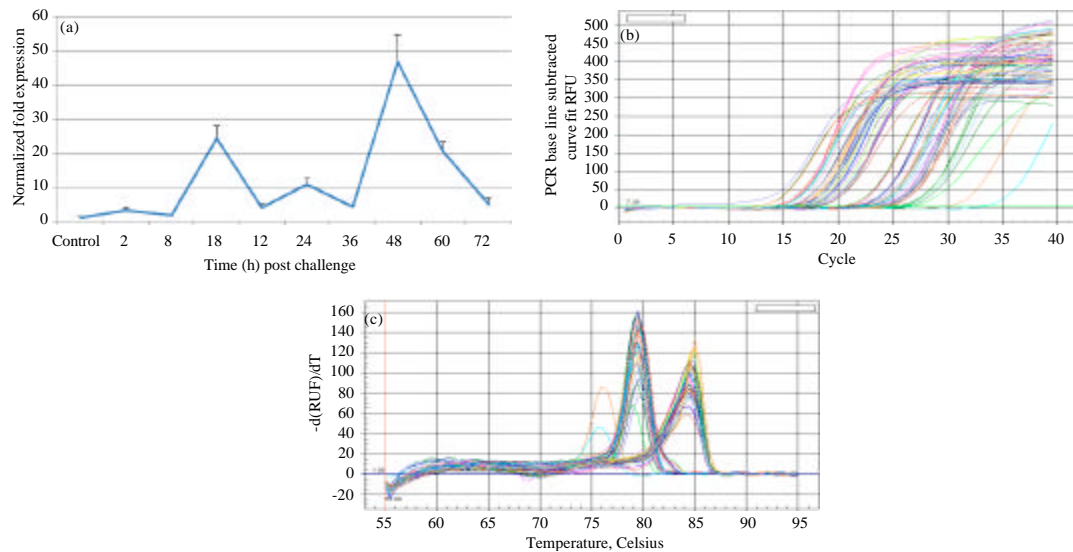


Fig. 8(a-c): (a) Dynamics of CCR6 mRNA in mammary gland of mice challenged with *S. aureus*, and Real-time PCR amplifications (b) Curve and (c) Melt peak chart of CCR6 expression in mammary gland of mice challenged with *S. aureus*

showed minor indications of illness and no obvious signs of infection. Food and water uptake was not diminished obviously all through the experiments as well. However, the infiltrated cells were found in mammary gland tissues and their number was increasing gradually in 72 h following challenge. The mammary epithelium had no any damage in this study. The results demonstrated that mice chronic mice model was induced by *S. aureus*.

The $\gamma\delta$ T cells represent a minor percentage of the peripheral lymphocyte pool in most animals, however they represent a major lymphocyte subset in cattle and can constitute up to 60% of the circulating T-cells in calves (Guzman *et al.*, 2011). However, the role of $\gamma\delta$ T cells in mammary gland infections was unraveled. Understanding the $\gamma\delta$ T cells response to the bacterial causes of mastitis is very important in mastitis prevention and control (Guzman *et al.*, 2011). In this study, $\gamma\delta$ T cells in mammary gland of mice chronic mastitis were analyzed through cytometry.

$\gamma\delta$ T cells were categorized into $\gamma\delta$ T1 and $\gamma\delta$ T17 based on the effector cytokines IFN- γ or IL-17 (Korn and Petermann, 2012). $\gamma\delta$ T cells that had encountered their cognate antigen in the thymus acquired the capacity to produce IFN- γ , while ligand Naive $\gamma\delta$ T cells produced IL-17 (Jensen *et al.*, 2008). In the present study, the percentage of $\gamma\delta$ T1 in $\gamma\delta$ T cells decreased significantly in challenged mice mammary gland, while the percentage of $\gamma\delta$ T17 in mammary $\gamma\delta$ T cells were increased significantly at 18 h, 48 h and 60 h post challenge significantly compared to control (Fig. 3). $\gamma\delta$ T cells are the first line of host defense controlling neutrophil-mediated innate immune responses (Shibata *et al.*, 2007; Kabelitz, 2011). Our results suggest $\gamma\delta$ T17 cells were activated rapidly and played a key role in mediating mammary inflammation caused by *S. aureus* and $\gamma\delta$ T1 cells had little role in inflammation of mammary gland. However, the ratio of $\gamma\delta$ T17 cells decreased at 24 and 36 h while increased at 18, 48 and 60 h which may display the impulse mode of $\gamma\delta$ T17 cells in the subclinical mastitis.

IFN- γ and IL-17 are the effector cytokines of $\gamma\delta$ T1 and $\gamma\delta$ T17 cells, respectively (Roark *et al.*, 2008; Haas *et al.*, 2009; Ribot *et al.*, 2009; Korn and Petermann, 2012). IFN- γ is one of proinflammatory cytokines in viral or intracellular infections, however, Satorres *et al.* (2009) found the IFN- γ plays a detrimental role of in murine defense against nasal colonization of *S. aureus*. In this study, although the folds of IFN- γ mRNA higher than the control group, the expression of IFN- γ decreased gradually after its peak value at 8 h (Fig. 4) which profiles were similar to the $\gamma\delta$ T1 in $\gamma\delta$ T cells of challenged mammary gland (Fig. 2). These results suggested that IFN- γ can be activated at the very early stage but may be not play the key role in mediating inflammation of *S. aureus* mastitis.

IL-17 is a major player in the immune responses at epithelial surfaces and important for efficient clearance of pathogenic infections via recruitment and activation of neutrophils (Laan *et al.*, 1999; Kolls and Linden, 2004; Marks and Craft, 2009). Increasing evidence demonstrated that IL-17 was associated with protective immune responses in the lung, gastrointestinal tract and skin (Lockhart *et al.*, 2006; Cooper, 2009; Cho *et al.*, 2010). Shibata *et al.* (2007) demonstrated that resident $\gamma\delta$ T cells control early infiltration of neutrophils after escherichia coli infection via IL-17 production and demonstrated a novel role of $\gamma\delta$ T cells as a first line of host defense controlling innate immune responses. In the present study, the IL-17 mRNA expression increased rapidly during very early stage (2 h) and the average folds of IL-17 were always higher than control group in mammary glands at all time points after challenge (Fig. 6) which was accordance with previous study in *S. aureus* mastitis of cow (Riollet *et al.*, 2006; Tao and Mallard, 2007). A previous experiment in our laboratory also displayed that the concentration of milk IL-17 increased in goat mastitis induced with *S. aureus* and *E. coli* experimentally (Jing *et al.*, 2012). The level of IL-17 mRNA appeared two peaks at 12 and 48 h post challenge (Fig. 6) which may be because the mice model was the chronic mastitis induced with *S. aureus* in this study and the weak antigenicity or biofilm of *S. aureus* (Seo *et al.*, 2008). Our results demonstrated that IL-17 play an important role during the crucial early phases of immune response in mammary gland infection with *S. aureus*.

The $\gamma\delta$ T17 cells represent a subset of $\gamma\delta$ T cells with similar characteristics as Th17 cells, such as expression of CCR6, IL-23R (Shibata *et al.*, 2007; Martin *et al.*, 2009). Multiple studies had shown that CCR6⁺ $\gamma\delta$ T17 cells express IL-23R and produce IL-17 in response to IL-23 without T cell receptor engagement (Sutton *et al.*, 2009). In this study, the mRNA level of IL-23p19 increased markedly, peaked at 8 and 60 h, respectively (Fig. 7) and the expression folds of CCR6 were always higher in glands challenged all through experiments and the peak folds appeared at 8 h and 48 h post challenge (Fig. 8) which profiles were similar to the $\gamma\delta$ T17 cells. The results suggested IL-17 in mammary gland mainly sourced from activated CCR6⁺ $\gamma\delta$ T17 cells as depending on IL-23 following intramammary injection with *S. aureus*.

To our knowledge, this is the first comprehensive report of a mouse model of *S. aureus* mastitis characterized by the increase of $\gamma\delta$ T17 in the mammary gland and histological alterations. This study provides important information about the cellular responses at the early stages in the mouse model of *S. aureus* mastitis and might possibly offer new cellular targets for improved therapy of persistent *S. aureus* mastitis.

CONCLUSION

In the present study, the systematic response of $\gamma\delta$ T cells is evaluated in mice chronic mastitis induced with *S. aureus*. The results showed that CCR6⁺ $\gamma\delta$ T17 cells played an important role in mediating inflammation by producing effector cytokine IL-17 as depending on IL-23 in mammary gland. In conclusion, the characterization of $\gamma\delta$ T17 cells in the early inflammatory response upon *S. aureus* mouse mastitis no longer remains unaddressed and the obtained knowledge may contribute to the field of mastitis research.

ACKNOWLEDGMENTS

We are grateful for the dedicated assistance by Wei Zhao. This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201103038).

REFERENCES

- Al-Graibawi, M.A., V.K. Sharma and A.J. Al-Shammari, 1986. Microbial pathogens from goat mastitis and phage-typing of *Staphylococcus aureus* isolates. Comp. Immunol. Microbiol. Infect. Dis., 9: 23-28.
- Berry, E.A. and J.E. Hillerton, 2002. The effect of selective dry cow treatment on new intramammary infections. J. Dairy Sci., 85: 112-121.
- Brouillette, E. and F. Malouin, 2005. The pathogenesis and control of staphylococcus aureus-induced mastitis: Study models in the mouse. Microbes Infection, 7: 560-568.
- Brouillette, E., G. Grondin, B.G. Talbot and F. Malouin, 2005. Inflammatory cell infiltration as an indicator of *Staphylococcus aureus* infection and therapeutic efficacy in experimental mouse mastitis. Vet. Immunol. Immunopathol., 104: 163-169.
- Chandler, R.L., 1970. Experimental bacterial mastitis in the mouse. J. Med. Microbiol., 3: 273-282.
- 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.
- Contreras, A., D. Sierra, A. Sanchez, J.C. Corrales, J.C. Marco, M.J. Paape and C. Gonzalo, 2007. Mastitis in small ruminants. Small Ruminant Res., 68: 145-153.
- Cooper, A.M., 2009. Il-17 and anti-bacterial immunity: Protection versus tissue damage. Eur. J. Immunol., 39: 649-652.

- Guzman, E., S. Price, H. Poulson and J. Hope, 2011. Bovine $\gamma\delta$ t cells: Cells with multiple functions and important roles in immunity. *Vet. Immunol. Immunopathol.*, 148: 161-167.
- Haas, J.D., F.H.M. Gonzalez, S. Schmitz, V. Chennupati and L. Fohse *et al.*, 2009. Ccr6 and NK1. 1 distinguish between IL-17a and IFN- γ -producing $\gamma\delta$ effector t cells. *Eur. J. Immunol.*, 39: 3488-3497.
- Jensen, K.D.C. and Y.H. Chien, 2009. Thymic maturation determines $\gamma\delta$ t cell function, but not their antigen specificities. *Curr. Opin. Immunol.*, 21: 140-145.
- Jensen, K.D.C., X. Su, S. Shin, L. Li and S. Youssef *et al.*, 2008. Thymic selection determines $\gamma\delta$ t cell effector fate: Antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon- γ . *Immunity*, 29: 90-100.
- Jing, X.Q., Y.Q. Zhao, C.C. Shang, Y.L. Yao, T.T. Tian, J. Li and D.K. Chen, 2012. 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. *J. Anim. Vet. Adv.*, 11: 475-479.
- Kabelitz, D., 2011. $\gamma\delta$ T-cells: Cross-talk between innate and adaptive immunity. *Cell. Mol. Life Sci.*, 68: 2331-2333.
- Kolls, J.K. and A. Linden, 2004. Interleukin-17 family members and inflammation. *Immunity*, 21: 467-476.
- Korn, T. and F. Petermann, 2012. Development and function of interleukin 17-producing $\gamma\delta$ t cells. *Ann. New York Acad. Sci.*, 1247: 34-45.
- Kotwal, G.J., 1997. Microorganisms and their interaction with the immune system. *J. Leukocyte Biol.*, 62: 415-429.
- Laan, M., Z.H. Cui, H. Hoshino, J. Lotvall and M. Sjostrand *et al.*, 1999. Neutrophil recruitment by human il-17 via c-x-c chemokine release in the airways. *J. Immunol.*, 162: 2347-2352.
- Lockhart, E., A.M. Green and J.L. Flynn, 2006. Il-17 production is dominated by $\gamma\delta$ t cells rather than cd4 t cells during mycobacterium tuberculosis infection. *J. Immunol.*, 177: 4662-4669.
- Marks, B.R. and J. Craft, 2009. Barrier immunity and il-17. *Semin. Immunol.*, 21: 164-171.
- Martin, B., K. Hirota, D.J. Cua, B. Stockinger and M. Veldhoen, 2009. Interleukin-17-producing $\gamma\delta$ t cells selectively expand in response to pathogen products and environmental signals. *Immunity*, 31: 321-330.
- Mork, T., S. Waage, T. Tollersrud, B. Kvitle and S. Sviland, 2007. Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. *Acta Vet. Scand.*, Vol. 49. 10.1186/1751-0147-49-23
- Notebaert, S. and E. Meyer, 2006. Mouse models to study the pathogenesis and control of bovine mastitis. A review. *Vet. Q*, 28: 2-13.
- 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.
- Ribot, J.C., A. de Barros, D.J. Pang, J.F. Neves and V. Peperzak *et al.*, 2009. Cd27 is a thymic determinant of the balance between interferon- γ -and interleukin 17-producing $\gamma\delta$ t cell subsets. *Nat. Immunol.*, 10: 427-436.
- Riollet, C., D. Mutuel, M. Duonor-Cerutti and P. Rainard, 2006. Determination and characterization of bovine interleukin-17 cDNA. *J. Interferon Cytokine Res.*, 26: 141-149.
- Roark, C.L., P.L. Simonian, A.P. Fontenot, W.K. Born and R.L. O'Brien, 2008. $\Gamma\delta$ T cells: An important source of IL-17. *Curr. Opin. Immunol.*, 20: 353-357.

- Satorres, S.E., L.E. Alcaraz, E. Cargnelutti and M.S. Di Genaro, 2009. Ifn- γ plays a detrimental role in murine defense against nasal colonization of staphylococcus aureus. *Immunol. Lett.*, 123: 185-188.
- Seo, Y.S., D.Y. Lee, N. Rayamahji, M.L. Kang and H.S. Yoo, 2008. Biofilm-forming associated genotypic and phenotypic characteristics of *Staphylococcus* spp. isolated from animals and air. *Res. Vet. Sci.*, 85: 433-438.
- Shibata, K., H. Yamada, H. Hara, K. Kishihara and Y. Yoshikai, 2007. Resident V δ 1+ $\gamma\delta$ T cells control early infiltration of neutrophils after *Escherichia coli* infection via IL-17 production. *J. Immunol.*, 178: 4466-4472.
- Shim, E.H., R.D. Shanks and D.E. Morin, 2004. Milk loss and treatment costs associated with two treatment protocols for clinical mastitis in dairy cows. *J. Dairy Sci.*, 87: 2702-2708.
- Sutton, C.E., S.J. Lalor, C.M. Sweeney, C.F. Brereton, E.C. Lavelle and K.H. Mills, 2009. Interleukin-1 and IL-23 induce innate IL-17 production from $\gamma\delta$ T cells, amplifying th17 responses and autoimmunity. *Immunity*, 31: 331-341.
- Tao, W. and B. Mallard, 2007. Differentially expressed genes associated with *Staphylococcus aureus* mastitis of Canadian holstein cows. *Vet. Immunol. Immunopathol.*, 120: 201-211.
- Tenhagen, B.A., I. Hansen, A. Reinecke and W. Heuwieser, 2009. Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *J. Dairy Res.*, 76: 179-187.
- Weigmann, B., I. Tubbe, D. Seidel, A. Nicolaev, C. Becker and M.F. Neurath, 2007. Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. *Nat. Protocols* 2: 2307-2311.
- Witherden, D.A. and W.L. Havran, 2011. Molecular aspects of epithelial $\gamma\delta$ T cell regulation. *Trends Immunol.*, 32: 265-267.
- 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- κ B in mammary epithelial cells and to quickly induce TNF α and interleukin-8 (CXCL8) expression in the udder. *Mol. Immunol.*, 45: 1385-1397.