Livestock Science 154 (2013) 215–223

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Immune response of the mammary gland during different stages of lactation cycle in high versus low yielding Karan Fries crossbred cows

Joydip Mukherjee [a,*], Nishant Varshney [b], Mandira Chaudhury [c], A.K. Mohanty [b], A.K. Dang [d]

[a] Department of Veterinary Physiology, West Bengal University of Animal and Fishery Sciences, Kolkata 700037, West Bengal, India
[b] Animal Biotechnology Centre, National Dairy Research Institute, Karnal 132 001, Haryana, India
[c] Animal Resources Development Department, Government of West Bengal, Malda, India
[d] Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal 132 001, Haryana, India

ARTICLE INFO

Article history:
Received 21 April 2012
Received in revised form
17 March 2013
Accepted 18 March 2013

Keywords:
Crossbred cows
Leukocyte activity
Real time PCR
IL-8
TNF-α
TLR-4

ABSTRACT

To investigate the magnitude of innate immunity of mammary glands during different stages of the lactation cycle, milk samples were collected from both high ($n=10$) and low ($n=10$) yielding Karan Fries (KF) crossbred cows during early, mid and late lactation stages. Milk somatic cells released during different stages of the lactation cycle were measured microscopically. In vitro immune activity of each milk leukocytes (viz. neutrophils, lymphocytes and macrophages) were also evaluated after isolating each of the cells from the total milk somatic cell pellet. Relative mRNA expression profiles of TNF-α, IL-8 and TLR-4 by RT polymerase chain reaction were also studied in total milk somatic cells during various stages of the lactation cycle in high yielding cows, whereas low yielding KF cows were taken as control. The results were analyzed and significance was tested by employing two way ANOVA, and the relative expression ratio of the target genes of high yielding cows was tested and analyzed for significance by Relative Expression Software Tool REST version 2009. Irrespective of the stage of lactation, high yielding animals were having higher ($P<0.01$) somatic cell counts (SCC) compared to low yielders. Milk SCC was found to be significantly higher ($P<0.01$) during mid-lactation compared to other stages in high yielding cows. In vitro immune response of milk leukocytes were lower in high yielders ($P<0.01$) compared to low yielders, irrespective of stage of lactation. In vitro immune response of all the milk leukocytes was found to be lower ($P<0.01$) in high yielding cows compared to low yielding cows. In vitro phagocytic index (PI) of milk neutrophils was higher ($P<0.01$) during mid lactation periods in both the groups of cows. PI of milk macrophages was found to be higher ($P<0.01$) during early lactation period in both high and low yielding cows. LPS induced milk lymphocyte blastogenic response was found to be higher ($P<0.01$) in early lactation period of low yielders. Abundance of IL-8, TNF-α and TLR-4 transcripts were up-regulated ($P<0.01$) during mid lactation of high yielders as compared to control cows (low yielding cows). Abundance of TNF-α and TLR-4 transcripts were down-regulated ($P<0.01$) during late lactation in high yielding cows. This study indicated that innate immune responses of milk somatic cells in KF cows are modulated by both lactation stage and milk yield. It was also found that mammary glands of high yielding cows have better magnitude of innate immune response during mid lactation stage compared to early and late lactation in terms of milk SCC and in vitro immune response of isolated milk leukocytes.

* Corresponding author. Tel.: +91 8902 386 001.
E-mail address: joyphy@gmail.com (J. Mukherjee).

1871-1413/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2013.03.017

1. Introduction

Selection of dairy cows with superior milk production traits has resulted in a steady increase in the incidence of clinical mastitis (Emmanuelson et al., 1988; Harmon, 1994; Owen et al., 2000). When mastitis and other diseases are frequent, it may be beneficial to identify sires and cows based on potential health-related markers. Selection for improved host defense could reduce the prevalence of mastitis and other infectious diseases (Detilleux et al., 1995; Owen et al., 2000; Wagter et al., 2000). A positive genetic correlation between selection for increased milk production and the increased rate of clinical mastitis (Emmanuelson et al., 1988; Owen et al., 2000) has shown that superior production is associated with unfavorable changes in host defense mechanisms that could result in an increased occurrence of mastitis. Thus, even cows from well managed dairy herds utilizing the most recent and effective control measures can experience a high rate of mastitis, especially during the first 90 days of lactation. Many of these intra mammary infections (IMI) originate during the dry or non lactating period and result in clinical and (or) subclinical mastitis during early lactation. Therefore the mammary gland of high yielding cows is under stress throughout the lactation cycle and some suitable and sensitive markers are required to assess the immune response of mammary gland throughout the lactation cycle other then routine somatic cell counts. In recent years, the role of cytokines in the patho-physiology of bovine mastitis has been the subject of many studies. Cytokines are naturally produced proteins that play an important role in essentially all aspects of host defense by regulating the activity of cells that participate in specific and nonspecific immunity. Cytokines can be produced both by the somatic cells and mammary epithelial cells but, the somatic cells are probably the main sources of cytokines in milk (Lee et al. 2006). Among all the bovine cytokines, tumor necrosis factor-α (TNF-α) and Interleukin-8 (IL-8) are the main attributors of mammary defense as they are considered to be important for accumulation of phagocytic leukocytes in the udder (Persson-Waller et al., 1997; Shuster et al., 1996). They mediate both neutrophil and lymphocyte function, allowing leukocytes to resolve bacterial infections by migrating through blood vessel walls and to the site of infection (Kehrli and Harp, 2001).

Toll like receptor-4 (TLR-4) is a cell-surface receptor that recognizes a common 'pattern' in structurally diverse LPS molecules (Park et al., 2009), resulting in increased expression of IL-6 and IL-8 (Sawa et al., 2008). Low concentrations of circulating TNF-α and IL-8 in blood or plasma make investigation of systemic cytokine patterns a difficult task and sensitive detection systems are required to monitor their expression and secretion during different physiological stages to evaluate the immune response of mammary glands. Therefore, the objective of this study was to investigate the innate immune response of mammary gland in terms of relative abundance of TNF-α, IL-8 and TLR-4 transcripts and milk leukocyte activity during different stages of lactation cycle in high yielding Karan Fries (KF) crossbred cows.

2. Materials and methods

2.1. Selection of experimental animals and milk sampling

Twenty clinically healthy Karan Fries (KF) interse mated crossbred (Holstein Fresian × Tharparkar) cows (third parity) were selected from the animal herd of the National Dairy Research Institute (NDRI). They were divided into two groups (High and Low yielders) based on expected producing ability (EPA) (Thomas and Chakravarty, 1999), i.e., high yielder (EPA > 5000 kg/305 d, $n=10$) and low yielder (EPA < 3500 kg/305 d, $n=10$)}. All the animals were screened weekly for clinical mastitis by assessing macroscopical examination (Visualization and palpation of the udder, Visualization of the milk, Electrical conductivity of milk, California Mastitis Test) and microscopical (individual cow somatic cell counts). Culturing milk from the individual quarter of experimental cows was also done for evaluating udder health.

All the cows were kept in loose housing system with brick flooring and managed as per the practices followed in the institute's herd. They were offered ad lib green (maize (*Zea mays*) and sorghum (*Sorghum bicolor*) was fed to them in summer and Oats (*Avena spp.*), Clover (*Trifolium spp.*) and winter maize (*Zea mays*) was fed during the winter season and calculated amount of concentrate mixture based on milk production (460 gm/kg of milk produced) only at the time of milking. Fresh tap water was available ad lib at all the time of the day.

Three milking were done from all the cows, i.e., morning, noon and evening. Each cow was machine milked in vario tandem milking parlors and the composite samples represented the entire udder filling. To maintain the sterility of milking machine, the machine is thoroughly cleaned with hot water, soap, acid and germicide solutions twice a day. Before collection, teat dipping was done with an effective teat dip (0.5% iodine or 4% hypochlorite) leaving the predip on the teat for at least 20–30 s before removal. Then the teats were carefully and vigorously scrubbed with a cotton or cloth gauze pad moistened (but not dripping wet) with 70% to 80% ethyl or isopropyl alcohol. Composite milk samples (representing all four quarters) were collected into sterile tubes (200 mL/cow) during early (15–105 days of lactation cycle), mid (120–195 days of lactation cycle) and late (210–300 days of lactation cycle) lactation stages with an interval of 15 days from all the experimental cows. Samples for early and mid lactation were taken from non pregnant animals and the late lactation samples were taken from pregnant animals (third trimester of pregnancy). A total of 21 milk samples were collected from each cow, i.e., a total of 420 milk samples (210 from high and 210 from low producers) were collected during the entire lactation period at fortnightly intervals. Milk was then subjected to subsequent processing as described below.

2.2. Counting of milk SCC and in vitro activity of milk leukocytes

Somatic cell counts (SCC) and differential leukocyte counts (DLC) of milk samples was measured microscopically