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Research paper

Differential levels of mRNA transcripts encoding immunologic mediators in mammary gland secretions from dairy cows with subclinical environmental *Streptococci* infections

Daniela R. Bruno[a], Paul V. Rossitto[b], Ralph.G.S. Bruno[a], Myra T. Blanchard[c], Tatjana Sitt[c], Bret V. Yeargan[c], Wayne L. Smith[b], James S. Cullor[b], Jeffrey L. Stott[c,*]

[a] *Texas Veterinary Medical Diagnostic Laboratory, Texas A&M, Amarillo, TX, USA*
[b] *Department of Population, Health, and Reproduction, Veterinary Medicine Teaching and Research Facility, School of Veterinary Medicine, University of California, Davis, USA*
[c] *Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, USA*

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ABSTRACT

Dry-off, and the period around parturition, are associated with increased susceptibility to intramammary infections in dairy cows. The immunological profiles of mammary gland secretions during these periods are not well described. The objective of the present study was to better characterize association(s) between chronic subclinical Environmental *Streptococci* infections at dry-off and relative levels of mRNA transcripts encoding multiple immunologic mediators present in cells derived from mammary gland secretions at dry-off and continuing through parturition. The chronic subclinical bacterial infections in the present study were characterized by multiple isolations of Streptococcus species and elevated SSC for a minimum of three weeks prior to dry-off. The majority of differences between principal and control quarters were identified at dry-off. Transcript levels of IL-17, IL2Rα and iNOS were increased while pro-inflammatory cytokine IL-6, and the regulatory cytokine IL-10, were reduced.

Following antibiotic treatment of mammary glands, IL-17 transcripts remained elevated over the course of the study, indicative of a persistent insult. IL-4 transcript levels were modestly elevated at 7 days following dry-off and significantly elevated at 14 days, consistent with activated T_H1 and T_H2 lymphocytes in the principal quarters, respectively. From a temporal perspective, transcript levels of IL-8 decreased in all animals through the dry-off period animals and returned to pre-dry-off levels at parturition; levels of iNOS peaked at parturition. Five of the six principal cows experienced recurrent bacterial mastitis during the subsequent lactation; four were in the same quarter as was initially infected with Streptococcus and three of these four were due to coliforms. Taken together, this apparent chronic susceptibility of select mammary glands to bacterial infection would suggest a physiologic and/or immunologic dysfunction. Identification of factor(s) that contribute to the predisposition of mammary glands to developing mastitis should facilitate development of new control strategies.

1. Introduction

Intramammary infections (IMI) are common in dairy cows and result in a typical inflammatory response characterized by an influx of somatic cells composed pri-

* Corresponding author at: Department of Pathology, Microbiology and Immunology, University of California, 4206 Vet Med 3A, Davis, CA 95616, USA. Tel.: +1 530 752 2543; fax: +1 530 752 3349.
E-mail address: jlstott@ucdavis.edu (J.L. Stott).

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marily of neutrophils accompanied by variable numbers of macrophages and lymphocytes (Rainard and Riollet, 2003). The response is driven by the action of a variety of inflammatory mediators including cytokines, chemokines, prostaglandins and leukotrienes, all of which play pivotal roles in mammary gland defenses by mediating and regulating inflammation and immunity. The ensuing inflammatory response induced by entry of bacteria into the mammary gland is variable, the intensity typically dictating an outcome ranging from successful elimination of the pathogen to establishment of chronic infection.

Temporal expression and release of inflammatory mediators, as a function of udder health, stage of lactation and role in resolution of infection, is not well defined (Taylor et al., 1997; Asai et al., 1998, 2003). A variety of immunologic profiles and associated responses have been described following infection of the mammary gland with gram-positive and gram-negative bacteria (Alluwaimi et al., 2003; Schmitz et al., 2004; Denis et al., 2006; Lahouassa et al., 2007; Corl et al., 2008; Griesbeck-Zilch et al., 2008; Petzl et al., 2008). The majority of these studies have focused on correlates of innate immunity with emphasis on measuring alterations in cytokine production using a combination of reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assays (ELISA). Multiple studies (Alluwaimi et al., 2003; Bannerman et al., 2004a,b) of experimental intramammary infections with gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus uberis*, described up-regulation of mammary gland leukocytes and a wide variety of associated cytokines and inflammatory mediators including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), C5a, IL-12, lipopolysaccharide binding protein (LBP), IL-8, soluble CD14 (sCD14), IL-2 and interferon gamma (IFN-γ). Alluwaimi et al. (2003) described decreased levels of IL-2 within 2 days post-infection and suggested *S. aureus* infection might be suppressing the immune system. In a similar vein, Bannerman et al. (2004a) identified increased IL-10, consistent with negative regulation of the immune system. Studies employing gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Serratia marcescens*, similarly resulted in the up-regulation of mammary gland leukocytes and a wide variety of immune response and regulatory genes including sCD14, IL1, TNF-α, IL1β, C5a, IL8, IL-12, LBP, IFN-γ, IL-10, transforming growth factor alpha (TGF-α), TGF-β1 and TGF-β2 (Bannerman et al., 2004a,b,c, 2005).

Environmental *Streptococci* (*Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Streptococcus* species other than *S. agalactiae*) are significant contributors to clinical and subclinical mastitis in both lactating and non-lactating cows. Some of these microorganisms, such as Streptococcus uberis, show characteristics of contagious mastitis organisms as they can be resistant to phagocytosis and killing by leukocytes, often progressing to chronic mastitis that is unresponsive to intramammary antibiotic therapy (Leigh, 1999).

Given the importance of environmental *Streptococcus*, and the fact that cows entering the dry period with an intramammary infection are at higher risk for developing mastitis on the next lactation (Bradley and Green, 2004), the current objectives were designed to measure relative levels of immune response gene transcripts in mammary gland secretions in the dry period and early lactation.

2. Materials and methods

2.1. Animals and milk sampling

Animal management and associated procedures were according to those approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California–Davis. Holstein cows from a single dairy farm in the San Joaquin Valley of California, in different parities, were included in this study. Composite milk samples from all quarters were pre-screened for infection by microbiologic culture and somatic cell count (SCC) at 21 days prior to dry-off. Cows identified as being positive for Environmental *Streptococcus*, and select "clean" cows, were subsequently screened by individual quarter at 14, 7 and 2 days prior to dry-off. Cow health was monitored throughout the study for any signs of illness.

Cows identified as being positive for Environmental *Streptococcus*, and free from concurrent non-strep infection, were assigned to the infected group (six cows). One infected quarter was enrolled on the study. All infections were established for a minimum of 3 weeks, and were subclinical with no gross effect on milk appearance. Cows with a negative culture result in the pre-screening, and SCC less than 200,000, were assigned to the control group (eight cows). Two non-infected quarters were selected to be enrolled on the study. After enrollment at dry-off, milk samples were collected at 7 days after dry-off (7 d), 14 days after dry-off (14 d), calving and 5 days after calving (5 days in milk; DIM) for SCC, microbiology, and RT-PCR analysis. The average dry period was 61 days with a minimum of 57 days and a maximum of 69 days.

2.2. Milk bacteriologic culture and SCC

Microbiological examinations of milk and SCC determinations were performed at all time points. Bacteria were initially cultured on sheep blood agar plates and confirmed as Environmental *Streptococci* by bench test results including being 3% KOH negative, catalase negative and gram-positive cocci (Rossitto et al., 2002). Isolates were stored in glycerin at −20 °C for future speciation. *Mycoplasma* culture was performed on all quarters of all cows to insure enrolled animals were not co-infected with *Mycoplasma spp*. Milk samples for SCC were submitted to the local Dairy Herd Improvement Association (DHIA, Tulare, CA 93274).

Dairy computer records were reviewed for six months following parturition to identify development of any clinical mastitis following parturition. Identification of mastitis was by dairy employees who entered the events into dairy management software. Samples, when collected by the dairy, were submitted to the Milk Quality Lab (Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, Univ. of Calif. Tulare, CA 93274) for routine milk microbiological examination of milk as prescribed