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Proteomic analysis of differentially expressed proteins in caprine milk during experimentally induced endotoxin mastitis

Z. Olumee-Shabon, T. Swain, E. A. Smith, E. Tall, and J. L. Boehmer[1,2]
United States Food and Drug Administration, Center for Veterinary Medicine, Laurel, MD 20708

ABSTRACT

The goal of the current study was to identify proteins in goat milk before and at 18 h following intramammary challenge with lipopolysaccharide (LPS). Initial evaluation of protein profiles generated using 2-dimensional gel electrophoresis on skim milk samples from a group of 6 goats collected before challenge and at 18, 24, and 48 h after LPS challenge revealed little change in the abundance of casein proteins, and minimal changes in the presence or abundance of the plasma protein serum albumin, which is known to leak into milk during coliform mastitis in dairy cattle. Proteins in baseline milk samples and in milk from the same goats 18 h post-LPS challenge were excised from the gels, and peptides were sequenced using nano-flow liquid chromatography coupled with tandem mass spectrometry. Despite the overwhelming presence of casein proteins and β-lactoglobulin, the lower abundance proteins β-2-microglobulin, fatty acid-binding protein, serum albumin, and retinol-binding protein were detected in skim milk samples from healthy goats. Skim milk samples 18 h postchallenge were characterized by the sustained presence and abundance of the casein proteins, and by the presence of haptoglobin, serum amyloid A, lactoferrin, cathelicidin-1, and cathelicidin-3. No marked differences in the intensity of the spot corresponding to serum albumin were observed in gels of skim milk samples 18 h postchallenge, which could indicate that the breakdown of the blood–milk barrier during endotoxin mastitis may not be as profound in goats as has been observed in dairy cattle. Nonetheless, the occurrence of an inflammatory response was supported by elevated somatic cell counts in the goat milk following inoculation with endotoxin, as well as by the presence of both antimicrobial and acute phase proteins. The results provide information about the composition of proteins in goat milk as well as added knowledge of the host response during endotoxin mastitis in goats.
Key words: proteomic analysis, endotoxin mastitis, milk protein, caprine

INTRODUCTION

Sustained economic losses, limited treatment options, and poor efficacy of available preventative therapies explain why a continued focus of ruminant mastitis research remains the elucidation of mechanisms and mediators involved in the host response to intramammary bacterial pathogen invasion. Additionally, the use of antimicrobial agents for the treatment and prevention of mastitis infections has introduced concerns regarding the safety and quality of dairy products. Specifically, the sustained use of antibiotics in food animals can result in the presence of drug residues in edible tissues and milk products, and can affect the continued emergence of resistant strains of bacteria.

Escherichia coli, a gram-negative environmental pathogen, is a prominent cause of clinical mastitis that is problematic to treat. Earlier studies have indicated that antibiotics offer little or no efficacy in resolving clinical or subclinical cases of mastitis caused by environmental pathogens (Hogan et al., 1989; Erskine, 2000). Further, the outer cell wall of *E. coli* contains LPS, a toxin that stimulates the upregulation of several proinflammatory cytokines, including IL-1β, IL-6, IL-8, and tumor necrosis factor α, that cause profound inflammation and potential tissue damage in the bovine mammary gland (Bannerman, 2009).

The soluble mediators of inflammation in bovine milk and plasma during clinical mastitis have been researched extensively (Bannerman, 2009), but most studies have been accomplished through the use of antibody-based strategies such as Western blots and ELISA. Although these methodologies are both quantitative and accurate, the use of such strategies can limit the detection of novel inflammatory mediators, as the techniques are highly dependent on the availability or development of specific antibodies. Conversely, mass spectrometry–based proteomics technologies allow for the simultaneous analysis of a larger number of proteins

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[1]Corresponding author: jamie.boehmer@va.gov
[2]Current affiliation: Department of Veterans Affairs Office of Research Oversight, 810 Vermont Avenue NW (10R), Washington, DC 20420.

without reliance on antibodies. Accordingly, the use of such strategies to characterize the bovine inflammatory response to gram-negative pathogens and to profile changes in the bovine milk proteome during mastitis has gained popularity in recent years (Smolenski et al., 2007; Boehmer et al., 2008, 2010a,b; Danielsen et al., 2010).

Milk protein modulation during bovine mastitis has been evaluated using several common proteomic approaches, including 2-dimensional gel electrophoresis (**2D-GE**), matrix-assisted laser desorption-ionization mass spectrometry (**MALDI-MS**), and liquid chromatography coupled with tandem mass spectrometry (**LC-MS/MS**; Hogarth et al., 2004; Smolenski et al., 2007; Boehmer et al., 2008, 2010a,b; Danielsen et al., 2010). Likewise, recent studies have sought to quantify differential milk protein expression using milk from clinically healthy cows and cows with experimentally induced coliform mastitis (Boehmer et al., 2010a,b; Danielsen et al., 2010). However, although our knowledge of modulation in the bovine milk proteome during coliform mastitis continues to expand, very little comparative data exist on lactating dairy goats.

At present, goat milk accounts for a small percentage of global milk consumption, although the popularity of goat milk-derived products has increased in recent years because of the inherent nutritional factor of goat milk and its suitability as a substitute for bovine milk when intolerance, allergies, and gastrointestinal disorders are a factor (Bellioni-Businco et al., 1999; McCullough, 2003; Sanz Ceballos et al., 2009). Like other ruminant species that are managed for milk production, goats are also affected by mastitis; however, much less is known about the goat innate immune response to mastitis pathogens or the subsequent changes in goat milk protein expression over the course of a clinical infection. Although limited, previous research aimed at characterizing the host response during mastitis in goats has revealed that goats exhibit clinical symptoms similar to those evidenced in dairy cattle during experimental induction of endotoxin (LPS) and *E. coli* mastitis, including increased rectal temperature, elevated SCC, reduced appetite and milk production, increased heart rates, and changes in blood chemistry parameters (Anderson et al., 1991; Massart-Leen and Vandeputte-Van Messom, 1991). Similarly, prior reports of proteomic evaluations of goat milk have been limited to the analysis of casein protein expression in goat milk, and the determination of the molecular weights of major goat milk proteins (Roncada et al., 2002; Ham et al., 2012). To date, no studies have focused on the proteomic analysis of goat milk protein modulation during clinical mastitis.

The goals of the current study were to identify proteins in goat milk and to qualitatively evaluate potential changes in protein expression in goat skim milk following experimental induction of endotoxin mastitis. Milk samples were obtained from a group of 6 lactating goats before and at 3 time points following intramammary challenge with LPS. Proteins present in baseline (0 h) and milk samples collected 18 h following LPS challenge were profiled using 2D-GE, and tryptic peptides resulting from in-gel digestion were identified by nanoflow (**n**)LC-MS/MS. The results of the study provide additional information regarding protein expression in goat milk, expand current knowledge of proteins present in both normal and mastitic goat milk, and may aid in the further characterization of soluble mediators of inflammation present in goat milk during disease.

MATERIALS AND METHODS

Goats

Six clinically healthy, mixed-breed dairy goats in their first lactation, approximately 100 to 150 DIM and ranging in weight from 49 to 63 kg, were used in these experiments. None of the goats had prior incidence or treatments for mastitis, and all had milk SCC <200,000 cells/mL at the onset of the study. Composite milk samples from all goats were cultured before the start of the study, and all cultures were negative for the presence of pathogens (Quality Milk Production Services, Cornell University, Ithaca, NY). The care and use of all animals in this study was approved by the Center for Veterinary Medicine Office of Research Institutional Animal Care and Use Committee.

Determination of Milk SCC

Goat milk can contain cytoplasmic masses, as well as a greater number of epithelial cells than are typically detected in bovine milk. Because electronic cell counters cannot accurately differentiate between cytoplasmic masses, epithelial cells, and white blood cells, goat milk SCC are routinely determined using the California Mastitis Test (**CMT**), which reacts with the genetic material of somatic cells, and provides a reasonably accurate SCC. A CMT score of 0 (negative) was used as an indicator to determine a SCC <200,000 cells/mL.

Intramammary Challenge with LPS

On the day of challenge, the right udder half of each of the 6 does was infused with 4 μg of LPS/kg in 2 mL of pyrogen-free saline using a sterile disposable teat cannula. The left half of each udder was infused with 2 mL of sterile PBS to serve as a control to the challenged half and to validate that any inflammation or