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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

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ABSTRACT

Streptococcus uberis is a prevalent causative organism of mastitis and resides naturally in the environment of the dairy cow making prevention of the disease difficult. A bovine cDNA microarray comprising approximately 22,000 expressed sequence tags was used to evaluate the transcriptional changes that occur in the mammary gland after the onset of clinical *Strep. uberis* mastitis. Five lactating Friesian heifers were intramammary infused in an uninfected quarter with approximately 1,000 to 1,500 cfu of a wild-type strain of *Strep. uberis.* Microarray results showed that *Strep. uberis* mastitis led to the differential expression of more than 2,200 genes by greater than 1.5-fold compared with noninfected control quarters. The most highly upregulated genes were associated with the immune response, programmed cell death, and oxidative stress. Quantitative real-time reverse transcription PCR analysis confirmed the increase in mRNA expression of immune-related genes complement component 3, clusterin, IL-8, calgranulin C, IFN-γ, IL-10, IL-1β, IL-6, toll-like receptor-2, tumor necrosis factor-α, serum amyloid A3, lactoferrin, LPS-bonding protein, and oxidative stress-related genes metallothionein 1A and superoxide dimutase 2. In contrast, a decrease of mRNA levels was observed for the major milk protein genes. Bovine mammary epithelial cells in culture challenged with the same *Strep. uberis* strain used to induce clinical mastitis in the in vivo animal experiment did not cause a change in the mRNA levels of the immune-related genes. This suggests that the expression of immune-related genes by mammary epithelial cells may be initiated by host factors and not *Strep. uberis*. However, challenging epithelial cells with different *Strep. uberis* strains and *Staphylococcus aureus* resulted in an increase in the mRNA expression of a subset of the immune-related genes measured. In comparison, an *Escherichia coli* challenge caused an increase in the majority of immune-related genes measured. Results demonstrate the complexity of the bovine mammary gland immune response to an infecting pathogen and indicate that a coordinated response exists between the resident, recruited, and inducible immune factors.

Key words: mastitis, bovine, microarray, *Streptococcus uberis*

INTRODUCTION

Bovine mastitis is an inflammation of the mammary gland and is usually a consequence of microbial infection (Jain, 1979). It is a highly prevalent and costly disease for the dairy industry worldwide (Blosser, 1979; DeGraves and Fetrow, 1993). *Streptococcus uberis* is a major causative agent of IMI in dairy cows both in countries with pasture-based management systems and seasonal calving (Hillerton et al., 1993; McDougall, 2002) and where cattle are housed and have year-round calving (Hillerton et al., 1993; McDougall, 2002). *Streptococcus uberis* resides naturally in the environment and has been isolated from the soil, races, and feces, in addition to various anatomical regions in the cow (Cullen, 1966; Khan et al., 2003; Lacy-Hulbert et al., 2005). Currently, it is not effectively controlled using existing control methods (Leigh, 1999), with the primary means being through administration of antibiotics after the identification of infection. However, with ongoing international pressure to decrease antibiotic use in agriculture due to its perceived link to increasing antibiotic resistance (Teuber, 2001; Phillips et al., 2004), an understanding of the bovine immune response to mastitis may elucidate alternate avenues of combating this disease.

The immune response associated with mastitis is a very complex biological process and involves resident, recruited, and inducible immune factors (Rainard and Riollet, 2006). The innate immune response is the predominant defense during the early stages of infection and is induced rapidly at the site of infection (Sordillo

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et al., 1997). Distinctive differences in the innate immune response have been reported after the infusion of gram-positive and gram-negative organisms into the mammary gland (Bannerman et al., 2004a,b). Although the systemic and innate immune response for gram-negative organisms appears similar, differences were evident in these responses for 2 prolific mastitis-causing gram-positive pathogens, *Staphylococcus aureus* and *Strep. uberis* (Bannerman et al., 2004a,b). These studies highlight the variability of the innate immune response to different pathogens and establish the importance of understanding the mediators involved in the recognition and signaling of invasion by specific pathogens. Understanding of the immune response of the bovine mammary gland during *Strep. uberis* mastitis is still very rudimentary. However, this broad-spectrum response offers potential avenues of research for increasing the control and prevention of mastitis through the numerous immune and epithelial cell-derived molecules, which respond to the presence of pathogens in the mammary gland (Paape et al., 2000; Bannerman et al., 2004a).

Microarray technology enables the examination of complex interactions between the host and bacterial pathogens (Zheng et al., 2006). It has become a valuable tool in experimental biology, because it enables the simultaneous generation of quantitative data relating to the differential expression of thousands of genes (Schena et al., 1995; Shalon et al., 1996). The identification of genes whose expression is altered in response to bacterial pathogens and the ability to classify these into biologically functional groups aid in the overall understanding of the host response to a specific pathogen (Zheng et al., 2006). The purpose of this study was to analyze the changes in gene expression in vivo in the bovine mammary gland in response to *Strep. uberis* and to compare this to other significant mastitis pathogens in vitro to identify the specific role of the epithelial cells using a bovine mammary epithelial cell (**bMEC**) model. The use of microarray technology allowed a comprehensive investigation into the complex transcriptional changes that occur after the onset of clinical *Strep. uberis* mastitis and subsequent comparative in vitro studies of *Strep. uberis* and other mastitis pathogens, highlighted differences in the immune response to these pathogens, and improves the overall understanding of this disease.

MATERIALS AND METHODS

Animals and Sample Collection

A healthy front and rear quarter of the mammary gland of each of 5 Friesian heifers (mid-late lactation) was infused with approximately 1,000 to 1,500 cfu of a wild-type strain of *Strep. uberis* (*Strep. uberis* 233, New Zealand clinical mastitis isolate, nonencapsulated) in 1 mL of quarter-strength Ringer's solution (Oxoid, Hampshire, UK) immediately after an afternoon milking (Milner et al., 1997). One of these quarters was selected for analysis (Table 1). The strain of *Strep. uberis* was in the log growth phase, and serial dilution plate counts were performed to enumerate the inoculum before infusion. The *Strep. uberis* strain used had been previously isolated from a cow with clinical mastitis. The cows were observed for visual signs of clinical mastitis. Animals had no previous episodes of clinical or subclinical mastitis. Quarter milk samples on the day of trial confirmed that the sampled quarters were free from mastitis pathogens and had less than 150,000 cells/mL. The animals were slaughtered at the Ruakura abattoir (Hamilton, New Zealand) using standard commercial procedures (electrical stunning followed by exsanguination). Mammary alveolar tissue (approximately 30 g) was collected from the middle of the upper one-third of one of the *Strep. uberis*-infused quarters displaying clinical signs of mastitis, specifically clots in the milk, and a control quarter that had the lowest SCC and negative bacteriology data (Table 1). Samples were snap-frozen in liquid nitrogen for subsequent RNA and protein extraction. All procedures were approved by the Ruakura Animal Ethics Committee in accordance with the 1999 Animal Welfare Act of New Zealand. Milk samples were taken for bacteriological examination and SCC to determine infection status 1 h before tissue sampling (Stelwagen and Lacy-Hulbert, 1996).

Array Preparation

Bovine cDNA libraries were generated at AgResearch from a variety of tissues from both dairy and beef breeds as described by Singh et al. (2008). Expressed sequence tags (**EST**) were amplified, and a microarray consisting of 21,378 spots was printed onto poly-L-lysine-coated glass slides using an ESI array robot (ESI, Toronto, Canada) at the University of Otago Genomics Facility (Dunedin, New Zealand) as described by Baird et al. (2004).

Target Preparation and Hybridization

Labeled cDNA was prepared as described in Singh et al. (2008). Briefly, total RNA from mammary alveolar tissue was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and purified using an RNeasy kit (Qiagen, Valencia, CA). Ribonucleic acid integrity