Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 125 (2008) 182–189
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Short communication

Escherichia coli inoculation of porcine mammary glands affects local mRNA expression of Toll-like receptors and regulatory cytokines

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Received 21 December 2007; received in revised form 27 April 2008; accepted 2 May 2008

Abstract

The expression of mRNAs for the Toll-like receptors (TLRs) TLR2 and TLR4, pro- and anti inflammatory cytokines and their receptors was evaluated in mammary gland biopsy material collected from sows intramammarily inoculated with *Escherichia coli* strain O127 at parturition. Quantitative real-time RT-PCR analysis showed increased mRNA levels for TLR2, the proinflammatory cytokines interleukin IL-1β and tumor necrosis factor-alpha TNF-α, and the anti-inflammatory cytokine IL-10 in the inoculated mammary glands 24 h after inoculation. Increased mRNA levels of the proinflammatory cytokine IL-6 were only observed in the inoculated mammary glands of sows that developed clinical signs of mastitis. In contrast, the expression of the anti-inflammatory cytokine, transforming growth factor-beta 1 (TGF-β1) mRNA was unaltered, as was mRNA expression for the IL-1 receptor type I (IL-1R1). Furthermore, IL-1β and IL-10 mRNA expression was higher in the inoculated mammary glands of sows that developed clinical signs of mastitis compared with sows that remained clinically healthy. Notably, sows that developed clinical signs of mastitis had significantly lower pre-inoculation levels of IL-1β mRNA than sows that remained clinically healthy. These findings suggest that development of coliform mastitis is associated with the level of local expression of regulatory cytokines in response to intramammary *E. coli* inoculation and infection.

Keywords: Cytokine; Toll-like receptor; Real-time RT-PCR; Pig; *Escherichia coli*

1. Introduction

The initial recognition of microbes by cells of the immune system, is largely based on pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs) (Aderem and Ulevitch, 2000; Hallman et al., 2001). Of these, TLR4 is the main PRR, for lipopolysaccharides (LPS) from Gram-negative bacteria, whereas TLR2 recognizes lipoteichoic acid (LTA) from Gram-negative bacteria, peptidoglycan and bacterial lipoproteins abundant in the cell wall of both Gram-positive and Gram-negative bacteria, and other bacterial products that are distinct from Gram-negative LPS (Brightbill et al., 1999; Grabig et al., 2006; Werts et al., 2001).

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doi:10.1016/j.vetimm.2008.05.002

Signalling via TLRs may commonly lead to early activation of the transcription factor nuclear factor-κB (NF-κB) and subsequent expression and the release of several proinflammatory mediators including interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α) (Aderem and Underhill, 1999; Akira and Takeda, 2004). To avoid detrimental inflammatory responses, a balance between activation and inhibition is needed throughout the immune response. Therefore, the expression of the anti-inflammatory mediators such as IL-10 and transforming growth factor-beta 1 (TGF-β1) that specifically inhibit the release of proinflammatory mediators, limit the acute inflammatory response and prevent the spread of inflammatory mediators into the bloodstream, is important for the regulation of responses (Finlay and McFadden, 2006; Navarre and Zychlinsky, 2000; Portnoy, 2005; Tracey, 2002). The inflammatory response and its regulation during bacterial-induced mastitis are sparsely characterized yet. Recently, the local cytokine response to LPS inoculation in the mammary gland of rats was described (Miao et al., 2007) and studies in cattle have shown a significant increase in the proinflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α at both the mRNA (Lee et al., 2006) and the protein level (Bannerman et al., 2004; Riollet et al., 2000) in either milk or mammary tissues collected from the infected mammary glands following intramammary inoculation with *Escherichia coli*. Likewise, both TLR2 and TLR4 mRNA expression increased in infected mammary glands of cows with mastitis caused by either *Staphylococcus aureus* or *E. coli* (Goldammer et al., 2004). Moreover, other studies have shown that the production of the anti-inflammatory cytokines IL-10 (Bannerman et al., 2004), TGF-β1 and TGF-β2 (Chockalingam et al., 2005) in bovine milk increased following intramammary *E. coli* inoculation. We have previously shown that the production of the proinflammatory cytokines IL-1β, IL-6 and TNF-α at both protein and the mRNA levels were up-regulated in the *E. coli*-inoculated mammary glands of sows that developed clinical signs of mastitis (Zhu et al., 2007a,b). Furthermore, results from these semi-quantitative RT-PCR analyses and immunohistochemical labelling indicated that sows that developed clinical signs of mastitis had lower pre-inoculation levels of IL-1β than sows that remained clinically healthy.

To further explore the innate immune recognition system and the cytokine network in the porcine mammary gland, qRT-PCR assays were established for the detection and quantification of mRNA expression for TLR2, TLR4, IL-1R1 and a number of regulatory cytokines that are likely to be involved in the early inflammatory response to invading bacteria.

2. Materials and methods

2.1. Animals

Twelve pregnant crossbred (Swedish Landrace × Yorkshire) primiparous sows were included in the study that was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden. Clinical signs of mastitis or other diseases were not observed in any of the sows during the 6–8 weeks period of observation before anticipated parturition.

The experimental model and categorization of sows have previously been described in detail (Osterlundh et al., 2002). In brief, each teat on the right side of mammary glands (inoculated glands) was inoculated with 0.5 ml of bacterial suspension (10^5 colony-forming units (CFUs)/ml) containing *E. coli* strain serotype O127 during the 24-h period before parturition. The contralateral mammary glands were used for sampling from non-inoculated glands. Four of the inoculated sows developed clinical signs of mastitis (i.e. fever of >39.5 °C, anorexia, lethargy, and swelling of two or more mammary glands) 24 h after intramammary inoculation with *E. coli* and were categorized as the affected group. Seven of the other eight inoculated sows that did not develop any signs of clinical mastitis and one sow only showed mild mammary gland affection. These animals were thus categorized as the non-affected group.

2.2. Biopsy procedure

The biopsy procedure was performed as previously described (Loving and Magnusson, 2002). Briefly, two biopsies per mammary gland were carried out using a human Bard® Magnum® Biopsies instrument and a Core Tissue Biopsy Needle (12G × 10 cm) (CR BARD Inc., Covington, GA). Mammary gland biopsy was performed immediately before inoculation (0 h) and from an inoculated and a contralateral non-inoculated gland 24 h later. Thus, each mammary gland was only sampled once. The specimens were immediately frozen in liquid nitrogen and stored at −80 °C until used for analyses.

2.3. Total RNA extraction

Total RNA was extracted from frozen mammary biopsy samples using TRIzol Reagent (Invitrogen Ltd.,