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Evaluation of cytokine expression by blood monocytes of lactating Holstein cows with or without postpartum uterine disease

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Abstract

Whereas neutrophils are the main phagocytic leukocytes, monocytes and macrophages are actively involved in immunomodulation after infection. Recent studies have demonstrated that neutrophil function is impaired by the state of negative energy balance around parturition, and that cows that develop uterine disease have a greater degree of negative energy balance than healthy cows. The objectives of this study were to compare monocyte gene expression and protein secretion of selected cytokines from calving to 42 d after calving in Holstein cows that did or did not develop uterine disease. Real time quantitative RT-PCR (Tumor necrosis factor-α (TNFα), Interleukin (IL)-1β, IL-6, IL-8 and IL-10) and ELISA (TNFα, IL-1β and IL-8) were used to evaluate cytokine response following *in vitro* stimulation of blood-derived monocytes with irradiated *E. coli*. Relative to unstimulated cells, *E. coli*-stimulated monocytes from cows with metritis had lower gene expression of key pro-inflammatory cytokines than healthy cows from calving to 14 d after calving (TNFα at 0, 7, and 14 d after calving, IL-1β and IL-6 at 7 and 14 d after calving; $P < 0.05$). There were no significant differences between groups for expression of IL-8 or the anti-inflammatory cytokine IL-10. This was due, in part, to higher gene expression in unstimulated monocytes (TNFα, IL-1β, IL-6 and IL-10) in early lactation from cows with metritis. Expression of mRNA in stimulated cells (relative to housekeeping genes) was lower for TNFα (7 and 14 d postpartum) and for IL-10 (7 and 14 d postpartum) in cows with metritis. Concentration of TNFα was lower in the culture medium of *E. coli*-stimulated monocytes from cows with metritis than healthy cows at calving and 7 and 21 d after calving ($P < 0.05$). Circulating cytokine concentrations were not different between groups for IL-8 and were below the limits of detection for TNFα and IL-1β. Cytokine gene expression and production were similar between healthy cows and cows that developed endometritis, diagnosed cytologically at 42 d after calving. We concluded that altered levels of expression and production of pro-inflammatory cytokines postpartum could contribute to impaired inflammatory response and predispose cows to development of metritis.

Keywords: Metritis; Endometritis; Cytokines; Gene expression; Dairy cows

1. Introduction

Uterine diseases are highly prevalent in Holstein dairy cows shortly after calving, with > 20% of the cows developing metritis (an acute puerperal disease characterized by fetid, serous red-brownish uterine dis-

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charge, uterine flaccidity, and systemic signs such as depression, inappetence, diminished milk yield, and fever) in the first 3 wk after parturition, and >20% of cows suffering persistent endometritis (generally in the absence of other signs) > 3wk after parturition [1,2]. Metritis is an acute condition associated with bacterial infection (mainly *Fusobacterium necrophorum*, *Prevotella melaninogenica* and *Escherichia coli*) shortly after parturition, whereas endometritis is a more chronic and dynamic condition where cows can be infected (mainly *Arcanobacterium pyogenes* and *E. coli*), cleared, and re-infected [3,4].

Innate immune response to bacteria depends on phagocytosis and killing of microorganisms by neutrophils, monocytes, and macrophages. Bacteria are recognized by specific pattern recognition receptors expressed by these cells [5–7] initiating signaling pathways leading ultimately to release of proinflammatory cytokines such as TNFα and IL-1β. The importance of TNFα in immune function is demonstrated by the range of infectious complications suffered by (human) patients on anti-TNF treatment [8,9]. Tumor necrosis factor-alpha is stimulated and secreted after activation of the NFκB pathway via toll-like receptors [6,7]. Interleukin-1β is produced as a procytokine upon initial stimulation of immune cells via toll-like receptors. Cleavage to the active secreted form generally requires caspase-1 activation. Caspase is activated after inflammasome activation in response to stimulation of intracellular NOD-like receptors [10]. These cytokines, along with interferon-gamma (IFNγ– mainly a product of natural killer cells and natural killer lymphocytes rather than monocytes [11]) and IL-18, stimulate phagocytosis by neutrophils and macrophages and bacterial killing by toxic oxygen and nitrogen intermediates. Interleukin-6 has complex roles in enhancing or limiting the immune response [12]. Although TNFα and IL-1β are associated with initiation of preterm labor in women, IL-6 is not [12]. In fact, IL-6, usually regarded as a pro-inflammatory cytokine, may inhibit migration to inflammatory sites [12,13]. Interleukin-8 (CXCL8) is an important chemokine for recruitment of neutrophils to inflammatory sites [14,15] and IL-10 is the prototypical anti-inflammatory Th2-associated cytokine [16]. The importance, complexity of interaction and multiplicity of roles of these and other cytokines make their deviation from normal levels of expression potentially important in pathogenesis of disease.

Whereas neutrophils are the main leukocyte type involved in bacterial clearance during uterine infection [3,17,18], monocytes and macrophages have a more important role in immunomodulation [19]. After contact with bacteria, they are stimulated to produce and release pro-inflammatory cytokines and chemokines, including TNFα, IL-1β, IL-6, and IL-8, and later to release anti-inflammatory cytokines such as IL-10 [19,20]. Pro-inflammatory cytokines (TNFα, IL-1β and IL-6) and the chemokine IL-8 stimulate neutrophil and monocyte diapedesis and chemoattraction and promote increased phagocytosis and bacterial killing [19]. Monocytes are the circulating pool of macrophages; they differentiate into macrophages after leaving the blood circulation [21].

Several studies reported a decrease in neutrophil function that developed around the time of calving, including decreased chemotaxis, phagocytosis, and killing ability in high-producing dairy cows [22–24], particularly those that developed uterine disease [25–28]. This decrease was associated with a greater degree of negative energy balance (energy intake < energy expenditure) in cows that developed uterine disease compared with healthy cows [4,27]. In addition to impaired neutrophil function, decreased lymphocyte function was observed around calving, indicating that other leukocytes such as monocytes and macrophages might also be affected [22,29].

Therefore, we hypothesized that postpartum monocyte function would be compromised in cows that developed uterine disease. Because the state of negative energy balance is believed to contribute to decreased leukocyte function [23,30], we evaluated monocytes to avoid the confounding effect of the long periods of culture (5–7 d) necessary for differentiation of monocytes into macrophages [31]. The objective of this study was to evaluate monocyte gene expression and protein production of the major pro- and anti-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, and IL-10) in cows that developed postpartum metritis or endometritis.

2. Materials and methods

2.1. Experimental design

This was a prospective cohort study. Cows were followed from calving to 42 d postpartum. Blood samples were obtained at calving and then every week for 6 weeks for isolation of blood-derived monocytes. Expression and secretion of key proinflammatory cytokines elaborated by monocytes (TNFα, IL-1β and IL-6), the major neutrophil chemokines (IL-8), and the anti-inflammatory cytokine IL-10 were measured as