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Purinergic signaling gene network expression in bovine polymorphonuclear neutrophils during the peripartal period[1]

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ABSTRACT

An effective immune response relies on efficient activation of polymorphonuclear neutrophilic leukocytes (PMNL). The PMNL release cellular ATP in response to inflammatory mediators. Although extracellular ATP is rapidly degraded to adenosine, both compounds can readily bind to either the purinergic receptor P1 (adenosine) or P2 (ATP). The P1 and P2 receptors are members of the G-protein-coupled receptor family. The peripartal period is characterized by marked changes in metabolic and inflammatory status that are functionally related with immune responses in the cow. We evaluated the mRNA expression of genes associated with purinergic signaling in PMNL during the peripartal period. Seven multiparous Holstein cows were dried off at d −50 relative to expected parturition and fed a controlled-energy diet (net energy for lactation = 1.24 Mcal/kg of dry matter) for ad libitum intake during the entire dry period. After calving, all cows were fed a common lactation diet (net energy for lactation = 1.65 Mcal/kg of dry matter) until 30 d in milk. Blood PMNL collected at −10, 3, and 21 d in milk were used to study the expression of 22 genes associated with adhesion to endothelium, chemoattractant binding at the plasma membrane, and purinergic signaling. Other blood samples around calving were used to analyze concentrations of insulin, metabolites, and whole-blood phagocytosis. The expression of purinergic receptor P2Y, G-protein coupled, 2 (*P2RY2*) increased on d 3 and then decreased on d 21. This response suggested that ATP could play a role in the amplification of chemotactic signals. In contrast, the expression of genes encoding cell adhesion [selectin L (*SELL*) and selectin P ligand (*SELPLG*)], chemoattractant receptors [complement component 5a receptor 1 (*C5AR1*), IL-8 receptor α (*CXCR1*), IL-8 receptor β (*CXCR2*), and platelet-activating factor receptor (*PTAFR*)], and adenosine receptors [adenosine A1 receptor (*ADORA1*) and adenosine A3 receptor (*ADORA3*)] decreased between −10 and 3 d. The decrease coincided with a marked increase in blood nonesterified fatty acids and hydroxybutyrate concentrations, and a decrease in glucose and insulin concentrations. The increase in metabolites also was associated with greater expression of leukotriene B4 receptor (*LTB4R*) on d 3 and 21 compared with d −10, which is involved in inflammatory prostaglandin synthesis. Most chemoattractant receptors increased by 21 d, but cell adhesion genes and blood leukocyte phagocytosis was lower. The expression of adenosine A2a receptor (*ADORA2A*), which is associated with immunosuppression of PMNL and that of adenosine uptake channels [solute carrier family 29 (nucleoside transporters), member 1 (*SLC29A1*) and member 2 (*SLC29A2*)] and the nucleotidase adenosine deaminase (*ADA*) was greater at 3 and 21 d compared with −10 d. The reduction in key immune responses, such as cell adhesion and chemotaxis, by bovine PMNL could partly be a function of changes in mRNA expression of genes associated with purinergic signaling.
Key words: inflammation, transition cow, adenosine, adenosine triphosphate

INTRODUCTION

Polymorphonuclear neutrophilic leukocyte chemotaxis is an important feature of the immune response to an invading pathogen (Amulic et al., 2012). At the level of PMNL, this process is partly regulated by extracellular nucleotides (e.g., ATP) and adenosine, which serve as intercellular messengers during the immune response (Chen et al., 2006; Chen et al., 2010). The PMNL release cellular ATP via pannexin 1 (**PANX1**) hemichannels upon activation (Chen et al., 2010). Once outside the cell, ATP has a half-life measured in seconds as a result of the action of several nucleotidases and hydrolytic enzymes causing the degradation of ATP to

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ADP, AMP, and adenosine (Picher et al., 2004). The ATP released into the extracellular space, and adenosine, can exert paracrine and autocrine effects that can regulate PMNL function partly via activation of purinergic receptors (Chen et al., 2010).

The purinergic receptors are classified into **P1** (or **ADORA**) adenosine receptors and **P2** (or **P2RY**) nucleotide receptors. Although both types of receptors share the overall topological structure that is typical of G protein-coupled receptors, it is now evident from sequencing analysis that P1 and P2 belong to 2 different groups of G protein-coupled receptors (D' Ambrosi and Volonte, 2013). These receptors are further subdivided into **P2X** (**P2RX**) and **P2Y** (**P2RY**) receptors. For instance, unlike P2X that work as ATP-gated ion channels, the P2Y encompass ATP, uridine triphosphate, and their related molecules (Chen et al., 2010).

The purinergic receptor P2Y, G-protein coupled, 2 (**P2RY2**) and adenosine A3 (**ADORA3**) receptors are considered the major purine receptors capable of stimulating chemotaxis by PMNL (Chen et al., 2006). In contrast, the adenosine A2 receptors (**ADORA2**) are involved in the inhibition of PMNL migration, recruitment to the infected cells, and adhesion and infiltration to the endothelium near infection sites (Barletta et al., 2012). The existence of nucleotide-hydrolyzing enzymes [e.g., adenosine deaminase (**ADA**)] also is an important factor in the context of PMNL chemotaxis because such enzymes can modulate the concentration of ATP and adenosine, thereby affecting PMNL chemotaxis (Junger, 2011).

The PMNL account for up to 70% of leukocytes in humans (Junger, 2008) and a review of the literature concluded that in bovine, the PMNL account, on average, for only 25% of leukocytes (Paape et al., 2003). However, instances exist when the PMNL become a more predominant component of leukocytes [e.g., during an inflammatory challenge (adrenocorticotropin), the PMNL accounted for up to 60% of leukocytes (Paape et al., 1974)], and also around parturition, the number of PMNL increases before calving and decreases soon after calving (Kimura et al., 1999). A recent study reported that PMNL accounted for 54 to 69% of total leukocytes on d −30 prepartum and at calving (Trevisi et al., 2010). The pattern of change in PMNL around calving was proposed to represent a compensatory effect due to the inherently lower phagocytic capacity of PMNL during this physiological stage (Ingvartsen et al., 2003). However, recent data provided evidence that overall PMNL phagocytic performance after calving is not always lower than that prepartum (Sander et al., 2011).

Several studies reported that both the innate and adaptive immune systems in peripartal cows are often compromised; for example, cytokine production is impaired (Sordillo and Babiuk, 1991; Ishikawa et al., 1994), oxidative burst activity is reduced (Dosogne et al., 1999), and consequently phagocytic activity by leukocytes is often (Ingvartsen et al., 2003), but not always (Sander et al., 2011; Graugnard et al., 2012), reduced. Those functional changes are often accompanied by alterations in PMNL gene expression patterns (Preisler et al., 2000; Madsen et al., 2004). However, to our knowledge, no information exists regarding the purinergic signaling network in PMNL or whether the pattern of expression is altered around parturition. We hypothesized that purinergic signaling gene networks in PMNL are altered during the periparturient period. Our objective was to investigate not only the mRNA expression of genes in the pathway but also genes related to adhesion and migration of PMNL.

MATERIALS AND METHODS

Animals and Management

Complete details of these procedures were previously reported by Ji et al. (2012). Briefly, 7 multiparous Holstein cows were used. All cows were dried off at d −50 relative to expected parturition and fed a controlled-energy diet (NE_L = 1.24 Mcal/kg of DM) containing wheat straw at 36% of DM for ad libitum intake for the entire dry period. After calving, all cows were fed a common lactation diet (NE_L = 1.65 Mcal/kg of DM) until 30 DIM. Blood was sampled from the coccygeal vein or artery every Monday and Thursday before the morning feeding from −26 to 30 d around parturition. Samples were collected into evacuated serum tubes containing clot activator (BD Vacutainer; BD and Co., Franklin Lakes, NJ). Serum was obtained by centrifugation at 1,300 × *g* for 15 min at 4°C and frozen at −20°C until later analysis.

Whole-Blood Phagocytosis

The phagocytic capacity of leukocytes in heparinized whole blood was determined using the Phagotest kit (Orpegen Pharma, Heidelberg, Germany; Ballou, 2012). In brief, 20 μL of bacteria *Escherichia coli* was added to 1 of 3 whole-blood samples (100 μL) in test tubes (Falcon; Becton Dickinson, Franklin Lakes, NJ) and incubated for 10 min at 37°C. The cells were resuspended in 200 μL of DNA-staining solution, and light-protected in an ice bath until analyzed by flow cytometry (LSR II; Becton Dickinson, San Jose, CA).

Isolation of PMNL

Neutrophils were isolated based on procedures described by Moyes et al. (2010), with modifications.