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Pegbovigrastim treatment affects gene expression in neutrophils of pasture-fed, periparturient cows

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ABSTRACT

Treatment with granulocyte colony-stimulating factor has been reported to increase circulating neutrophil count and enhance neutrophil function in the periparturient cow. It was hypothesized that a commercially available recombinant bovine granulocyte colony-stimulating factor product (pegbovigrastim) affects gene expression profiles of neutrophils and supports neutrophil function in periparturient cows. Hence this study was undertaken to analyze expression of genes involved in neutrophil functions, including migration, interaction with pathogens, and cell survival. It also assessed the hypothesis that gene expression profiles in neutrophils are modulated by negative energy balance in the peripartum period. Holstein-Friesian, Jersey, and mixed-breed cows on pasture were blocked by expected calving date and body condition score and randomly assigned in a 2 × 2 factorial design. Cows were fed to exceed energy requirements prepartum (122%) or restricted to approximately 85% of prepartum energy requirements. At approximately 7 d before expected calving date, half the cows in each feed group were randomly assigned to be injected with pegbovigrastim or saline. Treatments were repeated within 24 h after calving. Blood samples were collected pretreatment approximately 7 d (d −7) before calving. Blood, uterine flush, and milk samples were collected at 4 (d 4) and 7 d in milk (d 7) to measure the expression of a panel of 20 genes representing cell adhesion, pattern recognition, inflammation and cytokine response, antimicrobial capacity, and apoptosis functions in neutrophils using NanoString technology (NanoString Technologies Inc., Seattle, WA) to quantify RNA copy numbers. No effects were observed of prepartum feeding group or a feeding group × treatment interaction for any of the investigated genes. An effect was observed of time on expression of several genes in blood neutrophils. After calving, expression of 2 of 4 cell adhesion-related genes, 3 of 4 pattern recognition receptors, 2 of 4 inflammatory genes, 2 antimicrobial genes, and 2 of 4 cell survival genes was significantly greater at d 4 or 7 or both compared with before calving (d −7). Expression of *ICAM1*, *TLR2*, and *PTGS2* was significantly higher in blood neutrophils from animals treated with pegbovigrastim compared with untreated controls, suggesting greater migration, pattern recognition, and inflammatory response ability. Pegbovigrastim also affected RNA expression in uterine cells with *ICAM1*, *NOD1*, *CLEC6A*, *PTGS2*, *MPO*, *DEFB5*, and *CATHL6* being expressed at higher levels and *SELL*, *ITGB8*, *IL8RB*, and *IL10* at lower levels. Milk somatic cells showed a similar pattern but with fewer significant changes. In contrast to the reported decline in neutrophil function in the transition period, neutrophil gene expression was increased for many of the genes studied, an apparent attempt to compensate for reduced neutrophil function. Treatment with pegbovigrastim further increased expression of several genes involved in these processes in blood neutrophils and changed uterine cells to a phenotype with increased antimicrobial capacity, typical for neutrophils that have migrated into their target tissue.

Key words: transition cow, neutrophil, gene expression, pegbovigrastim

INTRODUCTION

During the peripartum period, dairy cows are at an increased risk of disease (Ingvartsen et al., 2003). A decrease in circulating neutrophil number and function has been reported during this time, which contributes to increased susceptibility to uterine and mammary infections (Kehrli et al., 1989; Gilbert et al., 1993; Mallard et al., 1998). Decreased neutrophil function has been linked to the incidence and outcome of peripartum-related diseases, including mastitis, displaced abomasum, and retained placenta (Goff and Horst, 1997; Kimura

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et al., 2002; Burton et al., 2005). These studies indicate that neutrophil function is affected by peripartum nutrition and by metabolic adaptation to negative energy balance (Hammon et al., 2006; Graugnard et al., 2012; Zhou et al., 2015) and the need to counteract these effects.

The production of neutrophils is regulated by granulocyte colony-stimulating factor (**G-CSF**; Semerad et al., 2002; Martin et al., 2003; Bendall and Bradstock, 2014). A long acting analog of bovine G-CSF (pegbovigrastim injection, Imrestor, Elanco Animal Health, Greenfield, IN) is commercially available. It has been reported that pegbovigrastim injection increased neutrophil count in peripheral circulation and increased neutrophil function in dairy cattle (Kimura et al., 2014; Hassfurther et al., 2015; Canning et al., 2017; McDougall et al., 2017).

A previous report of the current experiment confirmed that pegbovigrastim treatment resulted in significant increases in neutrophil count and enhanced neutrophil function as indicated by increased myeloperoxidase release (McDougall et al., 2017). The objective of the present study was to gain further insight into the effects of pegbovigrastim on neutrophils by analyzing gene expression profiles in neutrophils from blood, uterus, and milk from periparturient dairy cows. The study also aimed to assess the hypothesis that gene expression profiles in neutrophils are modulated by negative energy balance in the peripartum period. Therefore, genes were selected to represent key neutrophil functions, such as their ability to be recruited to sites of infection (cell adhesion), to recognize (pattern recognition) and phagocytose microbes, to alert other cells of the immune system (inflammation and cytokine response), to kill pathogens (antimicrobial capacity), and eventually to undergo programmed cell death (apoptosis).

MATERIALS AND METHODS

Animals and Treatment

All animal manipulations reported in this study were approved by the animal ethics committee of AgResearch, Ruakura, New Zealand. Multiparous cows (n = 99; 48 Holstein Friesians, 13 Jerseys, and 38 Holstein Friesian × Jersey) on pasture housed at a research dairy facility (Massey University, Palmerston North, New Zealand) were enrolled in the study. Cows were blocked by week of expected calving, ranked on BCS, and randomly assigned to treatment within block in a 2 × 2 factorial design, regardless of their breed.

As reported previously (McDougall et al., 2017), of the 99 cows that were originally enrolled, 17 were removed from the study for various reasons. For the 82 cows remaining in the study, no difference was present in the calving date or parity between the feeding or treatment groups. With 41 (50%) Friesian, 33 (40.2%) crossbred, and 8 (9.8%) Jersey cows no difference was present in the breed distribution between treatment groups. The actual day of enrolment (i.e., d −28) and the day of first treatment (i.e., d −7) did not differ by feed or treatment group. The timing of scheduled sampling was actually −10.9 ± 5.5, 0.0 ± 0.0, 3.7 ± 0.6, and 6.8 ± 0.7 d relative to calving for visits scheduled for d −7, 0, 4, and 7 relative to calving, respectively. The actual day of sampling did not vary between treatment ($P = 0.72$) or feed groups ($P = 0.64$).

On 3 occasions, at weekly intervals, cows that were 4 to 5 wk before anticipated calving were assessed by a veterinarian. Cows were assigned to either a full ration (**FR**) or restricted ration (**RR**) at 31.4 (SD = 5.5) and 30.9 (SD = 4.9) d before calving, respectively (details of the feeding treatment were reported previously; McDougall et al., 2017). The FR cows were offered a ration to provide approximately 120 MJ of ME per cow per day, or 122% of prepartum energy requirements. The RR group was offered a ration providing approximately 82 MJ of ME per cow per day, or 85% of prepartum energy requirements. Postpartum, the 2 precalving feed groups were together on the same pastures and were offered the same ration.

At approximately 7 d before anticipated calving, cows were injected subcutaneously with 15 mg in 2.7-mL injection volume of the pegylated form of recombinant bovine G-CSF (pegbovigrastim injection, Imrestor, Elanco Animal Health) or 3 mL of 0.9% NaCl (control). On d 0 (the day of calving), cows were milked for the first time between approximately 1500 and 1700 h and a second injection of the same treatment was performed.

Blood, Milk, and Uterine Samples

Blood samples (7 mL) were drawn from the tail vessels 7 (±3) d before calving (d −7) and 4 (±1) d (d 4) and 7 (±1) d (d 7) after calving into evacuated glass containers containing EDTA (Becton Dickinson and Company, Franklin Lakes, NJ). Blood samples were drawn in the morning before supplements were fed and before Imrestor or saline treatment (d −7) held and transported at ambient temperature, and processed at the laboratory within 2 h of collection. At 4 (±1) and 7 (±1) d postpartum, composite milk samples (~100 mL) were collected during morning milking. On the same days in the following morning milking, uterine fluid (~20 mL) was collected. The vulva of the cow was wiped with a paper towel if gross contamination with fecal material was present. A 75-cm flexible catheter