J. Dairy Sci. 99:1–14
http://dx.doi.org/10.3168/jds.2015-10877

Parturition in dairy cows temporarily alters the expression of genes in circulating neutrophils

M. A. Crookenden,*†[1] A. Heiser,†‡ A. Murray,† V. S. R. Dukkipati,† J. K. Kay,§ J. J. Loor,# S. Meier,§ M. D. Mitchell,‖ K. M. Moyes,¶ C. G. Walker,* and J. R. Roche§
*DairyNZ, c/o University of Auckland, Auckland 1010, New Zealand
†Institute of Vet, Animal and Biomedical Sciences, Massey University, Palmerston North 4442, New Zealand
‡AgResearch, Hopkirk Research Institute, Palmerston North 4442, New Zealand
§DairyNZ, Newstead, Hamilton 3284, New Zealand
#Department of Animal Sciences, University of Illinois, Urbana 61801
‖University of Queensland, Centre for Clinical Research, Herston, Queensland 4029, Australia
¶Department of Animal and Avian Sciences, University of Maryland, College Park 20742

ABSTRACT

Extensive metabolic and physiologic changes occur during the peripartum, concurrent with a high incidence of infectious disease. Immune dysfunction is a likely contributor to the increased risk of disease at this time. Studies using high-yielding, total mixed ration-fed cows have indicated that neutrophil function is perturbed over the transition period; however, this reported dysfunction has yet to be investigated in moderate-yielding, grazing dairy cows. Therefore, we investigated changes in the expression of genes involved in neutrophil function. Blood was collected from cows at 5 time points over the transition period: precalving (−1 wk; n = 46), day of calving (d 0; n = 46), and postcalving at wk 1 (n = 46), wk 2 (n = 45), and wk 4 (n = 43). Neutrophils were isolated by differential centrifugation and gene expression was investigated. Quantitative reverse transcriptase PCR with custom-designed primer pairs and Roche Universal Probe Library (Roche, Basel, Switzerland) chemistry, combined with microfluidics integrated fluidic circuit chips (96.96 Dynamic Array, San Francisco, CA) were used to investigate the expression of 78 genes involved in neutrophil function and 18 endogenous control genes. Statistical significance between time points was determined using a repeated measures ANOVA. Genes that were differentially expressed over the transition period included those involved in neutrophil adhesion (*SELL*, *ITGB2*, and *ITGBX*), mediation of the immune response (*TLR4*, *HLA-DRA*, and *CXCR2*), maturation, cell cycle progression, apoptosis (*MCL1*, *BCL2*, *FASLG*, and *RIPK1*), and control of gene expression (*PPARG*, *PPARD*, and *STAT3*). We noted reduced gene expression of proinflammatory cytokines (*IFNG*, *TNF*, *IL12*, and *CCL2*) on the day of calving, whereas anti-inflammatory cytokine gene expression (*IL10*) was upregulated. Increased gene expression of antimicrobial peptides (*BNBD4*, *DEFB10*, and *DEFB1*) occurred on the day of calving. Collectively, transcription profiles are indicative of functional changes in neutrophils of grazing dairy cows over the transition period and align with studies in cows of conventional total mixed ration systems. This altered function may predispose cows to disease over the transition period and is likely to be a natural change in function due to parturition.
Key words: PMN, Fluidigm, peripartum period, innate immune function

INTRODUCTION

The transition period in a dairy cow is a critical period of metabolic and physiologic change encompassing parturition and the onset of lactogenesis (Grummer, 1995). A considerable alteration in nutrient and metabolic demand is associated with an increased susceptibility to metabolic and infectious disease conditions during the peripartum period (Overton and Waldron, 2004; Ingvartsen, 2006). For example, the incidence of intramammary infections is increased in the 4 wk immediately postcalving both in TMR- (Østergaard et al., 2005) and pasture-based dairy systems (Compton et al., 2007). An estimated 30 to 50% of cows are reported to experience health disorders during the transition period, and the majority (75%) of infectious disease and metabolic disorders in dairy cows occur during this time (Ingvartsen, 2006; LeBlanc et al., 2006). In pasture-based systems an estimated NZD$300 per cow is lost annually due to costs associated with transition

Received December 9, 2015.
Accepted April 2, 2016.
[1]Corresponding author: mallory.crookenden@dairynz.co.nz

failure (i.e., cows that experience health disorders; Compton et al., 2007; Roche, 2014).

Previous studies have indicated that the high incidence of disease during the transition period is linked to a state of reduced immune function (Detilleux et al., 1995; Goff and Horst, 1997; Heiser et al., 2015). In these investigations, neutrophils have received much attention due to their essential role in defense against pathogens responsible for mastitis and uterine infections (Madsen et al., 2004; Hammon and Goff, 2006; Graugnard et al., 2012). Functional studies at the cellular level have indicated neutrophil dysfunction during the peripartum period; for example, studies have shown reduced superoxide production, phagocytosis, expression of cell surface adhesion molecules, and capacity for migration (Kehrli et al., 1989; Burton et al., 1995, 2005). Consequently, there is reduced efficiency of the neutrophil inflammatory response, thereby contributing to greater incidence of infection. The extent of this peripartum neutrophil dysfunction has not been fully elucidated.

Furthermore, the studies referred to were all undertaken using high-yielding, housed cows. However, milk production is 30 to 50% less in pasture-based systems (Kolver and Muller, 1998), the population density on farm is lower, different management systems may alter pathogen load, and the infectious disease-causing pathogens may differ. Therefore, we hypothesized that the changes in peripartum neutrophil function will be different in grazing dairy cows in the absence of stimulation. To investigate this, neutrophils were extracted over the transition period from moderate-yielding, grazing dairy cows and the expression of 78 genes involved in neutrophil function were investigated.

MATERIALS AND METHODS

Animal Handling

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry of Primary Industries, 1999). The experiment was undertaken at Scott Farm, Hamilton, New Zealand (37°46′S, 175°18′E), between July and October 2013.

Experimental Design and Treatments

A subset of cows of mixed age and breed (Holstein-Friesian, Jersey, and Holstein-Friesian × Jersey) were selected from a parent experiment, described in detail by Roche et al. (2015). Cows were 1 of 2 precalving BCS categories (4.0 or 5.0; based on a 10-point scale; Roche et al., 2004) and 1 of 2 levels of energy intake preceding calving (75 or 125% of estimated requirements). Blood was sampled by coccygeal venipuncture at 5 time points over the transition period: 1 wk before estimated calving date (−1 wk; n = 46), day of calving (d 0; n = 46), d 7 (1 wk; n = 46), d 14 (2 wk; n = 45), and d 28 (4 wk; n = 43) postcalving. Blood for neutrophil isolation (total of 36 mL per cow at each time point) was collected in 6 evacuated blood tubes (Vacutainer; BD Bioscience, Plymouth, UK) containing acid citrate dextrose (solution b; 22.0 g/L of trisodium citrate, 8.0 g/L of citric acid, 24.5 g/L of dextrose). Blood tubes were inverted 8 times and placed immediately on ice awaiting neutrophil isolation by differential centrifugation based on protocols (Osorio et al., 2013; Moyes et al., 2014), with modifications described herein. Blood for metabolite analysis was collected in evacuated blood tubes containing a lithium heparin anticoagulant, inverted, and placed immediately on ice; within 30 min, blood tubes were centrifuged at 1,500 × *g* for 12 min at 4°C, plasma aspirated, and stored at −20°C until assayed.

Liver Sampling for Triacylglycerol Analysis

Liver samples were collected from all cows by biopsy the week before calving (wk −1), and subsequently on wk 1, 2, and 4 postcalving. Briefly, the skin was shaved and disinfected and the area anesthetized with 7 mL of 2% lignocaine (Lopaine 2%, lignocaine hydrochloride 20 mg/mL, Ethical Agents, South Auckland, New Zealand). An incision was made through the skin in the right 11th intercostal space at the level of the greater trochanter, through which a 12-gauge × 20-cm biopsy needle was inserted into the liver and approximately 1 g (wet weight) of liver tissue was collected. Liver samples were snap-frozen in liquid nitrogen and stored at −80°C.

Neutrophil Isolation

Neutrophils were isolated according to procedures previously described (Moyes et al., 2010a; Osorio et al., 2013) with modifications. Blood collected in the 6 acid citrate dextrose evacuated blood tubes were inverted and pooled by transfer into a 50-mL conical tube. Tubes were centrifuged for 30 min at 850 × *g* at 4°C. The plasma, buffy coat, and up to one-third of the red blood cells were aspirated by pasture pipette and discarded, leaving two-thirds of red blood cells and neutrophils remaining. Water (milliQ, Millipore, Billerica, MA) was added (25 mL) and mixed for 10 to 30 s to lyse the red blood cells, and 5 mL of 5× concentrated PBS (pH 7.4, Life Technologies, Carlsbad, CA) was added to each tube to restore isotonicity. Tubes were spun at 330 × *g*