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Longitudinal characterization of bovine monocyte-derived dendritic cells from mid-gestation into subsequent lactation reveals nadir in phenotypic maturation and macrophage-like cytokine profile in late gestation

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ABSTRACT

Changes in monocyte and dendritic cell populations during bovine pregnancy and lactation remain poorly described despite the key roles these cells play in immune tolerance and activation. Using a prospective longitudinal study, we characterized CD14+ monocyte-derived dendritic cell (moDC) differentiation and maturation and captured monocyte composition dynamics from mid-gestation through calving and into the subsequent lactation in dairy cows (n = 7). First, we measured absolute counts of classical (CD14 + CD16−, cM), intermediate (CD14 + CD16+, intM), and nonclassical (CD14-CD16+, ncM) monocytes in the blood and determined proportions of individual subsets within the total monocyte population. We found the proportion of cM decreased and intM increased significantly by early lactation, whereas there was a nadir in the proportion of ncM in late gestation, two weeks prepartum. Monocyte composition appears to be regulated in pregnancy, possibly to limit the proportion of highly inflammatory monocytes i.e. intM. Ultimately, we found that moDC differentiated from CD14+ monocytes isolated in the early dry period of late gestation had impaired *E. coli*-induced maturation, with nadirs in upregulation of CD80 and MHC II, and downregulation of CD14. The moDC from late gestation also had altered cytokine profiles with greatest production of pro-inflammatory IL-1β and anti-inflammatory IL-10. These data suggest monocytes in late gestation, in contrast to other stages of pregnancy and lactation, differentiate and maturate into moDC less capable of eliciting strong T cell activation, and have macrophage-like cytokine profiles. These results provide insight into maternal immune modulation and elucidate potential immune changes necessary to facilitate bovine pregnancy.

1. Introduction

During pregnancy the maternal immune system is presented with the challenge of needing to concurrently tolerate the growth of a semi-allogenic fetus and respond to invading pathogens. Highly inflammatory, T helper 1 (Th1) and T helper 17 (Th17)-type responses in pregnancy have been shown to be detrimental to the health of the fetus, thus, the maternal immune system must be carefully regulated (Rosbottom et al., 2008; Betz, 2012; Krishnan et al., 2013). Maternal immune regulation is dynamic; dairy cattle show varying responses to infectious agents depending upon their stage of pregnancy and lactation (Innes, 2007; Rosbottom et al., 2008; Quesnell et al., 2012; Sipka et al., 2013). Previous work found cows in the third trimester of pregnancy, particularly during the non-lactating period of late gestation (known as the 'dry period'), had a heightened susceptibility to persistent infections caused by mastitis- and abortion-causing pathogens including *E. coli* and *N. caninum* with hindered pro-inflammatory, Th1-type responses relative to the postpartum period and earlier stages

Abbreviations: cM, classical monocyte; DC, dendritic cell; DIM, days in milk; ED, early dry period; EL, early lactation; intM, intermediate monocyte; LD, late dry period; Mφ, macrophage; MG, mid-gestation; moDC, monocyte-derived dendritic cell; MOI, multiplicity of infection; ncM, nonclassical monocyte; PBMC, peripheral blood mononuclear cell; PC, post-calving; Th1, T helper 1; Th17, T helper 17.

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of gestation (Williams et al., 2000; Green et al., 2002; Anderson, 2007; Rosbottom et al., 2008; Quesnell et al., 2012). Monocytes and monocyte derived cells play a major role in maternal immune regulation throughout pregnancy across multiple species; these cells are involved at all stages of pregnancy including implantation, maintenance of pregnancy, and parturition and show dynamic regulation by stage of gestation (Oliveira et al., 2012; Gomez-Lopez et al., 2014; Leno-Duran et al., 2014; Schumacher et al., 2014). Though previous work on dairy cattle indicated that certain immune cell populations have altered function in pregnancy, this area of research remains largely underdeveloped (Lamote et al., 2004, 2006; Maeda et al., 2013; Pomeroy et al., 2015). We lack an understanding of underlying mechanisms in immune tolerance, gestational stage-dependent changes in maternal immune function, and how this relates to overall health of the dam and fetus.

Bovine monocytes have only recently been classified into three major subsets, classical (cM), intermediate (intM), and nonclassical (ncM), based on CD14 and CD16 expression analogous to their human counterparts (Hussen et al., 2013, 2014; Corripio-Miyar et al., 2015; Hussen et al., 2016). Unlike CD14− ncM, both CD14+ subsets, cM and intM, have been shown to be highly responsive to LPS stimulation (Hussen et al., 2013, 2014). Changes in monocyte subset composition have been shown to influence disease susceptibility and pregnancy outcome in species such as mouse and human, but these changes have not been characterized in cattle (Al-Ofi et al., 2012; Melgert et al., 2012; Devevre et al., 2015; Tang et al., 2015). These monocyte populations circulating in the periphery migrate into tissues either during inflammation or steady-state conditions where they may differentiate into macrophages (Mφ) or dendritic cells (DC).

Across multiple species, DC are shown to be regulated in pregnancy and play a crucial role in generating maternal immune tolerance toward fetal antigens (Bachy et al., 2008; Della Bella et al., 2011; Negishi et al., 2012; Leno-Duran et al., 2014; Pomeroy et al., 2015). Recently we found key differences in monocyte derived DC (moDC) phenotype and cytokine production in a cross-sectional study comparing moDC from late gestation cows to moDC from non-pregnant cows in early lactation. We found moDC had limited Th1-type responses and were likely to have a poor ability to activate T cells during late gestation (Pomeroy et al., 2015).

Here we aim to expand upon our previous work and capture the dynamics of monocyte subsets, and the function and phenotype of respective moDC populations through a prospective longitudinal study in cows from mid-gestation through calving into the subsequent lactation. The objective of this study was to analyze differentiation and maturation with UV-killed *E. coli*-stimulation of moDC, derived from blood CD14+ across pregnancy and into early lactation. We investigated phenotype and function analyzing surface marker expression (CD14, CD40, CD80, MHC II) and cytokine production (IL-1β, TNFα, IL-10) following *E. coli* stimulation. Furthermore we measured monocyte subset composition (cM, intM, ncM) in blood at the designated time points to describe the composition of monocyte subsets in blood as a baseline and further understand the implications of starting monocyte population on moDC differentiation and maturation.

2. Materials and methods

2.1. Animals

Seven Holstein–Friesian cows, in the second trimester of pregnancy between 90 and 110 days carrying calf ('mid-gestation', MG, 90-110dcc/P-190-170d) as determined from insemination date were selected from the Cornell University Veterinary College Teaching Dairy Barn. No disease (i.e. metabolic, reproductive, mastitis) was recorded within 30 days prior to the time of first sampling for all enrolled animals. Peripheral blood was collected by jugular venipuncture from enrolled animals into 250 ml vacuum bottles containing EDTA and 10 ml vacutainer glass serum tubes with no additive (Becton Dickinson, Franklin Lakes, NJ). Each enrolled animal was sampled repeatedly throughout the remainder of pregnancy and into the subsequent lactation as depicted in Fig. 1. Blood was collected at MG, early dry period within the first week since dry-off ~60–53 days prior to expected calving date ('early dry', ED, 1–7d dry/P-60-53d), late dry period ~2 weeks prior to expected calving date ('late dry', LD, 43d dry/P-14d), 4–6 days following parturition ('post-calving', PC, P + 4-6d), and the final collection ~45–55 days following parturition before breeding ('early lactation', EL, P + 45-55d) (Fig. 1). Disease was monitored daily and recorded in DairyComp® by farm management; metritis cases were diagnosed within the first two weeks postpartum by abnormal vaginal discharge and foul odor of the discharge. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee (project number: 2007-0110).

2.2. Generation of peripheral blood CD14+ moDC

Monocyte derived DC were generated from CD14+ monocytes as described previously by Pomeroy et al. (2015); CD14+ positive selection was preformed based on the use of UV irradiated *E. coli* ECC-Z stimulation to induce moDC maturation in conjunction with previous work from Hussen et al. (2013, 2014) indicating CD14+ cM and intM are the monocyte subsets responsive to LPS stimulation. In brief, peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation. The PBMC were incubated with anti-human CD14 antibodies conjugated with paramagnetic microbeads (Miltenyi Biotech, Inc., Auburn, CA) in MACS buffer (PBS, pH 7.2, 0.5% bovine serum albumin, 2 mM EDTA), transferred to MACS LS magnetic separation column, and after repeatedly washing the column with MACS buffer the magnetically labeled cells were collected (Miltenyi Biotech, Inc.). Purity was determined by flow cytometry and was shown to be >90%. Monocytes were re-suspended in differentiation media (phenol-free complete RPMI with l-glutamine, supplemented with 10% autologous serum, 20 ng/ml recombinant bovine IL-4 and, 20 ng/ml recombinant bovine GM-CSF both from Kingfisher Biotech), plated in 6-well tissue culture plates at 1.5×10^6 cells in 3 ml differentiation media per well, and cultured for 5 days (37 °C, 5% CO_2) in presence of recombinant cytokines to generate immature moDC; at 2–3 d cultures were given 1 ml/well of fresh differentiation media.

2.3. Inactivation of E. coli ECC-Z

E. coli ECC-Z was inactivated as described by Pomeroy et al. (2015). In brief, frozen stock of the previously characterized *E. coli* ECC-Z strain known to cause mild persistent clinical mastitis (Dogan et al., 2006; Lippolis et al., 2014) was grown to log phase in LB liquid. Bacteria were pelleted and diluted in PBS. Bacterial suspension was plated on agar plates prior to inactivation to determine concentration of stock solution. The bacterial suspension was inactivated using UV irradiation in the laboratory of Dr. Randy W. Worobo (Department of Food Science and Technology, Cornell University). The UV irradiated *E. coli* suspension failed to produce colonies on LB agar plates. Aliquots of UV irradiated *E. coli* were resuspended in sterile 10% glycerol/PBS and stored at −80 °C.

2.4. Maturation of moDC with UV irradiated E. coli

Maturation of moDC was induced by bacterial stimulation through the addition of UV irradiated *E. coli* to immature moDC cultures in an MOI (multiplicity of infection) of 10 for 24 h based