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Association between polyunsaturated fatty acid-derived oxylipid biosynthesis and leukocyte inflammatory marker expression in periparturient dairy cows

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ABSTRACT

Peripheral blood mononuclear leukocytes from periparturient cows can have exacerbated inflammatory responses that contribute to disease incidence and severity. Oxylipids derived from the oxygenation of polyunsaturated fatty acids (PUFA) can regulate the magnitude and duration of inflammation. Although PUFA substrate for oxylipid biosynthesis in leukocytes is known to change across the periparturient period, the plasma oxylipid profile and how this profile relates to leukocyte inflammatory phenotype is not clear. The objective of this study was to determine if a relationship exists between the profile of pro- and antiinflammatory plasma oxylipids and the inflammatory phenotype of peripheral blood leukocytes during the periparturient period. Seven multiparous Holsteins were sampled from the prepartum period through peak lactation. Plasma oxylipids were measured by liquid chromatography-mass spectrometry, peripheral leukocyte mRNA expression was measured by quantitative PCR, and PUFA content of peripheral blood mononuclear cells was measured by gas chromatography-mass spectrometry. Concentrations of several hydroxyl products of linoleic and arachidonic acid changed over time. Linoleic acid and arachidonic acid concentrations in leukocytes increased during early lactation, suggesting that substrate availability for hydroxyoctadecadienoic and hydroxyeicosatetraenoic acid biosynthesis may influence the oxylipid profile. Leukocyte mRNA expressions of *IL-12B*, *IL-1B*, inducible nitric oxide synthase 2, and cyclooxygenase 2 were correlated with several plasma oxylipids. These are the first observations linking leukocyte inflammatory gene responses to shifts in oxylipid biosynthesis in periparturient dairy cows.

Key words: inflammation, periparturient, eicosanoid, oxylipid, lipid mediator

INTRODUCTION

A major factor contributing to decreased productivity and longevity of dairy cows is the incidence of disease in the first month after parturition (Pinedo et al., 2010). Dysfunctional inflammatory responses during the periparturient period can contribute to disease incidence and severity (Sordillo and Raphael, 2013). For example, bovine peripheral blood mononuclear cells (**PBMC**) have an exacerbated inflammatory response to LPS in the periparturient period, compared with cells from mid-lactation cows (Sordillo et al., 1995). Uncontrolled inflammatory responses also were associated with increased severity of clinical mastitis (Shuster et al., 1996). Control and prevention of periparturient disease is currently difficult, partly because it is unclear why dysfunctional inflammatory responses occur around parturition.

Oxylipids are defined as the class of PUFA-derived metabolites that regulate all aspects of the inflammatory response. The biosynthesis of oxylipids involves several complex pathways that start with the peroxidation of cell membrane-derived PUFA, including linoleic (C18:2n-6), arachidonic (C20:4n-6), eicosapentaenoic (C20:5n-3), and docosahexaenoic acids (C22:6n-3). Initial peroxidation of these PUFA can occur enzymatically through the cyclooxygenases (**COX**), lipoxygenases (**LOX**), and epoxygenases, but are also mediated by free oxygen radicals. Oxylipids produced by PUFA peroxidation include 9- and 13-hydroperoxyoctadecadienoic acid (**HPODE**), prostaglandin (**PG**) H_2, 5- and 15-hydroperoxyeicosatetraenoic acids, and 14- and 17-hydroperoxydocosahexaenoic acids (Serhan and Petasis, 2011; Smith et al., 2011). These are unstable lipid mediators that are quickly reduced to more stable hydroxyls, such as hydroxyoctadecadienoic acid (**HODE**) and hydroxyeicosatetraenoic acid (**HETE**). The hydroxyl oxylipids can serve as substrates to a vast number of downstream metabolites, such as oxooctadecadienoic acids (**OxoODE**; Ramsden et al., 2012), leukotrienes (**LT**; Nakamura and Shimizu, 2011), thromboxanes (**TX**), PGD_2, PGE_2, PGF_2, PGI_2 (Smith et al., 2011), 7-maresin 1 (**MaR1**), and protectins (**PD**; Serhan and Petasis, 2011). Thus, the rate

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of PUFA oxidation and the extent of metabolism into downstream products determine the relative abundance of oxylipids with different functional capabilities.

A major determinant of whether oxylipids initiate or resolve inflammation is the PUFA from which they are derived. Both C20:5n-3- and C22:6n-3-derived oxylipids predominantly abrogate inflammation. For example, MaR1 is produced from C22:6n-3 and reduces neutrophil recruitment during peritoneal inflammation (Serhan et al., 2009). In contrast to the n-3 PUFA-derived oxylipids, the downstream products of n-6 PUFA peroxides have multiple effects in inflammatory pathways. Different PG, for example, can stimulate (e.g., PGF_2) or abrogate (e.g., PGD_2 and PGE_2) inflammatory events (Serhan et al., 2008). The effects of some n-6-derived oxylipids are dependent on species and cell types. For example, HODE activates adhesion molecule expression on endothelial cells (Friedrichs et al., 1999), thus facilitating leukocyte extravasation during inflammation. However, HODE also binds to nuclear receptors, and abrogates tumor necrosis factor α (**TNF-α**)-induced inflammatory responses in human epithelial tissue (Altmann et al., 2007). Several studies showed that dietary changes in PUFA substrate supply could effectively change oxylipid profiles. (Raphael and Sordillo, 2013). For example, n-3 PUFA dietary supplementation in humans and rodents was shown to increase tissue phospholipid content of these PUFA and increase n-3 PUFA-derived oxylipids in tissue (Poulsen et al., 2008; Ramsden et al., 2012). Recent studies in periparturient dairy cows identified C18:2n-6 as more abundant in several tissues after parturition, including PBMC (Contreras et al., 2010; Akbar et al., 2013), but the influence of C18:2n-6 on oxylipid production and leukocyte inflammatory pathways in cows is not known.

Concentrations and relative potency of individual oxylipids (Nagy et al., 1998; Norling et al., 2012) determine the net effect of the tissue oxylipid profile on inflammatory processes. The abundance of proinflammatory oxylipids relative to proresolving oxylipids affects the chronicity and resolution of disease by initiating or resolving segments of the inflammatory pathway (Serhan and Petasis, 2011). Describing the plasma concentrations of oxylipids in periparturient cows, in particular those oxylipids that may affect circulating immune cells and vascular endothelium, may assist in explaining why periparturient PBMC may have enhanced inflammatory responses following exposure to bacterial agonists (Sordillo et al., 1995). The hypothesis of the current study was that a relationship exists between the profile of pro- and antiinflammatory plasma oxylipids and the inflammatory phenotype of peripheral blood leukocytes during the periparturient period.

MATERIALS AND METHODS

Animals

This study was conducted in the autumn months of 2012 in a 1,500-cow, intensively housed commercial dairy herd located in Michigan. The herd was fed TMR that contained corn silage and alfalfa haylage as predominant forages. Seven Holstein cows were randomly selected from all healthy cows within 8 to 12 d of expected parturition at study commencement. The median parity for the observed parturition was 3, and ranged from 2 to 4. Blood (50 mL) was aseptically collected in EDTA Vacutainers (Becton, Dickinson and Co., Franklin Lakes, NJ) by coccygeal venipuncture, and immediately stored on ice. The distribution of sampling time relative to parturition was 14 d prepartum (range = 6 to 17 d), immediately after parturition (range = 1 to 3 d postpartum), 10 d postpartum (range = 7 to 10 d), 28 d postpartum (range = 21 to 32 d), and 84 d postpartum (range = 77 to 88 d). Plasma NEFA were measured in 3 randomly selected cows by an enzymatic colorimetric method [Wako NEFA HR(2); Wako Chemicals USA, Richmond, VA] conducted at the Diagnostic Center for Population and Animal Health (Lansing, MI). The Michigan State University Animal Care and Use Committee preapproved all animal use and care.

Plasma Oxylipid Quantification

Oxylipid Extraction. Plasma (500 μL) was separated from whole blood before PBMC isolation (centrifugation at 931 × *g* for 30 min at 4°C). Formic acid (1.7 μL) and antioxidant/reducing agents were added [4 μL of combined 2.4 mg of EDTA in 3 mL of water, 2.4 mg of butylated hydroxytoluene (**BHT**) in 1.5 mL of ethanol, 24 mg of triphenylphosphine in 6 mL of methanol (Sigma-Aldrich, St. Louis, MO), and 24 mg of indomethacin in 1.5 mL of ethanol (Cayman Chemical Co., Ann Arbor, MI)]. Deuterated internal standards [LTB_4-d_4, TXB_2-d_4, $PGF_{2\alpha}$-d_4, PGE_2-d_4, PGD_2-d_4, 13(S)-HODE-d_4, 6-keto $PGF_{1\alpha}$-d_4, 12(S)-HETE-d_8, and 15(S)-HETE-d_8; Cayman Chemical Co.] were combined in ethanol:water (1:1), to achieve 0.1 ng/μL for each standard, and 200 μL of this mixture (20 ng of each standard) was added to samples. Methanol was then added to samples (1 mL; −20°C), and protein precipitated by brief vortexing and freezing (−20°C for 3 h). Samples were then centrifuged at 18,000 × *g* for 15 min at 4°C. The supernatant was diluted in 9.2 mL of water containing 0.1% formic acid (vol/vol). Oxylipids were extracted by solid-phase methods as previously reported (Farney et al., 2013), with the following modifications. Extraction columns

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