Lipid Mediators and Inflammatory Disorders of Dairy Cows

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Introduction

Dairy cattle are susceptible to increased incidence and severity of health disorders during the transition period. A common link contributing to the development of both metabolic and infectious diseases in transition cows is a dysfunctional inflammatory response (Sordillo and Mavangira, 2014). The purpose of the inflammatory response is to eliminate the source of infection or tissue injury and then return tissues to normal function. Aggressive or uncontrolled inflammatory responses, however, can cause damage to host tissues and contribute significantly to the pathophysiology of economically important diseases such as mastitis. A precarious balance between pro-inflammatory and pro-resolving mechanisms is needed to ensure optimal pathogen clearance and the prompt return to immune homeostasis. Therefore, inflammatory responses must be tightly regulated to avoid bystander damage to host tissues. Oxylipids are potent lipid mediators that can regulate all aspects of the inflammatory response. The biosynthetic profiles of oxylipids are dependent on both the availability of diverse polyunsaturated fatty acids substrates and their subsequent metabolism through various oxidizing pathways. Changes in lipid metabolism in dairy cows around parturition can profoundly change the composition and concentration of oxylipids in plasma and milk that may be responsible for dysfunctional inflammatory responses during this time. This paper will provide a brief overview of the bovine inflammatory response and the role that oxylipids play in contributing to the onset and resolution of inflammation especially as it pertains to mastitis. Factors associated with transition cows that can contribute to dysfunctional regulation of inflammation as a function of altered oxylipid biosynthesis also will be described. Understanding the role oxylipids may play in mediating the onset and resolution of inflammation is key to developing novel prevention and control programs for the dairy industry.

Inflammatory Response

Inflammation is a critical component of the innate defense system that involves complex biological responses following local tissue injury, trauma, or exposure to infectious pathogens. For example, initiation of the inflammatory response during mastitis results when resident cell populations located within tissues are able to sense the presence of bacteria through pattern recognition receptors (PRR) that can be located on the cell surface, secreted, or intracellular expressed. These PRR function by recognizing the diverse array of conserved motifs associated with different groups of microbes that are referred to as pathogen associated molecular patterns (PAMP) (Kumar et al., 2011). The Toll-like receptor (TLR) family of PRR where some of

the first to be discovered and are among the best characterized to date. There are 13 different TLR identified in mammals of which 10 are known to exist in cattle (Menzies and Ingham, 2006, Kumar et al., 2011). Both TLR2 and TLR4 are abundant PRR during intramammary infections as they are primarily activated in response to PAMPs associated with gram-positive (lipopeptides) and gram-negative (LPS) mastitis-causing pathogens (Porcherie et al., 2012). The initial PRR-PAMP interaction can trigger the release of potent pro-inflammatory mediators. Cytokines (TNF-□, IL1, and IL8) and oxylipids (prostaglandins, leukotrienes, and thromboxanes) are the principal soluble mediators produced during the initial stages of the inflammatory response. Depending on the type of invading pathogen, the number of soluble mediators produced and their timing of expression can vary considerably. For example, previous studies also showed that TXB2 and PGE2, known proinflammatory oxylipids, may have important roles in the enhanced severity of *E. coli* mastitis that occurs during the periparturient period (Vangroenweghe et al., 2005). Moreover, increased biosynthesis of PGE2 was related to bacterial growth and systemic disease severity during *E. coli* mastitis (Pezeshki et al., 2011). Both cytokines and oxylipids have the capacity to interact directly with blood vessels in the mammary gland to alter vascular tone and blood flow within the affected tissues, increase vasodilation of capillaries, and increase vascular permeability needed for the migration of blood leukocytes to the site of injury (Ryman et al., 2015). In the case of mastitis, neutrophils are the predominant leukocyte type found in milk and mammary tissues during the early stages of infection and the efficiency at which neutrophils are able to phagocytize and kill invading pathogens will influence the establishment of disease (Aitken et al., 2011). The collective responses of the vascular endothelium and infiltration of blood leukocytes into affected tissues can result in some of the classical signs of inflammation that include heat, swelling, redness, pain and loss of function.

As briefly outlined above, the onset of inflammation to bacterial invasion is a complex and tightly regulated response. Whereas a rapid and robust inflammatory response is protective, however, an uncontrolled acute or chronic inflammatory reaction can lead to extensive tissue damage that is associated with diseases pathogenesis. Therefore, a timely and natural resolution of inflammation is fundamental to overall dairy cattle health and well-being. The resolution of inflammation is an active event involving specific pro-resolving pathways and mediators that expedite the shutdown process by limiting leukocyte infiltration, modifying soluble mediator production, removal of cellular debris, and repairing damaged tissues (Tabas and Glass, 2013, Buckley et al., 2014). An essential requirement to turn off the inflammatory response is the removal of the invading pathogens that initiated the inflammatory cascade. The successful neutralization of the inciting pathogen will signal the cessation of proinflammatory mediator synthesis and lead to their catabolism. In dairy cattle with intramammary infections, studies have shown that TNF-□ and IL-1 are expressed rapidly during the initial stages of infection and have potent pro-inflammatory functions whereas IL-4, IL-10 and IL-17 actively promote the resolution of the inflammatory cascade (Bannerman, 2009).

A relatively new area of research into the termination of inflammation is the production of lipid mediators with potent anti-inflammatory and pro-resolving activities. Studies conducted in human and laboratory species showed that the resolution of inflammation is an active process governed by several distinct families of pro-resolving oxylipids, which include resolvins (RV), protectins (PD), and lipoxins (LX) (Bennett and Gilroy, 2016). Although metabolites derived from the COX pathway traditionally have been associated with driving the onset of the inflammatory response, there are several downstream metabolites including PGD2, PGJ2, and 15-deoxy-PGJ2 that have the capacity to suppress various pro-inflammatory signaling pathways. There is also considerable evidence to suggest that certain PG produced during the onset of inflammation, such ad PGE2, can serve as negative feedback signals to facilitate the resolution of inflammation (Ricciotti and FitzGerald, 2011). Although these oxylipids will be discussed in detail later in this review, it is important to note here that the active biosynthesis of pro-resolving lipid mediators play an essential role in limiting neutrophil infiltration into affected tissues, enhancing macrophage clearance of apoptotic cells within affected tissues, and facilitate the restoration of tissues to normal function (Tabas and Glass, 2013). Unfortunately, there is not a great deal of *in vivo* research available in dairy cattle to suggest how pro-resolving or anti-inflammatory oxylipids may contribute to the resolution of inflammation. The relative expression of plasma oxylipids with known roles in the resolution of inflammation was reported to decrease in transition cows when biomarkers of inflammation are often enhanced (Raphael et al., 2014). Others reported an imbalance between anti- (LXA4) and pro-inflammatory (LTB4) oxylipids in cows with chronic mastitis due to lower concentrations of LXA4 in the milk (Boutet et al., 2003). A broader understanding of how oxylipids profiles shift to facilitate both the onset and resolution of inflammation is required to design efficacious intervention strategies to optimize bovine inflammatory responses especially during times of increased susceptibility to disease.

Oxylipid Biosynthesis

In general, oxylipids are rapidly synthesized when needed and are not stored within cells. Although the biosynthetic pathways involved in the production of different oxylipids can be complex, the production of oxylipids typically shares some common steps. The initial step in oxylipid biosynthesis is the release of PUFA substrates from membrane phospholipids. The hydrolysis of esterified PUFA is catalyzed by members of the phospholipase (PL) enzyme family, especially the calcium-dependent PLA2 (Burke and Dennis, 2009). When cells become activated as a consequence of pathogen exposure or tissue damage, PLA2 is mobilized to release key PUFA precursors that include the n6 fatty acids (linoleic acid , LA; arachidonic acids, AA) and the n3 fatty acids (□-linolenic acid, ALA; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA). These PUFA are bound to ester linkage to the SN2 position of membrane phospholipids and PLA2 acts as an esterase to facilitate their release (Raphael and Sordillo, 2013). The amount and proportion of specific PUFA substrates can impact the balance of pro- and anti-inflammatory signals.

The oxygenated derivatives of PUFA are produced by either enzymatic or non-enzymatic pathways. There are 3 major classes of enzymes involved in the initial oxygenation of PUFA substrates including cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) epoxygenases. These enzymatic pathways function in general by removing susceptible hydrogen atoms from the PUFA structure with the subsequent insertion of oxygen molecules. The COX pathway is composed of 2 major isoforms (COX-1 and COX-2). The COX-1 isoform is constitutively expressed in most tissues and synthesizes low levels of PG, such as prostacyclin (PGI_2), that mainly function in the maintenance of normal physiological functions. Conversely, COX-2 is highly inducible in response to NF-kB activation and is primarily associated with the biosynthesis of pro-inflammatory mediators such as PGE_2, PGF2, and TXA_2. The relative expression profile of PGs and TX is important to the character of the inflammatory response since COX-derived metabolite are known to have opposing actions on vascular tone. For example, PGI_2 causes vasodilation and inhibits platelet aggregation whereas TXA_2 is capable of promoting aggregation and vasoconstriction. Previous assumptions that all COX2 metabolites are solely responsible for propagating the inflammatory response is no longer supported by the current literature. For example, AA-derived PGE_2 derived from the COX-2 pathway is recognized as having many proinflammatory activities including inducing pain and fever. Whereas increased COX-2 expression during the onset of inflammation is typified by PGE_2 production, enhanced COX-2 expression during the resolution of inflammation is associated with the presence of other COX-2-derivide AA metabolites, PGD_2 and 15d-PGJ_2. Both PGD_2 and its dehydration end product 15d-PGJ_2 can inhibit leukocyte adhesion to endothelial cells and decrease cytokine expression by blocking NF-kB activation.

There also are several different LOX isoforms, including 5-, 8-, 12- and 15-LOX, where the nomenclature is defined by the capability of each enzyme to introduce molecular oxygen on a specific carbon of the fatty acid structure (Kuhn and O'Donnell, 2006). The family of LOX enzymes utilize a non-heme Fe2+ to form ferrous hydroxide that extracts hydrogen and inserts molecular oxygen to form a peroxyt radical. The unstable peroxy radical is then rapidly reduced to a peroxy fatty acid. For example, metabolism of AA by the 5-LOX pathway gives rise to hydroxyl and hydroperoxy derivatives (5-hydroxyeicosatetraenoic acid (HETE) and 5-hydroperoxyeicosatetraenoic acid (HPETE) respectively, that are often elevated during inflammation. The 15-LOX isoform is characterized as an inducible enzyme expressed in endothelial cells, epithelial cells, reticulocytes, and macrophages with the ability to oxygenate PUFA during inflammation. The initial oxygenated product formed during AA metabolism by 15LOX is 15HPETE, which is the biosynthetic precursor of 15-HETE and other LTs (Natarajan and Nadler, 2004). Within the larger family of CYP isozymes, there is a subset of CYPs (CYP4A series) that uses their heme iron radicals in mediating the epoxygenation and hydroxylation of PUFA to form oxylipids directly or to further metabolize some of the COX-derived metabolites such as PGE2 and PGD2 (Spector et al., 2004).

Non-enzymatic pathways of oxygenation are driven by both free radical and non-radical molecules that are often collectively referred to as oxidants. Although the majority of oxidants are reactive oxygen species (ROS), a portion of the total oxidant pool can consist of reactive nitrogen species (Halliwell, 2007). Non-enzymatic oxygenation of PUFA follows the same general mechanisms associated with the major enzymatic pathways of oxylipid biosynthesis. The main difference with the non-enzymatic pathway, however, is that the oxidant-mediated proton removal lacks the stereo specificity demonstrated by each of the enzymatic pathways. Whereas the enzymatic pathways of oxylipid biosynthesis primarily utilizes hydrolyzed free PUFA, the main targets of non-enzymatic oxidation pathways are fatty acids that remain esterified to cell membrane phospholipids (Milne et al., 2015). An important endogenous source of ROS is the by-product of energy production in the mitochondria as part of the electron transport chain resulting in increased superoxide formation. Periparturient cows often suffer from oxidative stress due to enhanced ROS production during this time of increased metabolic demands (Bernabucci et al., 2005, Sordillo et al., 2007, Sordillo, 2013). Another major source of ROS is generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in phagocytic cells during an inflammatory reaction (Halliwell, 2007). Consequently, cows with mastitis also will experience increased incidence of oxidative stress (Mavangira et al., 2016). Finally, it should be recognized that certain aspects of oxylipid biosynthesis during inflammation also could be a contributing factor to oxidative stress. For example, oxidation of PUFA by COX, LOX , and CYP can produce superoxide in the process that contributes to the total ROS pool (Mavangira and Sordillo, 2017). In addition, initial oxygenation products of enzymatic oxidation are themselves potent hydroperoxides. Metabolism of AA through the 15-LOX pathway, for example, results in the biosynthesis of 15-hydroperoxyeicosatetraenoic acid (15-HPETE), which is a potent ROS that has the capacity to cause oxidative-damage to cells involved in bovine inflammatory responses (Weaver et al., 2001, Sordillo et al., 2005). Thus, it is not surprising that bovine plasma and milk profiles of oxylipids generated from autoxidation pathways change with respect to lactation stage and during mastitis (Mavangira et al., 2015, Kuhn et al., 2017).

Another important control point in the biosynthesis of oxylipids is the degree to which the initial oxygenation product is subsequently metabolized. The initial product resulting from either enzymatic or non-enzymatic fatty acid oxidation is subjected to additional downstream metabolism to form a diverse network of oxylipids. As an example, the intermediate product from the metabolism of AA through the either the COX-1 or COX-2 pathway is PGH2. This intermediate metabolite serves as the common substrate for a series of specific isomerase and synthase enzymes that ultimately produce PGE2, PGI2, PGD2, PGF2□, TXA2, and TXB2. Since all prostanoid are derived from a single precursor metabolite (PGH2), the relative expression of PGs and TXs during health and disease will largely be determined by the differential expression and activity of their respective biosynthetic enzymes. Changes in the redox status of cells also can influence the degree to which initial oxygenation products of PUFA are metabolized to form downstream lipid mediators. Oxidation of linoleic acid by 15-LOX-1 results in the initial

product, 13-hydroperoxoctadecaienoic acid (13-HPODE), which can be reduced to 13-hydroxyoctadecadienoic acid (13-HODE) by antioxidants and reducing agents such as glutathione (Kuhn et al., 2015). The 13-HODE then can be metabolized further to 13-oxooctadecadienoic acid (13-oxoODE) by the actions of NADPH-dependent fatty acid dehydrogenase (Altmann et al., 2007). The significance of the sequential oxidation and metabolism of LA is that the pro-inflammatory 13-HPODE is required for the subsequent generation of the anti-inflammatory 13-oxoODE. The CYP epoxygenase pathways is another example of how oxylipid profiles are a reflection of the degree of downstream metabolism following biosynthesis of the initial oxidation product. Isoforms of the CYP epoxygenase pathway (CYP2J and CYP2C) metabolize arachidonic acid to form 4 epoxyeicosatrienoic acid (EET) regioisomers that are thought to play a significant role in regulating inflammatory responses. Soluble exopide hydrolase (sEH) can rapidly hydrolyze EETs to their corresponding dihydroxyeicosatrienoic acid (DHET) metabolites that generally have much less biological activity. The significance of the further metabolism of EETs by sEH is that many of the benefical anti-inflammatory functions the initial epoxygenase metabolites is lost. Indeed, there are several in vivo and in vitro murine models that utilize sEH inhibitors to potentiate the anti-inflammatory properties of specific EETs (Deng et al., 2010, Gabbs et al., 2015).

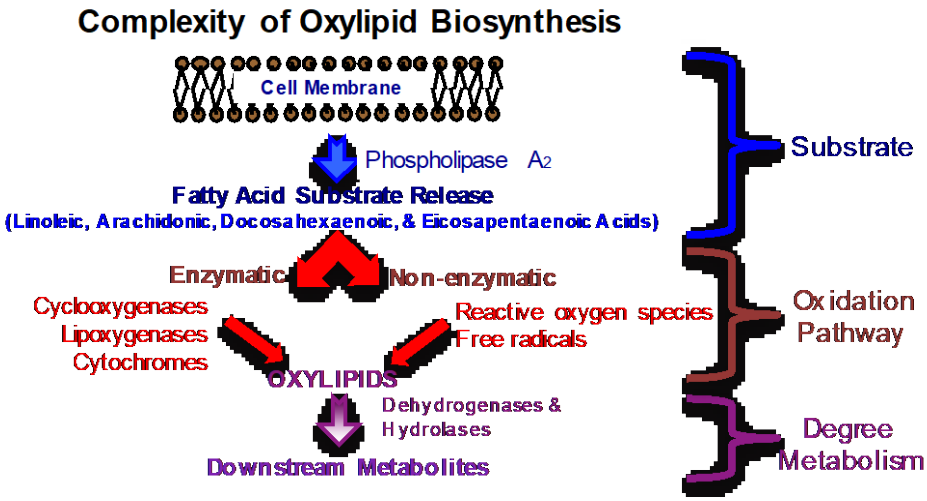

Figure 1. Complexity of Oxylipid Biosynthesis. Polyunsaturated fatty acids (PUFA) including linoleic acid, linolenic acid, and their respective derivatives serve as the substrates for oxylipid biosynthesis. Esterified PUFA are released from the membrane phospholipid through the actions of phospholipase A2. Once released from the membrane, PUFA are oxidized through enzymatic pathways (cyclooxygenase, lipoxygenase, or cytochrome P450 epoxygenase) and non-enzymatic pathways (reactive oxygen species or free radicals). The initial oxygenation

products may then be subjected to additional metabolism by stereospecific enzymes, dehydrogenases, or hydrolases. Various end products can have either pro-inflammatory or pro-resolving impacts on vascular inflammatory responses.

Conclusion

During uncontrolled inflammation, a likely combination of enhanced production of pro-inflammatory oxylipids and reduced expression of anti-inflammatory oxylipids prevents the full resolution of inflammation and the return to immune homeostasis. The oxylipid network is complex, highly interactive, and often cell-specific in orchestrating the onset or resolution of inflammatory responses. At present, there is ample evidence in dairy cattle to suggest that oxylipid biosynthesis is controlled at several levels including the amount and availability of PUFA substrates, the relative activity and substrate preference of oxidizing enzymes, and the degree to which intermediate metabolites are catabolize to their end products. The biosynthetic profiles of oxylipids and the subsequent impact that these metabolites may have on the character of the inflammatory response is also likely depend on the timing of their subsequent metabolism through various oxidizing pathways. A greater understanding of the factors that can regulate the delicate balance between the initiation and resolution of inflammatory responses is needed in order to diminish the morbidity and mortality associated with health disorders of dairy cattle such as mastitis.

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