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Targeting antimicrobial defenses of the udder through an intrinsic cellular pathway[1]

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ABSTRACT

The bovine innate immune system has a strong repertoire of antimicrobial defenses to rapidly attack infectious pathogens that evade physical barriers of the udder. Exploration of the intracrine vitamin D pathway of bovine macrophages has improved understanding of the signals that initiate antimicrobial defenses that protect the udder. In the intracrine vitamin D pathway, pathogen recognition receptors upregulate *CYP27B1* mRNA that encodes for the enzyme that converts 25-hydroxyvitamin D [$25(OH)D_3$] to the active vitamin D hormone, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. The $1,25(OH)_2D_3$, in turn, is generally known to increase antimicrobial activity and decrease inflammatory responses of immune cells. In cattle specifically, $1,25(OH)_2D_3$ increases nitric oxide and β-defensin antimicrobial responses of bovine monocytes. Immune activation of the intracrine vitamin D pathway, including induction of inducible nitric oxide synthase and β-defensin gene expression by $1,25(OH)_2D_3$, has been documented in the mammary glands of lactating dairy cows. Furthermore, intramammary $25(OH)D_3$ treatment decreased bacteria counts and indicators of mastitis severity in cows experimentally infected with *Streptococcus uberis*. We propose that vitamin D signaling in the udder contributes to containment of bacterial pathogens and inflammatory responses of the udder. Verification of vitamin D-mediated defenses of the mammary gland potentially provides a path for development of alternative solutions (i.e., nutritional, genetic, therapeutic) to increase mastitis resistance of dairy cows. Continued exploration of the intrinsic cellular pathways that specifically promote antimicrobial defenses of the udder, such as the vitamin D pathway, is needed to support mastitis control efforts for dairy cows.

Key words: macrophage, vitamin D, mastitis, dairy cow

INTRODUCTION

Mastitis is an inflammatory disease of the mammary gland most commonly caused by IMI of opportunistic bacterial pathogens that are ubiquitous in the environment of dairy cows (Ruegg, 2012). Inflammation of mammary tissue has long-term effects on productivity in addition to the immediate effects of inflammation on milk quality, productivity, and well-being of dairy cows. Prevention of IMI by proper care, management, and nutrition of dairy cows is the most effective approach for mastitis control; however, implementation of best management practices for mastitis prevention does not eliminate the occurrence of mastitis. Currently, antimicrobial drugs are the most effective therapeutic option available for treatment of IMI in dairy cows, but they have limited efficacy, require disposal of milk from treated cows, and raise concerns of antimicrobial resistance (Oliver and Murinda, 2012). Nutritional and therapeutic strategies that bolster antimicrobial defenses of the mammary gland offer a promising alternative for prevention and treatment of mastitis. Recent reports have documented a role for vitamin D in activation of antimicrobial defenses via intracrine and paracrine vitamin D signaling pathways (Liu et al., 2006; Nelson et al., 2010a,b). This paper will review evidence for vitamin D-mediated antimicrobial defenses and potential strategies to boost antimicrobial defenses of the udder through targeting the vitamin D pathway for the prevention and treatment of mastitis.

INNATE IMMUNE DEFENSES OF THE UDDER

Multiple defense systems are in place to defend the mammary gland against IMI. These include the

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physical barriers of the teat, constitutive cellular and humoral defenses, and activated cellular and humoral defenses (Sordillo and Streicher, 2002; Rainard and Riollet, 2006). Maintenance of the physical barriers of the teat by use of proper milking procedures and hygiene is by far the most important factor in prevention of IMI. Nevertheless, the humoral and cellular defenses of the gland provide a critical role in defense against opportunistic pathogens that inevitably evade the physical barriers of the teat. Macrophages and mammary epithelial cells provide innate surveillance of the mammary gland for bacterial pathogens. Upon pathogen recognition, they activate antimicrobial and inflammatory responses, which include antimicrobial peptides, lactoferrin, chemokines, and cytokines (Riollet et al., 2000; Schmitz et al., 2004). Neutrophils are recruited to the mammary gland if the resident defenses fail to prevent establishment of infection and play a critical role in resolution of IMI by release of granules loaded with antimicrobial peptides, phagocytosis, production of superoxides, and release of neutrophil extracellular traps (Lippolis et al., 2006; Rainard and Riollet, 2006). The lethal neutrophil activity, however, comes at a cost of damaged mammary tissue. Therefore, optimization of the resident defenses of the mammary gland against bacterial pathogens is desired to prevent establishment of IMI and subsequent need for massive neutrophil recruitment.

Macrophages and mammary epithelial cells provide innate surveillance of the mammary gland for pathogens via receptors such as toll-like receptor 2 (**TLR-2**), TLR-4, and TLR-5, which recognize lipoteichoic acids, LPS, and flagella, respectively (Kopp and Medzhitov, 2003; Ibeagha-Awemu et al., 2008). The pathogen recognition receptors initiate antimicrobial, cytokine, and inflammatory responses via intracellular signaling pathways. Certain pathways, such as the mitogen-activated protein kinase (**MAPK**) and nuclear factor κB (**NFκB**) pathways, are primary pathways that prime immune functions and initiate transcription of genes involved in the immune response. Secondary pathways, such as prostaglandin, oxylipid, and vitamin D signaling pathways, are activated by MAPK or NFκB signaling and modulate TLR-induced antimicrobial and inflammatory responses (Schmitz et al., 2004; Liu et al., 2007a; Samuchiwal et al., 2017). The secondary pathways provide opportunities for specific modulation of innate immune responses via therapeutic or nutritional approaches (Ryman et al., 2017). Vitamin D signaling, in particular, has been identified as a key activator of antimicrobial defenses in human macrophages (Adams et al., 2007). Vitamin D signaling is activated in the bovine mammary gland during mastitis as part of the innate response to bacterial infection and enhances immune responses associated with antimicrobial defense in cattle (Nelson et al., 2010a; Lippolis et al., 2011; Merriman et al., 2017).

VITAMIN D METABOLISM AND MECHANISM OF FUNCTION

Vitamin D was originally discovered almost a century ago as a factor in butterfat that prevented rickets (McCollum et al., 1922). In the years to follow, it was also found to be synthesized in the skin exposed to sunlight and to be critically involved in calcium homeostasis. Long before the discovery that vitamin D prevented rickets, sunlight and cod liver oil, which are good sources of vitamin D, were prescribed as a therapy for tuberculosis in human patients (Cassidy and Martineau, 2014). As it turns out, vitamin D supports antimicrobial mechanisms of macrophages via an intracrine antimicrobial pathway (Liu et al., 2006).

Vitamin D_3 primarily exerts biological activity upon enzymatic conversion to a potent ligand for intracellular vitamin D receptors (**VDR**; Haussler et al., 2013). Vitamin D_3 undergoes 2 successive hydroxylation steps to become a biologically active molecule as depicted in Figure 1, a process that is very tightly controlled. First, vitamin D_3 is hydroxylated to 25-hydroxyvitamin D_3 [(25(OH)D_3] by cytochrome P450 enzymes CYP2J2, CYP2R1, CYP27A1, and CYP3A4 in the liver (Jones et al., 2014). Conversion of vitamin D_3 to 25(OH)D_3 is not tightly regulated and concentrations of 25(OH)D_3 circulating in blood are relatively stable over time (Sommerfeldt et al., 1983). Next, the 25(OH)D_3 metabolite is converted to 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the biologically active metabolite, by the cytochrome P450 1α-hydroxylase enzyme, CYP27B1. Both 25(OH)D_3 and 1,25$(OH)_2D_3$ are inactivated by CYP24A1, another cytochrome P50 enzyme with 24-hydroxylase activity. The ratio of CYP27B1 to CYP24A1 is a major determinant of vitamin D's physiological actions. The vast majority of CYP27B1 activity is in the kidneys, which is homeostatically controlled by an endocrine mechanism as a function of calcium and phosphorus concentrations in blood (Horst et al., 2005). The CYP24A1 is strongly induced by 1,25$(OH)_2D_3$ in target tissues such as the intestines and kidneys to provide feedback control of 1,25$(OH)_2D_3$ concentrations in target tissues. Immune cells also have CYP27B1 and CYP24A1 activity that is dictated by immune activation (Nelson et al., 2010b). The immune control of 1,25$(OH)_2D_3$ metabolism in infected tissues is a critical feature of vitamin D-mediated immunity.