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Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows

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ABSTRACT

The dairy industry continues to suffer severe economic losses due to the increased disease incidence cows experience during the transition period. It has long been the classical view that the major contributing factor to the development of these periparturient diseases is the considerable increase in nutritional demands for milk production. This classical view, however, fails to account for the substantial correlation between both metabolic and infectious diseases and the detrimental effects that can occur with the provision of high-energy diets to support these nutritional demands. Currently, increasing evidence implicates bacterial endotoxins in the etiopathology of most periparturient diseases. Bacterial endotoxins are components of the outer cell wall of gram-negative and gram-positive bacteria that are highly immunostimulatory and can trigger proinflammatory immune responses. The ability of endotoxins to translocate from the mucosal tissues, including the gastrointestinal tract, mammary gland, and uterus, into the systemic circulation has been observed. Once they have entered the circulation, endotoxins potentially contribute to disease either directly, through eliciting an inflammatory response, or indirectly through other factors such as the overreaction of the natural protective mechanisms of the host. Although the evidence implicating a role of endotoxins in the pathogenesis of transition diseases continues to grow, our current knowledge of the host response to mucosal endotoxin exposure and pathogenic mechanisms remain largely unknown. Developing our understanding of the connection between endotoxemia and dairy cattle disease holds significant potential for the future development of preventative measures that could benefit the productivity of the dairy industry as well as animal welfare.

Key words: bacterial endotoxins, dairy cows, periparturient diseases

INTRODUCTION

It is well known that during the transition period of dairy cows, defined as 3 wk before to 3 wk after calving, a marked increase in the incidence of both metabolic and infectious diseases occurs (Mallard et al., 1998). These diseases continue to cause severe economic losses within the dairy industry regardless of the ongoing attempts to prevent, manage, and treat them. Our current understanding of diseases commonly present around the time of calving, also known as periparturient diseases, is lacking, as half of all dairy cows within a herd are affected by one or multiple diseases, especially during the postpartum period. As such it is necessary to explore new possible mechanisms of pathogenesis to benefit not only dairy industry profitability but also animal welfare and wellbeing.

Efforts have increased from multiple labs worldwide with regard to improving our understanding of the etiopathogenesis of periparturient diseases and developing new prevention technologies. Interestingly, a growing body of evidence supports the possible role of endotoxins in many of these highly detrimental diseases (Andersen, 2003; Ametaj et al., 2010). Endotoxins are membrane components of gram-negative bacteria (**GNB**) and gram-positive bacteria (**GPB**) that strongly elicit an immune response when present in the circulation (Draing et al., 2008; Knirel and Valvano, 2011). The physiological changes (i.e., immunosuppression), dietary shift to high grains, and increased exposure to endotoxins [both LPS and lipoteichoic acid (**LTA**)] at many mucosal tissues during the transition period may be facilitating the development of these periparturient diseases (Ametaj et al., 2010; Mallard et al., 1998).

High-grain diets play a significant role in the development of endotoxemia through the depression of ruminal pH, which eventually leads to the lysis of many ruminal microbiota and subsequently increases the concentration of free endotoxins in the rumen fluid (Mao et al., 2013). Increased endotoxin concentrations are also present at the mammary gland and uterus due to infectious diseases that commonly affect cows postpartum. The ability of endotoxins to translocate into

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the systemic circulation from multiple mucosal sites has been observed, and once an endotoxin has entered the host the natural detoxification processes and the onset of a systemic immune response against endotoxins can contribute to the pathogenesis of multiple diseases simultaneously (Hakogi et al., 1989; Mateus et al., 2003; Ametaj et al., 2010). The aim of the current review is to present the current knowledge of endotoxins, their translocation into the circulation, and their possible role in the etiopathology of multiple periparturient diseases of dairy cows.

FUNCTIONS OF BACTERIAL ENDOTOXINS

LPS in GNB

Lipopolysaccharide, also commonly referred to as endotoxin (**ET**), is a vital component of the outer cell wall of GNB. Lipopolysaccharides contain strong negative charges within their core polysaccharide region that are stabilized by divalent cations present within the membrane. Studies have shown that the structure of LPS influences the architecture and dynamics of naturally forming LPS aggregates, suggesting the differences in LPS among various bacterial species may play a central role in the structure and function of the outer membrane (Knirel and Valvano, 2011). Gram-negative bacteria are also able to release outer membrane vesicles, formed from a bulging out and pinching off of the outer membrane, that are composed mainly of outer membrane components, including LPS, and thus contribute to the release of LPS from live GNB (Mashburn-Warren et al., 2008).

Functions of Lipoteichoic Acid in GPB

Lipoteichoic acid is a vital component in the cell wall of GPB and shares many pathogenic properties with LPS (Ginsburg, 2002; Draing et al., 2008). Although the term endotoxin is used synonymously with LPS alone, LTA is considered as the endotoxin of GPB. Whereas the functions of LPS in GNB are well documented, those of LTA in GPB are largely unknown at this point. It has been suggested that LTA is likely involved in the proper growth and physiology of bacteria with a role in homeostasis and virulence as evidenced by defects observed in bacteria either lacking LTA or containing mutated forms (Percy and Grundling, 2014). Although less information is available regarding the effects of LTA, as those of LPS have been more extensively studied, compelling evidence exists of its contributing role in bovine disease, which will be further discussed.

SOURCES OF ET

Gastrointestinal Tract

The main source of ET in dairy cattle is the gastrointestinal tract (**GIT**) due to the microbiome of the rumen, which is rich in both GNB and GPB species, and its sensitivity to dietary changes. Notable changes in the microbial community occur as a result of the shift from high-forage to high-grain diets following parturition, which is common practice within the dairy industry to support high milk production (Tajima et al., 2001; Overton and Waldron, 2004; Mao et al., 2013). Significant evidence has shown that high-grain diets and the resulting depression of ruminal pH are accompanied by an increase in ruminal LPS concentrations. The reported increases to ruminal LPS vary significantly from 2 to 13.5 fold (Emmanuel et al., 2010; Zebeli et al., 2011a; Zhou et al., 2014).

During high-forage diets, the ruminal pH remains within a normal range and the microbial community is dominated by the phyla *Bacteroidetes* and *Firmicutes* (Jing et al., 2014; Zhang et al., 2014). It has been shown that SARA, induced by feeding rations containing either alfalfa or grain, is associated with a decrease in the proportion of GNB, mainly phylum *Bacteroidetes*, and an increase in phylum *Firmicutes*, which are mainly GPB (Khafipour et al., 2009c). Interestingly, it has been shown that intravenous administration of LPS alone can cause the same shift in rumen microbiota from *Bacteroidetes* to *Firmicutes*. It was suggested that this shift may be indirectly caused by systemic LPS via VFA accumulation and lowered rumen buffering, leading to depressed ruminal pH. Thus, translocation of LPS into the systemic circulation during SARA may aggravate acidosis and initiate a vicious cycle impairing rumen health (Jing et al., 2014).

Some controversy exists regarding the ability of LPS to translocate from the rumen into the host circulation due to the inconsistency of LPS detection in the peripheral circulation during SARA (Plaizier et al., 2012). Whereas some studies of SARA reported no increase to peripheral LPS (Li et al., 2012; Plaizier et al., 2014), others have observed strong associations between rumen and peripheral LPS concentrations (Khafipour et al., 2009b) as well as increased rumen permeability (Minuti et al., 2014). Early studies addressing ruminal absorption of LPS in steers administered ^{51}Cr-labeled *Escherichia coli* LPS into the rumen (Lassman, 1980; Anderson, 1984) found no evidence of its absorption in the lymphatic system or portal blood and concluded that the ruminal epithelium is impermeable to ET. Interestingly, a prior study by Ravin et al. (1960) in-