TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Pathogen-specific immune response and changes in the blood–milk barrier of the bovine mammary gland[1]

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ABSTRACT: Because of the decreasing use of antimicrobial drugs in animal food production, new treatments of infectious diseases such as mastitis are needed. This includes strategies to optimize the function of the animal's immune system. The present review discusses the components of the mammary immune response and the involvement of the blood–milk barrier during infections with different bacteria, strategies to manipulate the blood–milk barrier, and the potential to increase the efficiency of the animal's immune response. The mammary immune response is widely based on the cellular components of the innate immune system, which can be detected as an increase of the somatic cell count (SCC). During infection with Gram-negative bacteria such as *Escherichia coli*, characterized by severe clinical symptoms, there is a considerable transfer of soluble blood components including immunoglobulins from blood into milk. This is not typically observed during intramammary infection with Gram-positive bacteria such as *Staphylococcus aureus*, which is typically observed as a chronic subclinical infection. We have simulated these different types of mastitis by administering cell wall components of these bacteria (i.e., lipopolysaccharide [LPS] from *E. coli* and lipoteichoic acid [LTA] from *S. aureus*). Dosages of these 2 components intramammarily administered were adjusted to induce a comparable increase in SCC. Treatment with LPS caused a comprehensive transfer of blood components including immunoglobulins into milk, whereas in the LTA-induced mastitis, only a small increase of blood components in milk occurred. The blood–milk barrier can be manipulated. Glucocorticoids such as prednisolone reduced the transfer of blood components from blood into milk while reducing the general inflammatory reaction. It is possible that this treatment also inhibits the transfer of immunoglobulins into milk, likely reducing the efficiency of the immune response. In contrast, an opening of the blood–milk barrier could be achieved by an extremely high dosage of oxytocin (e.g., 100 IU). We assume that the myoepithelial hypercontraction increases the epithelial permeability that allows an increased flux of blood components including immunoglobulins into milk. The potential for manipulating the blood–milk barrier permeability as a treatment for mastitis is possible if specific antibodies against pathogens can be efficiently transported to the infected mammary gland.

Key words: blood–milk barrier, cow, mastitis, pathogen-specific immune response

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INTRODUCTION

Mastitis is one of the most critical production-related diseases in the dairy industry because of its direct connection to product quality and farm income. Clinical mastitis is painful for the animal, making it also an animal welfare issue (Fitzpatrick et al., 2013). Treatment strategies of infections including mastitis

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are increasingly needed. Both public health policies and consumer opinion aim to reduce or completely stop the increased use of antibiotics in food producing animals (Guterbock et al., 1993; Oliveira and Ruegg, 2014; Santman-Berends et al., 2015). Because the use of antibiotics has been established for decades as the most important tool for mastitis management in dairy cows, there has been decreased focus on the function of the cow's immune system. More recently, there has been some emphasis placed on manipulation of the cow's immune system to combat pathogens. The administration of various immunostimulatory components and vaccination against various mastitis pathogens are being used (Erskine, 2012; Scali et al., 2015). The contribution of the specific immune system to support the innate immune response during intramammary infection depends of the transfer of immunoglobulins from the blood circulation through the blood–milk barrier to the infected gland. This transfer is independent of the development of these immunoglobulins through previous pathogen contact or through vaccination.

The course of disease and the contribution of the different components of the immune system differ among the mastitis-causing pathogens (Bannerman et al., 2004b; Wellnitz et al., 2011). These differences are influenced by different patterns of the opening of the blood–milk barrier during the inflammatory process. The present review demonstrates the pathogen-specific difference of the immune response to Gram-negative and Gram-positive bacteria with special emphasis on the regulation of the blood–milk barrier, mainly based on studies with pathogen-specific cell wall components of *Escherichia coli* and *Staphylococcus aureus*.

CONSTRUCTION AND ORGANIZATION OF THE BLOOD–MILK BARRIER

An intact barrier between blood and milk is crucial for mammary function. This barrier prevents the uncontrolled exchange of components between blood and milk, allowing water, which is necessary for the suckled offspring, to move along an osmotic gradient from blood into milk, and prevents the loss of nutrients into blood, which are specifically secreted into milk to nourish the offspring. As a prerequisite of mammary gland function, the blood–milk barrier mainly forms during lactogenesis and achieves full integrity during the first days of lactation (Wall et al., 2015). The blood–milk barrier consists of several functional structures: endothelial cells, connective tissue, the basal membrane, and epithelial cells. The epithelial cells are closely connected by different structures such as adherens junctions, desmosomes, and tight junctions. These structures control the cell-to-cell adherence and also the regulation of the actin cytoskeleton and intracellular signaling (Wei and Huang, 2013). Although adherens junctions and desmosomes mainly provide a connection that helps to resist shearing forces, the tight junctions are generally accepted to present the most important structures that are critical for the prevention of uncontrolled exchange of blood and milk constituents by separating the milk-containing compartments of the gland from the surrounding vasculature (Nguyen and Neville, 1998; Stelwagen and Singh, 2014). Although most of the changes of blood–milk barrier permeability seem to be based on changes in tight junction integrity, other functional structures seem to be involved in the reduced integrity of the barrier (Wellnitz et al., 2016). Therefore, we use the phrase blood–milk barrier throughout this review whenever the involved barrier structure is not clearly defined.

HOST–PATHOGEN INTERACTION: COMPONENTS OF THE MAMMARY IMMUNE RESPONSE

Components of the innate immune system and of the adaptive immune system are both involved in the mammary immune response. The innate immune system provides the most important defense mechanisms because it is activated without having previous exposure to a pathogen. The innate defensive is represented by phagocytosing leukocytes and different humoral factors. Only if mastitis pathogens circumvent the innate immune system does a specific adaptive immune response gain in importance. The components of the adaptive immune system recognize specific antigenic determinants of the pathogens and are primarily represented by lymphocytes and immunoglobulins.

THE CONTRIBUTION OF THE INNATE IMMUNE SYSTEM IN MAMMARY IMMUNE DEFENSE

The innate immune response is mostly represented by an increase in somatic cells in milk (reviewed by Paape and Capuco [1997] and Wellnitz and Bruckmaier [2012]). Somatic cells in milk are, besides exfoliated epithelial cells, immune competent leukocytes. These leukocytes consist mainly of macrophages, polymorphonuclear neutrophils, and lymphocytes. In healthy cows, macrophages are the predominant cell population in milk (Sarikaya et al., 2006). In particular, in the cistern milk fraction (i.e., close to the potential entry

point of the pathogens), macrophages represent the highest percentage of milk cell populations (Sarikaya et al., 2006). After pathogen recognition, polymorphonuclear neutrophils are recruited in large amounts from the blood into the milk (Harmon, 1994; Kehrli and Shuster, 1994; Wellnitz et al., 2015). Their recruitment from blood into milk is important for successful combat against pathogens because they phagocytose and kill mastitis-inducing microorganisms (Kehrli and Shuster, 1994; Sordillo and Streicher, 2002). The amount of cells in milk (i.e., the somatic cell count [**SCC**]) is very important for the mammary immune response, and the immune response in udder quarters with lower SCC compared with that in udder quarters with normal SCC is less efficient (Wellnitz et al., 2010). In addition, the number of recruited phagocytic cells is dependent on the pathogen (de Haas et al., 2004).

Besides cellular components of the mammary immune system, soluble components that are nonspecifically directed against all invaded microorganisms are either locally synthesized within the mammary gland (e.g., lactoferrin and lysozyme) or transferred from blood into the milk, and the extent of their increase in milk can depend on the type of pathogen involved (Wellnitz et al., 2011, 2013).

The innate immune response is initiated when specific receptors (pattern recognition receptors) on the surfaces or in intracellular host cells bind particular molecules of microorganisms. These molecules are conserved motifs that are shared by groups of microorganisms (pathogen-associated molecular patterns [**PAMP**]). Several Toll-like receptors, a type of pattern recognition receptor, are present in the mammary gland on leukocytes and in mammary epithelial cells (Goldammer et al., 2004; Strandberg et al., 2005; Menzies and Ingham, 2006). As soon as the PAMP molecule binds to the Toll-like receptors, a signaling pathway is initiated that stimulates the host defense. Mainly, the resulting translocation of nuclear factor-κB into the nucleus induces the increased transcription of various immunomodulators. Important cytokines that are involved in the mammary gland are tumor necrosis factor α (TNF-α), IL-8, or RANTES (regulated on activation, normal T cell expressed and secreted; Nishimura, 2003).

HOST FACTORS AND PATHOGEN SPECIFICITY OF THE INNATE IMMUNE RESPONSE

The mammary immune response is influenced by a number of host-related factors such as the cow's genetic background (Griesbeck-Zilch et al., 2009); the metabolic stage of the animal, which is responsible for a particularly high mastitis risk at certain lactational stages (Burvenich et al., 2003; Burton and Erskine, 2003; Zarrin et al., 2014); or the level of SCC in the milk before infection (Suriyasathaporn et al., 2000; Wellnitz et al., 2010). These host-specific differences are mainly related to the initial immune response of the mammary gland against pathogen exposure, which is provided by the innate immunity (reviewed by Rainard and Riollet [2006] and Wellnitz and Bruckmaier [2012]). In addition, the type of mastitis-causing bacteria influences the mammary immune response (Bannerman et al., 2004b; Lee et al., 2006; Lahouassa et al., 2007; Bannerman, 2009). Therefore, the level and mode of activation of the immune system is influenced by both host and pathogen and is assumed to be directly linked to the clinical pattern and the cure rate of mastitis.

A broad range of pathogens, mostly bacteria, can successfully establish intramammary infections. Bacterial pathogenic factors and growth rate considerably determine the severity of mastitis (Zecconi et al., 2005). Several recent studies have shown differences in the immune response of the mammary gland toward different bacteria or their endotoxins that usually cause different mastitis severity. Mastitis is more severe during infection with Gram-negative bacteria compared with infections with Gram-positive bacteria (reviewed by Wellnitz and Bruckmaier [2012]). One well-investigated pathogen, although not the most frequent mastitis pathogen, is the Gram-negative *E. coli*. *Escherichia coli* can often be isolated from milk of animals suffering from severe clinical mastitis (Hogan and Smith, 2003), which is usually accompanied by a very fast and intense increase of SCC (Bannerman et al., 2004b). In contrast, *S. aureus* mastitis is often isolated from subclinical and chronical mastitis cases (Sutra and Poutrel, 1994) that are characterized by moderate and delayed SCC increase (Bannerman et al., 2004b) in the absence of clinical inflammatory symptoms. *Escherichia coli* and *S. aureus* have been shown to induce quantitatively and qualitatively different immune responses in the mammary gland that are related to the different course of the respective types of mammary infections. Several studies (Riollet et al., 2000; Bannerman et al., 2004b; Lee et al., 2006) described no increase of the cytokines in milk after intramammary challenge with *S. aureus* but a significant elevation of these factors after *E. coli* challenge. In these studies, the rapid and pronounced increase in cytokines after infection with *E. coli* was also characterized by a faster and more intense increase of SCC compared with infection with *S. aureus*. However, intramammary growth of *S. aureus* was shown to be much slower than that of *E. coli*, even though the infection was performed with the same cfu/quarter.

To avoid the influence of different bacterial growth in individual experimental setups, we used a defined constant challenge with the same concentration (multiplicity of infection) of heat-inactivated bacteria to stimulate isolated mammary epithelial cells in vitro for an optimal comparison of the immune stimulatory effects of different types of bacteria (Griesbeck-Zilch et al., 2008). This study resulted in a more pronounced mRNA abundance of cytokines such as TNF-α, IL-1β, IL-6, or IL-8 or the chemokine RANTES by *E. coli* than by *S. aureus*.

The variation in bacterial growth can also be prevented in experimental setups by the use of pathogenic components of bacteria to investigate differences of the immune response toward these pathogens. In Gram-negative bacteria, a crucial molecule that induces an immune response is the cell wall component lipopolysaccharide (**LPS**). It is involved in the development of many clinical signs after *E. coli* infection (i.e., fever, pain, increasing SCC, and changes in milk composition; Jain et al., 1978; Hill, 1981; Guidry et al., 1983).

In Gram-positive bacteria, several molecules interact with the immune system (reviewed by Henderson et al. [1996]). One important pathogenic component besides lipoproteins (Hashimoto et al., 2006) and peptidoglycans (Girardin et al., 2003) is lipoteichoic acid (**LTA**), which is a cell wall component in the murein capsule that can be recognized by the host (Schröder et al., 2003).

Strandberg et al. (2005) showed that LTA from *Streptococcus pyogenes* induced lower mRNA expression of several cytokines in mammary epithelial cells in vitro, reflecting a weaker immune response than that induced by LPS from *E. coli*. However, 20 μg LTA/mL medium compared with 50 μg LPS/mL medium was used in our studies. In these investigations (Wellnitz et al., 2011, 2013), in vivo dosages of LPS from *E. coli* and LTA from *S. aureus* isolated from bacteria-causing intramammary infections were established that induced similar increases of SCC as a reference level. Experiments demonstrated a different stimulation of various immune factors despite a comparable increase of SCC after stimulation with LPS and LTA. Lipopolysaccharide was a stronger inducer of increased intramammary mRNA abundance of the cytokines TNF-α, IL-1β, and IL-8 and the chemokine RANTES. Fever was induced only in LPS-challenged cows but not in LTA-challenged cows, which was most likely induced by the considerable TNF-α induction in LPS-challenged cows, which was not observed in response to LTA. Despite a comparable SCC increase, LPS isolated from *E. coli* is a more potent inducer of cytokines and other inflammatory factors than LTA isolated from *S. aureus*. These differences clearly demonstrate the involvement of the mammary immune system in the differential development of mastitis severity after infection with the different bacteria. The specific triggers causing the differential mammary immune response to different bacteria types are currently not known.

Experiments exploring differences in mammary immune response to different pathogens other than *E. coli* and *S. aureus* are rare. Bannerman et al. (2004a) showed that Gram-positive *Streptococcus uberis* that induced clinical mastitis and Gram-negative *Serratia marcescens* both induced increased milk concentrations of TNF-α, IL-1β, IL-8, and other cytokines in milk; however, most of the factors were more sustained in *S. uberis* infection. These differences were proposed to be involved in a slower recovery of milk production level and an extended increase of SCC in *S. uberis* infection.

Even different strains of pathogenic bacteria that induce different severities of mastitis were shown to stimulate the mammary immune system to a different extent. In vitro, it was shown that different strains of *S. uberis* that were isolated from acute and severe mastitis compared with a chronic and difficult-to-treat mastitis induced different severities of mastitis along with activation of differential cytokine mRNA expression patterns in mammary epithelial cells (Wellnitz et al., 2012). For *S. aureus*, it was shown that strains containing different genes that are accompanied with the induction of a range of severities of mastitis disparately induce the activation of cytokines in vitro (Zbinden et al., 2014) and in vivo (Zecconi et al., 2005).

COMPONENTS CHARACTERIZING BLOOD–MILK BARRIER PERMEABILITY

Various blood-derived factors are elevated in milk when the permeability of the blood–milk barrier is increased. Factors including milk L-lactate, lactate dehydrogenase (**LDH**), serum albumin (**SA**), and IgG as well as chloride and sodium ions (Bruckmaier et al., 2004; Lehmann et al., 2013; Wellnitz et al., 2015) are altered in the milk when the blood–milk barrier is compromised and can often contribute to the defense of the mammary gland. Milk proteins, such as α-lactalbumin (**α-LA**), can also be used as indicators of increased barrier permeability when present in the blood (Wall et al., 2016a,b). Milk LDH is also being commercially used as a mastitis indicator, especially on farms with automatic milking systems. Lactate dehydrogenase has been proposed as a marker for blood–milk barrier integrity and, hence, IgG transfer from blood into milk (Lehmann et al., 2013; Hernández-Castellano et al., 2017) because IgG measurement on farm is not

possible. Serum albumin, a major blood protein, has no known immune function in the mammary gland but is also indicative of a leaky barrier when present in milk (Stelwagen et al., 1994; Ben Chedly et al., 2010; Wall et al., 2016a,b). Immunoglobulin G1 and IgG2 both have important immune functions in the mammary gland. Colostrum is rich in IgG1, which is actively transported until parturition by the neonatal FcRn receptor located on the basolateral side of the mammary epithelium (Ghetie and Ward, 2000). During mastitis, an influx of total IgG (both IgG1 and IgG2) into the milk occurs and is indicative of a permeable barrier (Wellnitz et al., 2013). During an infection, IgG2 becomes the predominant immunoglobulin being transferred into milk through the permeable barrier and serves as the major opsonin for phagocytosis in the mammary gland (Burton and Erskine, 2003). The transfer of IgG2 from blood into milk is much more pronounced during infection with Gram-negative bacteria than during infection with Gram-positive bacteria (Wellnitz et al., 2013).

Lastly, the expression of α-LA, a whey protein and subunit of lactose synthase, is related to the intensity of lactose synthesis and, hence, a marker of the rate of secretory activity of the mammary gland (Larson, 1985; Bleck et al., 2009). This protein can be detected in blood during increased barrier permeability, including both the colostral phase and during mastitis (McFadden et al., 1987; Wellnitz et al., 2015). We have recently discovered that α-LA in blood increases during the course of machine milking, which indicates a reduced integrity of the blood–milk barrier by the mechanical load of machine milking or by the oxytocin (**OT**)-induced myoepithelial contraction and milk ejection (R.M. Bruckmaier and O. Wellnitz, unpublished data).

DIFFERENTIAL OPENING OF THE BLOOD–MILK BARRIER DURING MASTITIS

In a healthy mammary gland, the blood–milk barrier prevents a free interchange of blood components into milk and vice versa. During mastitis, the integrity of the blood–milk barrier is impaired as a result of tissue damage and invading immune cells (Burton and Erskine, 2003). The exchange of constituents between blood and milk through open tight junctions and potentially also other structures forming the blood–milk barrier leads to considerable changes in milk composition. In addition, the detection of changes in concentration of specific blood constituents can be used as mastitis indicators such as LDH, lactate, or SA, although these factors do not likely contribute to the defense against pathogens (Lehmann et al., 2013). During a mammary immune response to endotoxins of different mastitis pathogens, the blood–milk barrier is differentially opened. Even if the dosage of LPS and LTA is chosen to induce a comparable SCC increase, the increase of other blood constituents such as SA, LDH, or IgG in milk is much more pronounced if the immune response is induced by LPS from *E. coli* compared with the challenge by LTA from *S. aureus* (Wellnitz et al., 2013). To achieve this quantitatively similar response (based on SCC), much more LTA needs to be administered than LPS (Wellnitz et al., 2011). However, in cases of spontaneous mastitis, *E. coli* resulted in a large increase of IgG and LDH in milk compared with mammary infection with Gram-positive bacteria (Hernández-Castellano et al., 2017). Therefore, components of both the innate and adaptive immune system including immunoglobulins were transferred in response to LPS administration or *E. coli* infection, whereas in response to LTA administration or *S. aureus* infection, primarily the components of the innate immune system were activated and immunoglobulins were not transferred from blood into milk (Wellnitz et al., 2013). A specific role for IgG transferred from blood into milk in the immune defense has not been demonstrated; however, there is likely a role for this. We demonstrated that the transferred IgG is not necessarily directed only against mastitis pathogens but rather reflects the whole spectrum of antibodies present in blood (Lehmann et al., 2013). During mastitis, mainly IgG2 increases in milk. The primary role for IgG2 is opsonization of pathogens. It is also known that neutrophils invading the mammary gland during mastitis upregulate their IgG2 Fc receptors (Burton and Erskine, 2003). These mechanisms are most likely the reason why vaccination against mastitis pathogens has been demonstrated to positively affect the course of the disease and the cure rate (reviewed by Erskine [2012]).

In vitro treatments with LPS from *E. coli* resulted in a faster and more pronounced opening of the mammary epithelial barrier of primary mammary epithelial cells cultured on permeable supports (transwells) compared with LTA from *S. aureus* (Wellnitz et al., 2016). Interestingly, in this study, it was shown that LPS additionally caused considerable cell damage, whereas the opening of the epithelial barrier due to LTA challenge seemed to be a result of the opening of the epithelial tight junctions. Comparable results were shown earlier in vivo where LPS-induced mastitis resulted in epithelial cell damage, which is known to contribute to the breakdown of the blood–milk barrier (Wagner et al., 2009). These results confirm a pathogen-specific impairment of the blood–milk barrier, which has

considerable consequences on milk composition and, hence most likely, on mastitis severity.

MANIPULATIONS OF THE BLOOD–MILK BARRIER

The permeability of the blood–milk barrier can be manipulated using different pharmacological agents. Glucocorticoids, such as prednisolone (**PRED**), are known to stabilize tight junction structures. They have been shown to increase transepithelial electrical resistance representative of formation of tight junctions (Zettl et al., 1992) as well as increase the expression of the tight junction protein occludin in mouse mammary epithelial cells (Stelwagen et al., 1998). These results were also demonstrated in dairy cows using treatments of PRED (Wellnitz et al., 2014; Wall et al., 2016a). Treatment with PRED leads to decreased permeability of the blood–milk barrier. In combination with either LPS or LTA treatment, the differential reaction of the treatment was clear. The increase of SCC by LTA was fully abolished by PRED, whereas the similarly high SCC increase in response to LPS was not affected by PRED (Wall et al., 2016a). In contrast, PRED caused a reduction in the increase of LDH seen in both LPS and LTA challenges. The concentrations of SA and IgG in milk increased only in response to LPS but not LTA. This increase was abolished by the additional administration of PRED (Wall et al., 2016a). The differential effect of PRED indicates that the transfer of blood constituents into the milk is pathogen dependent and uses different pathways. Additionally, it seems likely that glucocorticoids bind to the glucocorticoid receptor, resulting in anti-inflammatory action. Intramammary administration of PRED during mastitis was shown to reduce inflammation and restore milk quality more rapidly than with antibiotics alone (Sipka et al., 2013). If glucocorticoids are administered during mastitis (e.g., if added to antibiotic injectors), it should be also considered that parts of the immune response may be impaired. Wellnitz et al. (2014) also examined the influence of PRED on the blood–milk barrier in quarters treated with LPS. This study showed that there was no effect of the PRED on the mRNA expression of TNF-α, IL-1β, or IL-8 in LPS-challenged quarters and also no effect of the PRED on cell migration or the concentration of TNF-α in the milk. However, there was an influence of PRED on the blood–milk barrier permeability, as there were decreased concentrations of both SA and LDH compared with LPS-treated quarters (Wall et al., 2016a).

The opening of the blood–milk barrier can be of interest in mastitis treatment, in particular if immunorelevant factors are not spontaneously transferred from blood into milk. Oxytocin, a hormone synthesized in the hypothalamus and released from the pituitary, induces myoepithelial cell contraction and alveolar milk ejection in the mammary gland (Bruckmaier and Blum, 1998). When cows are administered OT at an extremely high supraphysiological dosage such as 100 IU, the blood–milk barrier is disrupted (Allen, 1990; Wall et al., 2016b). This disruption is likely due to mechanical stress on the cell-to-cell contacts as a result of the maximum myoepithelial contraction (Stelwagen and Singh, 2014). Allen (1990) also observed a decrease in milk lactose concentrations. This result could be explained by a disruption of tight junctions causing an increase in sodium and chloride and decrease of potassium ion concentrations in the milk. Although sodium and chloride can replace the osmotic effect of lactose in milk, leading to a decline of lactose concentration, the increased intracellular Na:K ratio could also have decreased the synthesis of lactose. Additionally, OT has been examined as an alternative for antibiotic treatment for mastitis. Knight et al. (2000) experimentally infected quarters with a low virulence strain of *S. aureus* and observed that 100 IU OT intramuscularly injected could reduce bacterial load and that OT treatments did not differ in efficacy to an antibiotic treatment. Guterbock et al. (1993), who also used 100 IU OT intramuscularly injected to treat naturally occurring mastitis, also observed that there were no differences in clinical or bacterial cure rates between antibiotic treatment and OT treatment. Knight et al. (2000) hypothesized that the clearing of bacteria is likely due to milk ejection, which promotes the complete emptying of the udder and thus removal of the pathogen. Our own studies have demonstrated that an intravenous injection of the same supraphysiological dosage of OT (100 IU) increased the permeability of the blood–milk barrier (Wall et al., 2016b). Together with an intramammary LPS challenge, OT caused higher SCC, IgG, LDH, and SA concentrations in milk compared with the milk of quarters treated with only LPS. In quarters treated with LTA, the additional OT administration caused a greater transfer of IgG, LDH, and SA from blood into milk (Wall et al., 2016b). Remarkably, the treatment with an extremely high OT dosage can have implications as an alternative mastitis therapy. In particular, if the presence of specific antibodies against mastitis pathogens are assumed to be present in the blood, OT potentially can aid in the bringing these antibodies to the location where they are needed for the immune defense. This seems particularly necessary during subclinical infections with Gram-positive bacteria where a spontaneous increase of IgG in milk is very low (Hernández-Castellano et al., 2017).

CONCLUSIONS

The differential pathogen-dependent immune response to Gram-positive vs. Gram-negative intramammary infection is obviously linked to the characteristic course of disease, including cure rate. Gram-negative bacteria cause a comprehensive activation of components of the innate and adaptive immune response, leading to a severe disease but considerable chance of complete healing. In contrast, Gram-positive bacteria induce an increase of SCC but no considerable transfer of other blood components including immunoglobulins into milk, leading mostly to subclinical but chronic mastitis. This difference indicates that an increased permeability of the blood–milk barrier could support the immune response via the transfer of specific antibodies into milk and, hence, a better abolishment of infections with Gram-positive pathogens. The induction of a myoepithelial hypercontraction through injection of an extremely high dosage of OT appears to be a possibility for this goal.

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