J Mammary Gland Biol Neoplasia (2011) 16:291–304
DOI 10.1007/s10911-011-9230-4

Immunopathology of Mastitis: Insights into Disease Recognition and Resolution

Stacey L. Aitken · Christine M. Corl · Lorraine M. Sordillo

Received: 1 August 2011 / Accepted: 1 September 2011 / Published online: 22 September 2011

Abstract Mastitis is an inflammation of the mammary gland commonly caused by bacterial infection. The inflammatory process is a normal and necessary immunological response to invading pathogens. The purpose of host inflammatory responses is to eliminate the source of tissue injury, restore immune homeostasis, and return tissues to normal function. The inflammatory cascade results not only in the escalation of local antimicrobial factors, but also in the increased movement of leukocytes and plasma components from the blood that may cause damage to host tissues. A precarious balance between pro-inflammatory and pro-resolving mechanisms is needed to ensure optimal bacterial clearance and the prompt return to immune homeostasis. Therefore, inflammatory responses must be tightly regulated to avoid bystander damage to the milk synthesizing tissues of the mammary gland. The defense mechanisms of the mammary gland function optimally when invading bacteria are recognized promptly, the initial inflammatory response is adequate to rapidly eliminate the infection, and the mammary gland is returned to normal function quickly without any noticeable clinical symptoms. Suboptimal or dysfunctional mammary gland defenses, however, may contribute to the development of severe acute inflammation or chronic mastitis that adversely affects the quantity and quality of milk. This review will summarize critical mammary gland defense mechanisms that are necessary for immune surveillance and the rapid elimination of mastitis-causing organisms. Situations in which diminished efficiency of innate or adaptive mammary gland immune responses may contribute to disease pathogenesis will also be discussed. A better understanding of the complex interactions between mammary gland defenses and mastitis-causing pathogens should prove useful for the future control of intramammary infections.

Keywords Mastitis · Mammary gland · Immunology · Immunity · Inflammation · Immunopathology

Abbreviations

Ig	immunoglobulin
LPS	lipopolysaccharide
IL	interleukin
IFN	interferon
TNF	tumor necrosis factor
TGF	transcription growth factor
PAMPs	pathogen-associated molecular patterns
TLR	toll-like receptor
TIR	toll-interleukin 1 receptor
MyD88	myeloid differentiation primary response gene 88
TIRAP	toll-interleukin 1 receptor domain containing adaptor protein
TRIF	toll-receptor-associated activator of interferon
TRAM	toll-receptor-associated molecule
NF-κB	nuclear factor kappa B
CD	cluster of determination
LBP	lipopolysaccharide binding protein
COX	cyclooxygenase
LOX	lipoxygenase
PG	prostaglandin
TX	thromboxane
PUFA	polyunsaturated fatty acid
HETE	hydroxyeicosatetraenoic acid
HPETE	hydroperoxyeicosatetraenoic acid
ICAM1	intercellular adhesion molecule 1
LX	lipoxin
LT	leukotriene
MDP	muramyl dipeptide

S. L. Aitken · C. M. Corl · L. M. Sordillo (✉)
G300 Veterinary Medical Center, College of Veterinary Medicine,
Michigan State University,
East Lansing, MI 48824, USA
e-mail: sordillo@msu.edu

LTA	lipoteichoic acid
NET	neutrophil extracellular trap
NOD	nucleotide-binding oligomerization domain
PMN	polymorphonuclear leukocyte

Introduction

Mastitis is an inflammatory condition of the mammary gland that is most often associated with lactating mammals and is mainly caused by bacterial infection. Epidemiological studies in humans found that up to a third of all lactating women will suffer from mastitis, where the clinical form of the disease is a primary reason why mothers will stop breast feeding [1, 2]. Adequate diagnosis and treatment of the disease in women are necessary to avoid lactation failure, recurrent mastitis, breast abscess, and even death in some situations [2]. Intramammary infections are an important health concern in food-producing animals such as dairy cattle, goats, and sheep [3, 4]. Mastitis is the most costly disease to the dairy industry as a consequence of decreased milk production and quality, treatment costs, replacement animal costs, and reduced ability to market dairy products. Despite ongoing research efforts to develop more effective preventative or curative control measures, mastitis remains a significant health problem in both human and veterinary medicine.

The development of mastitis is partially related to the degree that mammary glands are exposed to bacterial pathogens. A broad range of gram-positive and gram-negative pathogens cause mastitis. The establishment and severity of infection can be influenced by the expression of bacterial virulence factors. Increased susceptibility to mastitis and the extent of disease severity also is influenced by several host-related factors including nutrition, genetics, oxidative stress, environmental stressors, and physiological transition of the mammary gland from involution to lactation [5–8]. Indeed, the incidence and severity of mastitis in most mammals is more pronounced around the time of parturition. In humans, mastitis is most common within the first 3 weeks of lactation, and approximately 74–95% of all new mastitis cases occur within 12 weeks postpartum [2]. Dairy cattle also are especially susceptible to new intramammary infections beginning 3 weeks prior to parturition through early lactation [9]. The ability to control mastitis, especially during the periparturient period, is ultimately reliant upon an efficient immune system that rapidly clears bacterial pathogens and restores mammary gland function and milk production.

Innate and Adaptive Immune Responses

The mammary gland immune system consists of a diverse array of physical, cellular, and molecular factors that function within innate or adaptive (acquired) immune responses. The innate immune system constitutes the primary line of defense during the initial stages of infection and is a key determinant of mastitis outcome. Innate defense mechanisms can be pre-existent within the mammary gland, but are activated quickly upon exposure to bacteria. Depending on the efficiency of the innate defense mechanisms, pathogens may be eliminated within minutes to hours following invasion. Rapid elimination of bacteria often will not result in any noticeable changes in mammary gland function or milk quality. Components of the innate defense system include nonspecific physical barriers of the teat end, pattern recognition receptors, phagocytes (i.e., neutrophils and macrophages), and various soluble factors (i.e., cytokines, complement, and lactoferrin). The efficiency of the innate arm of the immune system not only determines if new intramammary infections occur, but also influences the severity and duration of mastitis by influencing the nature of the adaptive immune response.

The adaptive immune response is triggered when innate immune mechanisms fail to eliminate a pathogen. The adaptive immune response is characterized by the generation of antigen-specific lymphocytes and memory cells with the ability to recognize specific antigenic determinants of a pathogen. When the mammary gland is re-exposed to the same antigen, a heightened state of immune reactivity occurs as a consequence of immunological memory and clonal expansion of antigen-specific effector cells. A memory immune response would be much faster, considerably stronger, longer lasting, and often more effective in clearing an invading pathogen when compared to a primary immune response. In contrast to the innate immune response, adaptive immunity can take days to develop because of the clonal expansion of B and T lymphocytes specific to the invading pathogen. Antigen-specific B lymphocytes synthesize and secrete antibodies that recognize and counteract specific bacterial virulence factors. Effector functions of T lymphocytes include the production of cytokines that facilitate cell-mediated immunity by regulating the magnitude and duration of the immune response. The unique features of the adaptive immune response form the basis of mastitis vaccine protocols. Both innate and adaptive immune defenses of the mammary gland must be highly interactive and coordinated in order to provide optimal protection from mastitis.

Pathogen-Dependent Immune Responses

The outcome of host-pathogen interactions within the mammary gland is variable and can result in acute or chronic symptoms that may present as a range of severity from subclinical to clinical mastitis. Gram-positive and gram-negative pathogens are known to elicit different mammary gland immune responses during intramammary infections [10]. Differences in the magnitude and duration of host

responses are determined, in part, from specific bacterial virulence factors. For example, *Staphylococcus aureus* adheres to and internalizes within host cells enabling evasion of the initial innate immune response which often results in subclinical, chronic infections. Alternately, coliform infections are associated with rapid bacterial multiplication, toxin release, eicosanoid biosynthesis, and cytokine production that may result in acute, clinical mastitis. The host response to each bacterial pathogen is the result of bacterial recognition and communication between various cell types within the mammary gland. The ultimate goal of the immune response is to eliminate invading pathogens and restore the mammary gland to normal function. However, an overly robust immune response may cause tissue damage, so it is important that the mastitis-causing pathogen is neutralized and eliminated rapidly before extensive bystander tissue damage can occur. Therefore, a delicate balance of pro-inflammatory and inflammatory-resolving activities is critical to prevent inadvertent damage to the mammary gland and restore homeostasis to the immune system. Understanding critical host-pathogen interactions during mastitis pathogenesis will enable the development of novel interventions aimed at optimizing natural immune defenses of the mammary gland and avoiding immune-related pathology.

Mammary Immunobiology

Physical and Chemical Barriers of the Teat

The port of entry for bacterial pathogens is the teat canal. The teat sphincter and keratin lining provide a physical and chemical barrier to invading pathogens. Sphincter muscles surrounding the teat end opening can hinder bacterial penetration by maintaining tight closure between periods of milk removal. Patency of these muscles is directly related to increased susceptibility to new intramammary infections [11]. The stratified squamous epithelium lining the teat duct produces a keratin layer that also is crucial to maintaining the barrier function of the teat end between milking periods. The teat canal can become completely occluded during the nonlactating period when there is creation of a keratin plug. Bacteria are physically trapped within the keratin lining preventing subsequent migration into the gland cistern. Removal of keratin from the teat end was correlated to increased bacterial invasion and colonization in dairy cattle [11, 12]. The lipid components of keratin, including esterified and nonesterified fatty acids, were shown to have bacteriostatic properties [13]. Whereas the precise mechanisms of antibacterial activity are unknown, some evidence suggests that the long chain fatty acids found in keratin may disrupt the lipid integrity within bacterial cell walls and result in perforation and death of invading pathogens. Despite the ability of the teat to trap pathogens, intramammary infections still occur and the mammary gland must rely on additional antimicrobial defense mechanisms to inhibit bacterial growth.

Endogenous Soluble Defenses

Bacteria that are able to traverse the teat canal and enter the gland cistern are confronted with a number of soluble antibacterial factors (i.e., peptides, proteins, enzymes) that can target the invading organism. In the healthy mammary gland microenviroment, some of these pre-existing factors include lactoferrin, complement, lysozyme, cytokines, immunoglobulins (Ig), and other soluble molecules with known bactericidal and bacteriostatic properties. The presence of these factors changes during different stages of lactation and have variable efficacy against different mastitis-causing pathogens.

Lactoferrin is among the well-characterized antimicrobial proteins of the mammary gland. Produced by epithelial cells and leukocytes, lactoferrin is an iron-binding protein with known bacteriostatic capabilities. In the presence of bicarbonate, lactoferrin can sequester free ferric ions present in milk and therefore hinder the growth of bacteria which have iron requirements, such as staphylococci and coliforms. In ruminants, lactoferrin and Ig were shown to act synergistically to inhibit the growth of certain gram-negative bacteria. [14]. A recent in vitro study using a bovine mammary epithelial cell line found that lactoferrin may marginally neutralize the cytotoxic effects of endotoxin by binding the lipid A portion of lipopolysaccharide (LPS), thus potentially altering the course of gram-negative intramammary infections [15]. Certain bacteria, however, are resistant to the antibacterial effects of lactoferrin. For example, *Streptococcus agalactiae* can utilize lactoferrin as an iron source following binding with citrate or cell surface receptors. More recent studies showed that *Strep. uberis* also may utilize lactoferrin as a molecular bridge between adhesion molecules on the bacterial surface and lactoferrin receptors on mammary epithelial cell surfaces [16]. The lactoferrin bridge leads to internalization by mammary epithelial cells and protection from the action of local immune defense mechanisms. Lactation stage greatly influences the amount and effectiveness of lactoferrin's antibacterial properties. For example, the concentration of lactoferrin is low in the milk of healthy lactating mammary glands, but increases dramatically during involution and inflammation [17]. Lactoferrin, in the presence of other milk proteins such as β-lactoglobulin, appears to have synergistic antibacterial effects against mastitis-causing pathogens such as *S. aureus* and *Strep. uberis* [18].

Complement is another component of the innate defense system and consists of a collection of proteins present in

serum and milk. The proteins that comprise the complement system are synthesized mainly by hepatocytes, but other cellular sources include monocytes and tissue macrophages. Concentrations of complement are highest in colostrum, inflamed mammary glands, and during involution [19]. Activation of the complement system results in the generation of several pro-inflammatory fragments, of which the C5a fragment is especially associated with mastitis [20]. Direct bactericidal activities of complement result from the deposition of pore-forming complexes onto the surface of bacteria. Gram-negative mastitis-causing pathogens, such as *Escherichia coli*, are especially sensitive to complement-mediated lysis [19]. Other important biological functions of complement that contribute to early microbial killing include opsonizing bacteria and priming or activating host immune cells for phagocytosis and intracellular killing [20]. Complement also is a potent chemoattractant for neutrophils and monocytes during the early stages of infection [20]. Activation of the complement cascade in the milk of healthy mammary glands, however, is thought to play only a minor bactericidal role due to its relatively low concentrations.

The role of cytokines in the physiology and immunology of the mammary gland has been studied extensively over the last several decades. Cytokines not only are critical for normal physiological functions, but also are known to play a central role in essentially all aspects of inflammation and immunity [10, 21–24]. The cytokine network consists of a diverse group of proteins produced by both immune and non-immune cells throughout the entire body and under diverse circumstances. The physiological and immunomodulatory capacity of the cytokine network is complex. Individual cytokines can interact with other cytokines synergistically, additively, or antagonistically on multiple cell targets. Several different cytokines can affect biological processes in the same way, as there is considerable functional redundancy within the cytokine network. Most cytokines have very short half-lives, so their synthesis and function usually occurs in bursts of activity. Cytokines are able to influence cellular functions through high affinity receptors for each cytokine located on mammary gland cells. Therefore, the activity of any responder cell is a function of not only the quantity and type of cytokine in the mammary gland microenvironment, but also the relative expression of cytokine receptors.

The concentration and composition of cytokines expressed within tissues and secretions changes dramatically during different physiological transitions of the mammary gland. For example, expression of IL2 and IFN was lower during the periparturient period compared to the fully involuted bovine mammary glands [25]. In contrast, expression of IL4, IL10, and TNFα were reported to be higher in milk and mammary tissues [26, 27]. The increased expression of TNFα and TNF receptors during pregnancy and early lactation was suggested to play a role in the growth and development of rat mammary glands [28]. Higher expression of TGFα and TGFβ1 in bovine mammary glands during mammogenesis and involution were correlated to periods of active proliferation and reorganization of mammary tissues [29]. In addition, TGFβ1 was shown to play a critical role in regulating mammary gland regenerative capacity during successive cycles of lactation and involution in mice [30]. More recent studies showed that TNFα, IL6, IL10 and TGFβ1 also play an important role in remodeling of mammary tissues [31–33].

In contrast to other soluble defenses within the mammary gland, there is no evidence that cytokines have direct antibacterial functions. Instead, cytokines play an essential role in host defense by orchestrating the antimicrobial functions of mammary gland effector cell populations following exposure to invading pathogens. Therefore, pre-existing concentrations of cytokines in the healthy mammary gland likely exert their biological effects by influencing normal physiological functions and maintaining immunological homeostasis [21, 24]. As such, cytokines exert their diverse effects on mammary gland defense mechanisms by escalating innate and adaptive immune responses, activating inflammation, and initiating the migration of leukocytes from blood into infected tissues following bacterial recognition by local cell populations. The pattern of cytokine expression by cells in the mammary gland will differ depending on the mastitis-causing pathogen that elicits their response [10]. In general, however, gram-negative bacteria initiate a greater magnitude of pro-inflammatory cytokine responses (i.e., IL1, IL6, IL8, and TNFα) when compared to gram-positive bacteria that tend to express a weaker and slower cytokine response during the early stage of infection.

In addition to several pre-existing innate defense mechanisms, Ig also can function in the surveillance and early elimination of mastitis-causing pathogens from the mammary gland. Immunoglobulins are produced by antigen-activated B lymphocytes that subsequently proliferate and differentiate into antibody-secreting plasma cells. Concentrations of Ig in lacteal secretions are synthesized locally, are selectively transported, or present in transudate from serum [34]. Recent findings also reported the expression of Ig heavy and light chain transcripts by mammary gland epithelial cells of lactating mice [35]. In addition to plasma cells, these data suggest that at least some Ig found in the colostrum and milk may be produced by mammary gland epithelial cells. There are 4 classes of Ig that are known to influence mammary gland defense against mastitis-causing pathogens, namely IgG_1, IgG_2, IgA, and IgM, which all differ in their physiochemical and biological properties. In general, Ig concentrations are maximal during colostrogenesis and during intramammary infections. In bovines, IgG_1 is the predominant isotype in healthy mammary glands, while IgG_2 increases substantially during mastitis. Indeed, there is some

evidence to suggest that low concentrations of the IgG_2 isotype correlated to an increased incidence of bovine mastitis [7]. In the milk of women, however, IgA is found in highest concentrations especially during the immediate postpartum period. Increased susceptibility to mastitis in women is correlated to concentrations of IgA in milk. Indeed, the concentrations of IgA in the normal milk of women that ultimately developed mastitis was significantly lower when compared to women that remained mastitis-free, suggesting that IgA deficiency is a risk factor for human mastitis [36]. The presence of all Ig isotypes in colostrum and milk can facilitate antimicrobial defenses of the mammary gland. For example, several Ig isotypes (IgG1, IgG2, and IgM) can act as opsonins to enhance phagocytosis by neutrophils and macrophages. In addition to its role in opsonization, IgM is efficient at complement fixation. Whereas IgA does not aid in bacterial opsonization, it does function in bacterial agglutination that can impede the ability of certain pathogens to spread throughout the mammary gland. Another important role of IgA in the defense of the mammary gland is its ability to neutralize some bacterial toxins. Clearly, both the concentration and isotype composition of Ig found in lacteal secretions can have a profound influence on the establishment of new intramammary infections.

Pattern Recognition Receptors

The ability to sense the presence of bacteria within the mammary gland is essential for early host defense. Both immune and non-immune cell populations within the healthy mammary gland play a significant role in surveillance and activation of the innate immune response to invading pathogens via pattern recognition receptors (Table 1). Pattern recognition receptors can be expressed on the cell surface, secreted, or expressed intracellularly and function to recognize a diverse array of conserved motifs unique to specific microbes that are referred to as pathogen associated molecular patterns (PAMPs) [37, 38]. These PAMPs can differentiate a range of bacterial factors associated with mastitis-causing bacteria including lipopepetides of gram-positive bacteria and LPS of gram-negative bacteria. After binding to their ligand, pattern recognition receptors can initiate intracellular signaling cascades that result in initiation of immune responses or can facilitate antimicrobial activity directly.

The Toll-like receptor (TLR) family of pattern recognition receptors was among the first to be discovered and are pertinent to bacterial intramammary infections. To date, 10 TLRs were identified in humans and 12 in mice, with agonist specificity varying between species [37, 38]. The TLRs may work independently, antagonistically, or synergistically upon stimulation to modulate the immune response [39]. The TLR consists of a leucine-rich repeat area that recognizes the PAMP, a transmembrane domain, and an intracellular toll-interleukin 1 receptor (TIR) domain for downstream signaling. Toll like receptors recruit TIR domain-containing adaptor molecules, including MyD88, TIRAP, TRIF, and TRAM, to activate downstream signaling pathways. The adaptor molecule, MyD88, is used by all TLRs except TLR3 and activates NFκB to initiate the production of inflammatory cytokines. Multiple intracellular signaling pathways may be upregulated in response to the activation of TLR in addition to pro-inflammatory mediator production, including apoptotic pathways. Both TLR2 and TLR4 are the most significant during bacterial mastitis infections and are primarily activated in response to gram-positive and gram-negative infections, respectively.

The recognition of LPS from gram-negative pathogens by TLR4 is facilitated by additional proteins, CD14, LPS-binding protein (LBP), and myeloid differentiation protein 2. Myeloid differentiation protein-2 is a secreted protein that is associated with the extracellular portion of TLR4 and is critical for LPS signaling. LPS-binding protein is an acute phase protein in serum that facilitates the subsequent binding of LPS to membrane bound CD14 (mCD14). In addition, LBP may facilitate the transfer of LPS to soluble CD14 (sCD14) aiding the complex recognition and activation in endothelial cells [40]. CD14 is a glycosylated phosphatidylinositol-anchored protein located on the membranes of monocytes, macrophages, and neutrophils. Cells lacking mCD14, such as endothelial and epithelial cells, utilize sCD14 present in serum and milk to aid in LPS recognition by TLR4. Soluble CD14 may compete with mCD14 for the binding of LPS to prevent activation of monocytes and macrophages [41]. Studies of murine mastitis revealed that treatment with recombinant bovine sCD14 not only resulted in the recruitment of neutrophils to the mammary gland, but also increased survival of mice following *E. coli* infusion [42, 43] [44]. Transgenic mice expressing sCD14 in milk also were shown to be less susceptible to edema induced by *E. coli* mastitis [45]. Infusion of a plant-derived recombinant bovine sCD14 following experimental bovine *E. coli* mastitis resulted in decreased viable bacterial numbers and decreased clinical severity [46]. Collectively, these studies support the contention that CD14 plays a central role in the pathogenesis of gram-negative pathogens. Strategies to increase sCD14 in milk may reduce the severity of coliform mastitis.

Inflammation

Inflammation is a critical component of the innate defense system that involves complex biological responses of vascular tissues to harmful stimuli such as bacterial pathogens. The inflammatory process is initiated by cells already present within the mammary tissues. Resident cells

Table 1 Pathogen recognition receptors

Factor	Role	Reference
Innate Immunity		
CD14	Binds LPS. Membrane version is expressed on several cells including monocytes, macrophages, neutrophils, dendritic cells, and B cells. The soluble version may compete with mCD14 for LPS and is essential in the activation of non-mCD14 expressing cells, including epithelial and endothelial cells, by LPS.	[44, 120]
PGRP	Expressed in differentiated, lactating epithelium where it binds and hydrolyzes peptidoglycans.	[121]
TLR2	Recognizes peptidoglycan and LTA from gram+bacteria and lipoarabinomannan from mycobacteria. May form a heterodimer with TLR1 to recognize triacylated lipopeptides from gram - bacteria and mycoplasma or with TLR6 to recognize diacylated lipopeptides from gram+bacteria and mycoplasma.	[84, 88]
TLR3	Detects double-stranded RNA.	[84, 88]
TLR4	Recognizes LPS of gram - bacteria, heat-shock proteins, fibrinogen, and polypeptides.	[84, 88]
TLR5	Recognizes bacterial flagellin.	[84, 88]
TLR9	Intracellular recognition of CpG-containing oligodeoxynucleotides (ODNs).	[84, 88]
Acquired Immunity		
Memory B Cells	Produced during B cell division along with plasma cells following pathogen recognition. Their presence allows for a rapid response to a previously encountered antigen.	[7, 21]
Memory T Cells	Rapidly divide upon recognition of a previously encountered antigen. Longer life-span than memory B cells.	[7, 21]
Fc Receptor	Expressed on macrophages, neutrophils, and natural killer cells and recognize antibodies of infected cells or pathogens.	[7, 21]

that express pattern recognition receptors are activated by bacterial PAMPs and release various inflammatory mediators including cytokines and eicosanoids that initiate the inflammatory cascade. These mediator molecules initially increase vasodilation that enhances blood flow. The permeability of blood vessels also changes, causing the leakage of plasma components (i.e., serum albumin, complement, and acute phase proteins) into localized areas of affected tissues resulting in edema. Cytokines and other mediator molecules act directly on vascular endothelial cells to enhance the adhesion and migration of leukocytes from the blood to the site of injury. Neutrophils are the predominant cell type to undergo extravasation during the early stages of inflammation. Neutrophils first marginate and then adhere to the local endothelium near the site of infection. Cytokines and eicosanoids stimulate adherent neutrophils to move between endothelial cells and pass across the basement membrane into the damaged tissue. The movement of neutrophils is facilitated by chemotaxis gradients created by inflammatory mediator molecules at the localized site of infection.

Eicosanoids

Eicosanoids can regulate several inflammatory processes such as vascular permeability, leukocyte infiltration, localized edema, and fever [47] and eicosanoid concentrations are increased in the milk and plasma of mastitic cows [48, 49]. Eicosanoid biosynthesis occurs from the oxygenation of fatty acids through the cyclooxygenase (COX), lipoxygenase (LOX), or P450 enzymatic pathways. Depending on the timing and magnitude of expression, certain eicosanoids can either enhance or resolve the inflammatory response. The COX pathway is composed of 2 major isoforms. COX1 is constitutively expressed in most tissues and synthesizes low levels of prostaglandins (PG), such as prostacyclin (PGI_2), that functions in the maintenance of normal physiological functions and vascular homeostasis. Conversely, COX2 is highly inducible in response to pro-inflammatory stimuli and it is primarily associated with the biosynthesis of pro-inflammatory mediators such as PGE_2, $PGF_{2\alpha}$ and thromboxane B_2 (TXB_2). Increased PGE_2, $PGF_{2\alpha}$ and TXB_2 concentrations in milk were detected in both experimental and natural cases of mastitis caused by *Strep. uberis* as well as other mastitis-causing pathogens [50–53]. Increased COX2 expression during the resolution of inflammation, however, is associated with the presence of metabolites, such as PGD_2 and 15 d-PGJ_2, which can inhibit leukocyte adhesion to endothelial cells and decrease cytokine expression by blocking NF_kB activation [54]. Non-steroidal anti-inflammatory drugs can inhibit PG biosynthesis by targeting COX activity and are used widely to treat a variety of inflammatory-based diseases including mastitis in dairy cows, but with variable results [55–58].

LOX is a heterogeneous family of non-heme enzyme dioxygenases with the ability to oxidize polyunsaturated fatty acids (PUFA). There are several different LOX isoforms including 5LOX and 15LOX where the nomenclature is defined by the capability of each enzyme to

introduce molecular oxygen on a specific carbon of the fatty acid structure [59]. Metabolism of arachidonic acid by the 5LOX 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 15LOX1 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 arachidonic acid metabolism by 15LOX1 is 15HPETE, which is the biosynthetic precursor of 15HETE and other leukotrienes [60]. Increased 15LOX1 activity is recognized as an important factor in the development of certain inflammatory-based diseases such as atherosclerosis [61]. Metabolites of the 15LOX1 pathway enhanced intercellular adhesion molecule 1 (ICAM1) expression and monocyte adhesion in vessel walls during disease progression in humans [62, 63]. These data suggest that 15LOX1 may facilitate pathogenesis by enhancing the pro-inflammatory phenotype of endothelial cells. Increased expression of 15HPETE is found in bovine mammary endothelial cells as a result of selenium deficiency [64] and this metabolic product upregulates the expression of ICAM1 in other bovine endothelial cell types [65]. Conversely, evidence suggests that the LOX pathways also play a significant role in the biosynthesis of lipoxins (LX) that are a unique class of eicosanoids with dual anti-inflammatory and pro-resolving functions [66]. Relative to mastitis, an imbalance of LXA_4:LTB_4 occurs during chronic mastitis and was reportedly due to the dramatic reduction in LXA_4 biosynthesis within infected mammary glands [52]. The gene expression of 15LOX1 is increased in mammary tissues in early lactation dairy cows [67], however, its contribution to mammary gland health or disease during this time period is unknown and should be a focus a future research.

Mammary Vascular Endothelium

The mammary vascular endothelium has received relatively little attention with regards to bovine mastitis despite the significant role endothelial cells play in the pathogenesis of inflammatory-based diseases. The vascular endothelium is composed of a single layer of cells lining blood vessels that provides a barrier between blood components and extravascular tissues. Anatomical features of the mammary microvasculature were described extensively in rodents and to a lesser extent in the bovine [68]. The mammary capillary network forms a basket-like structure around alveoli and the endothelium lining these vessels is the primary site of exchange of metabolites from blood to tissue. The mammary gland depends upon an adequate supply of blood-derived nutrients and hormones to both initiate and sustain milk synthesis. Microscopic examination of human [69, 70] and rodent [71, 72] mammary glands suggest that the capillary endothelium is largely continuous, with some minor areas of fenestration. Rodent and bovine mammary gland capillaries exhibit numerous marginal folds and microvilli beginning from late pregnancy to peak lactation, thus increasing the surface area for the exchange of molecules from blood to tissue [71–74]. Despite the critical role of the vasculature to mammary physiology, its complex functions in milk-producing mammals, such as dairy cows, are not fully understood.

In addition to its role in supplying nutrients to the mammary gland, the mammary vascular system actively participates in the inflammatory response to infectious pathogens. Mammary vascular endothelial cells are activated in response to numerous stimuli released from cell populations in the mammary gland, bacterial toxins, reactive oxygen and nitrogen species, and potent lipid mediators [64, 75–78]. The endothelium responds in various ways including changes in vascular tone and blood flow to accommodate leukocyte slowing and migration, production of pro-inflammatory cytokines and adhesion molecules, and production of reactive oxygen and nitrogen species important in intracellular signaling [5]. Under normal physiological conditions the vascular endothelium is able to maintain its integrity. During prolonged or excess inflammation, however, a disturbance of endothelial homeostasis may occur, resulting in the loss of vascular barrier functions and the influx of serum components into lacteal secretions [17, 40, 79, 80]. Consequently, the loss of vascular integrity may contribute to the development of severe or chronic mastitis. The outcome of new intramammary infections may be dependent upon on the functional integrity of mammary endothelial cells.

Localized Cellular Components of Inflammation

Epithelial cells lining the teat canal, gland cistern and alveoli are among the first cells to recognize pathogens and participate in triggering an inflammatory response. Recently it was shown that the teat canal provides an early, active immune response to pathogens aside from the physical and chemical barrier [81, 82]. Rinaldi et al. (2010), reported rapid and intense immune gene changes within teat tissue following experimental *E. coli* infection. Genes shown to change within 12 h following infection were involved with pathways participating in the inflammatory response and leukocyte recruitment, antimicrobial peptide production, apoptosis, acute phase response, and bacterial recognition receptors [81]. Although the extent of participation of the teat canal in host defense is not completely known at this time, epithelial cells and leukocytes present within the teat end tissues have the capacity to respond to invading pathogens and may be important to the initiation and resolution of infection. Future studies that investigate how the teat tissues respond to

pathogen invasion earlier than 12 h seem prudent in light of these recent findings.

Alveolar epithelial cells also are involved in bacterial recognition and initiation of the innate immune system. A bovine mammary epithelial cell line, MAC-T, was found to express both TLR2 and TLR4 following exposure to LPS [83]. Others showed that primary bovine mammary epithelial cells responded robustly to *E. coli*-induced activation of TLR4-dependent signaling pathways with the enhanced expression of pro-inflammatory cytokines such as TNFα and IL8 [84, 85]. Whereas *S. aureus* also could properly induce TLR2 signaling in mammary secretory epithelial cells, the expression of TNFα and IL8 was muted due to inadequate NFκB activation [84]. Bougarn *et. al.* (2010) also found that some staphylococcal PAMPs (i.e. muramyl dipeptide (MDP) and lipoteichoic acid (LTA)) failed to increase the protein expression of pro-inflammatory cytokines in the culture supernatant of bovine mammary epithelial cells. However, they did find that MDP and LTA synergized to induce the production of several neutrophil chemoattractants by mammary epithelial cells that was dependent on NFκB activation [86]. Bacterial pathogens can upregulate TLR on mammary epithelial cells as well as produce non-specific bactericidal factors, such as cytokines, chemokines, and β-defensins [87, 88]. Collectively, these studies suggest that mammary epithelial cells could differentially affect the overall inflammatory response depending on how they recognize and respond to different bacterial PAMPs. Therefore, the severity and duration of mastitis may be related not only to TLR expression, but also how TLR-induced signaling pathways become activated in mammary alveolar epithelial cells.

Milk somatic cells from healthy glands are primarily composed of macrophages, but also include lymphocytes, neutrophils, and mammary epithelial cells. These cells function to survey the mammary gland for invading pathogens. The composition of milk somatic cells changes upon bacterial recognition and infection development. A shift to a predominately neutrophil population in the mammary gland occurs following bacterial recognition and release of chemoattractants (i.e., complement components, cytokines and eicosanoids) by macrophages and epithelial cells. Neutrophils function to eliminate pathogens primarily by phagocytosis and intracellular killing. Neutrophils can kill pathogens by several antibacterial mechanisms: neutrophil extracellular trap (NET) formation, respiratory burst, antibacterial peptides and defensins. Following binding of complement components and Ig on neutrophil receptors, neutrophils are activated and initiate a respiratory burst releasing high concentrations of oxidizing agents to kill ingested bacteria. Neutrophil granules also contain antibacterial peptides, such as cathelicidins, hydrolases, proteases, and lysozyme. The phagocytic and oxidative burst functions of neutrophils are drastically reduced in the presence of milk due to the ingestion of fat and casein [89]. Alternate neutrophil functions, such as the release of NETs, do not appear to be affected in the presence of milk [90]. Activated neutrophils release nuclear and granular material in a web-like fashion, called NETs, which physically trap bacteria. Bacterial entrapment by NETs contains the pathogen and places them in an environment with a high local concentration of antimicrobial agents released by neutrophils to enhance bacterial kill [91]. NETs may be important factors in neutrophil bacterial death during intramammary infection [90].

Macrophages, in addition to neutrophils, are responsible for bacterial phagocytosis. Upon bacterial recognition, macrophages activate the immune system by release of cytokines and other pro-inflammatory mediators and facilitate the innate immune response, including neutrophil migration and bactericidal functions. Macrophages appear to play a role in *E. coli* mammary epithelial invasion and colonization in a murine model of mastitis [92]. Addition of TLR4 expressing macrophages into milk spaces of TLR4 depleted mice was able to significantly decrease bacterial invasion of epithelial cells. Additionally, macrophages present bacterial antigens to lymphocytes for initiation of a specific immune response. Macrophages have a role in resolution of infection as well by phagocytizing aged neutrophils to minimize cellular and tissue damage by toxic antibacterial components released by neutrophils [93]. Macrophages are involved at multiple levels during mastitis and are indispensible to bacterial recognition and elimination.

Cellular communication between somatic and epithelial cells propagates the innate immune response. Maintaining the health of each cell type is necessary to rapidly resolve infection without causing irreversible mammary gland damage that will affect subsequent milk production.

Immunopathogenesis

Mammary gland innate and adaptive immune responses are complex, interconnected, and crucial for defense against mastitis-causing pathogens. There are some situations, however, when some mammary immune responses may facilitate disease pathogenesis and lead to deleterious consequences. There are several ways that host defense mechanisms can fail and subsequently lead to immunopathogenesis. The inability of local mammary gland defenses to adequately detect and eliminate pathogens can facilitate wide-spread immune evasion and the development of chronic inflammation. Conversely, the uncontrolled recruitment and activation of inflammatory cell types, especially neutrophils and macrophages, can result in the accumulation of toxic levels of cytokines, lipid mediators, and reactive oxygen species that

can severely damage host tissues with possible systemic complications leading to death. The delicate balance between a robust immune response needed to eliminate mastitis-causing pathogens during the early stages of infection and the generation of anti-inflammatory mechanisms needed to restore mammary gland immune homeostasis influence the extent of immunopathology and the outcome of disease (Figure 1).

An effective immune response occurs when pathogens are promptly detected and eliminated without excessive or prolonged inflammation. Various bacterial species, however, are capable of epithelial invasion and colonization and contribute to the severity and chronicity of mastitis. Subclinical, chronic *S. aureus* mastitis, for example, is associated with a suboptimal innate immune response and the ability to evade adaptive mammary gland defenses. Experimental intramammary infection with *S. aureus* decreased expression of intracellular receptors, NOD1 and NOD2, in bovine teat canal tissue that may be important to detection of this pathogen because of its ability to invade epithelial cells [82]. Cytokine gene expression for IL1β and TNFα did not significantly change across tissues within the mammary gland, consistent with other reports of subclinical *S. aureus* infection [82]. Gunther et al. (2011) demonstrated an inability of *S. aureus* to upregulate inflammatory genes through TLR MyD88-dependent activation in primary bovine mammary epithelial cells. Both *S. aureus* and *E. coli* were able to activate TLR2/4 signaling in mammary epithelial cells; however, neither *S. aureus* nor its active component LTA were able to activate NFκB. Conversely, a strong induction by *E. coli* and LPS was able to induce gene expression of IL8 and TNFα by an NFκB-mediated pathway (Yang et al., 2008). Some suggest that the lack of NFκB signaling by staphylococcal PAMPs may be due to the increased production of TGFβ in the mammary gland by *S. aureus,* thereby blocking the MyD88-dependent signaling as found by Naiki et al. (2005). Additionally, exotoxin production is an *S. aureus* virulence factor that facilitates immune evasion. Leukotoxins form pores in leukocytes inhibiting bacterial phagocytosis and permitting bacterial persistence within the mammary gland [94]. Causing a suboptimal immune response may be a bacterial adaptation

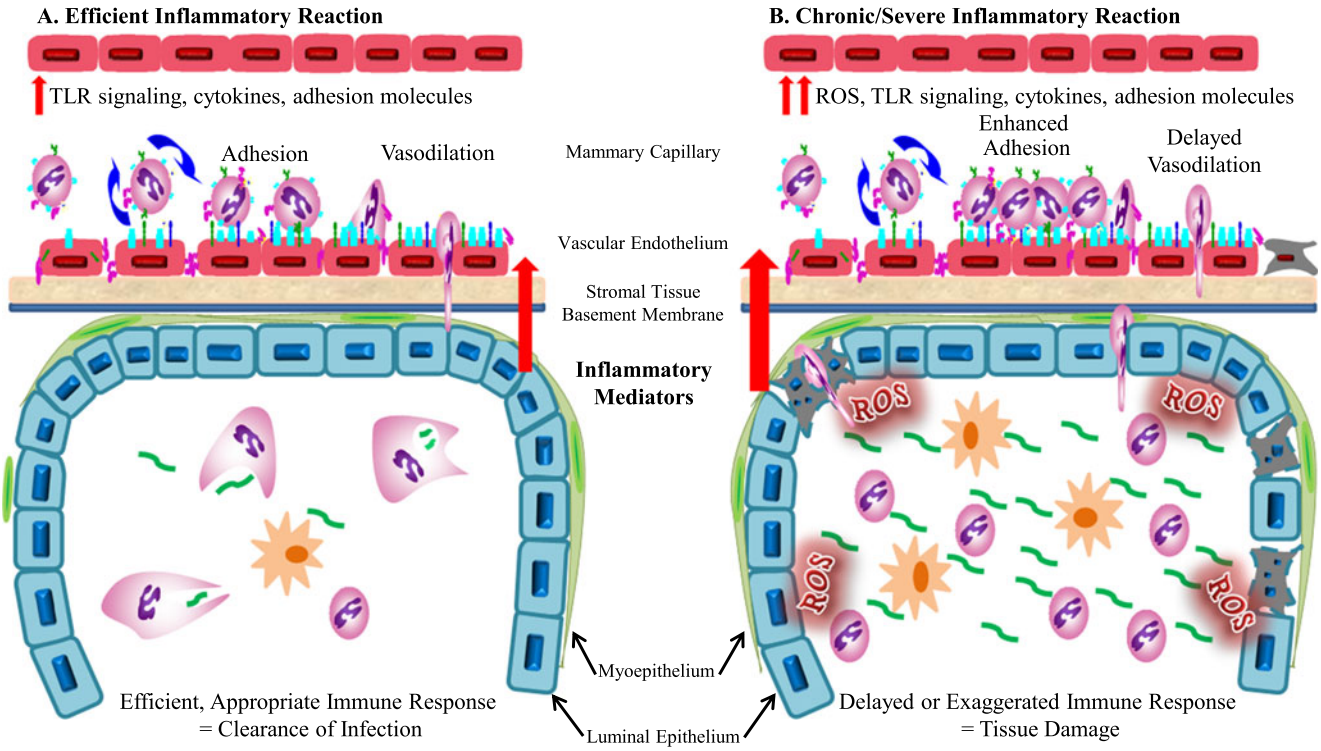

Figure 1 The mammary gland immune response to bacterial invasion results in prompt elimination and resolution of infection or delayed detection, rapid bacterial replication/growth, overproduction of inflammatory mediators and subsequent tissue damage. **a** Resident milk macrophages and neutrophils phagocytize invading pathogens. The immune system is activated following bacterial recognition by mammary macrophages and epithelial cells. A regulated release of cytokines and inflammatory mediators recruit leukocytes from the vasculature to the infection site and facilitate prompt bacterial removal with minimal damage to surrounding tissue. **b** Delayed or exaggerated immune responses, as occurs during the periparturient period, results in chronic or severe inflammation with subsequent tissue damage. Altered leukocyte migration or function promotes bacterial propagation. Excess release of bacterial toxins, inflammatory mediators, and leukocyte proteases, lysozymes and reactive oxygen species results in the breakdown of the blood-milk barrier and leads to irreversible epithelial and endothelial damage and ultimately lost milk production or death

to evade host bactericidal responses and promote bacterial survival within the mammary gland. Decreased ability to recognize pathogens alone or in combination with a reduced immune response to bacterial invasion may contribute to the ability of some mastitis-causing pathogens to evade the innate immune response and therefore result in subclinical, chronic mastitis.

Alternate to bacterial immune evasion with weakened host responses is an excess inflammatory response that causes local tissue damage or systemic consequences. Severe coliform infections are associated with an exacerbated inflammatory response following rapid bacterial growth. Mammary tissue damage may occur as a consequence of the release of bacterial toxins or bactericidal components from infiltrating inflammatory cells. Lipopolysaccharide triggers cellular signaling and upregulation of pro-inflammatory factors that, when produced in excess, may ultimately result in cellular damage or death [95]. For example, activation of NFκB in lactating murine mammary epithelium decreased milk production and was associated with apoptosis in involuting mammary epithelial cells [96]. A study by Long et al. (2001) reported increased expression of apoptotic factors in mammary tissue following experimental *E. coli* infection and is consistent with the findings of others that mammary tissue damage is induced by apoptosis or necrosis [97, 98]. Excess LPS release and NFκB activation during coliform mastitis may have an impact on decreased milk production through mammary epithelial apoptosis. Additionally, massive leukocyte influx during acute mastitis results in the release of reactive oxygen species, proteases and lysozymes causing indiscriminate damage to surrounding mammary tissue [97]. Mammary epithelial cell damage occurs secondary to activated leukocytes and release of reactive oxygen species such as myeloperoxidase and free radicals. Antioxidant supplementation reduced the severity of PMN cytotoxicity in mammary epithelial cells as well as MAC-T cells [99]. Preventing excessive leukocyte influx into the mammary gland will minimize mammary tissue damage and milk loss.

The vascular endothelium is a target of LPS released from replicating or dying gram-negative bacteria and is implicated in the pathogenesis of sepsis. Endothelial cells can respond to LPS ligation with the TLR4 signaling complex by either production of pro-inflammatory factors or initiation of endothelial apoptosis. Excess LPS can result in enhanced production of IL1, IL6 and TNFα. Many of the severe clinical symptoms of coliform mastitis are attributed to the actions of TNFα [100, 101]. Vascular dysfunction may be a result of excess exposure of the endothelium to TNFα resulting from enhanced leukocyte adhesion and transmigration, increased reactive oxygen and nitrogen species production, and ultimately apoptosis [102]. Although some cell types are susceptible to the apoptotic effect of TNFα, bovine mammary endothelial cells appear to have some resistance [75]. Recent studies suggest that upregulation of anti-apoptotic proteins may be a protective mechanism [103]. Prolonged exposure to high doses of TNF-α or in combination with other cytokines may leave bovine mammary endothelial cells vulnerable to apoptosis.

Vascular oxidative stress is associated with many human inflammatory-based diseases and may be another factor contributing to the severity of mastitis. An imbalance between reactive oxygen species and antioxidant production or availability results in oxidative stress. Excess reactive oxygen species production results in direct cellular and tissue damage from unstable molecules interacting with cellular nucleic acids, lipids, and proteins or may indirectly trigger intracellular signaling and promote a pro-inflammatory or pro-apoptotic cellular phenotype. Oxidative stress occurs in transition dairy cattle when several inflammatory-based diseases, such as mastitis, are most prevalent [104, 105]. Vitamin E and selenium, micronutrients with antioxidant functions, were related to decreased mastitis duration and severity in the periparturient period [106, 107]. Antioxidant supplementation decreases mammary epithelial cell cytotoxicity as previously discussed and also may protect the vascular endothelium against direct or indirect oxidant-induced damage.

Impacts of reactive oxygen species on the endothelium include increased permeability, increased adhesion of leukocytes, as well as altered cellular signaling and redox-regulation of transcription factors [108]. Neutrophil migration is a multi-step process that requires the upregulation of adhesion molecules on both the immune cell and the endothelium. Adhesion molecule gene expression changes in bovine mammary tissue during the transition period and is positively correlated with expression of several antioxidant enzymes, including selenoproteins [67]. Moreover, delayed neutrophil migration is associated with the severity of coliform mastitis [109]. Adhesion molecules in neutrophils have been studied extensively in the context of mastitis, however, little is known regarding their role in endothelial cells [110]. Selenium supplementation increased the speed of neutrophil migration into the bovine mammary gland during *E. coli* infection compared to selenium-deficient animals [106]. In bovine mammary and aortic endothelial cells, selenium deficiency causes oxidative stress and results in greater ICAM1 expression and neutrophil adherence [65, 78]. An immediate oxygenation product of 15LOX1 metabolism of arachidonic acid, 15-HPETE, upregulates ICAM1 expression in selenium-deficient bovine aortic endothelial cells [65]. Interestingly, 15LOX1 mRNA expression is significantly upregulated in early lactation cows compared to pre-partum cows [67]. Neutrophil-endothelial adhesion initiates intracellular signaling through adhesion molecules, such as ICAM-1, and enhanced expression of this adhesion molecule is associated with pathologic pro-inflammatory conditions [111]. Increased

expression of adhesion molecules and tight neutrophil-endothelial binding may indicate an inability to rapidly migrate to the infection site, thereby allowing bacterial growth and endotoxin release contributing to the severity of coliform mastitis.

Transmigration of leukocytes across the endothelium is necessary for mounting a proper immune response to infection. However, activation of immune cells in the process may have negative consequences on endothelial integrity. For example, activated neutrophils are able to kill pathogens by NET formation [112]. It is possible that release of these substances during the transmigration process may inadvertently cause endothelial damage. Activated human umbilical vein endothelial cells stimulated NET formation that consequently resulted in endothelial injury [113]. Excess neutrophil migration, NET formation and endothelial activation may contribute to endothelial damage during mastitis.

The speed of leukocyte influx into the mammary gland affects the outcome of mastitis. Downregulation of neutrophil adhesion molecules is known to contribute to periparturient immunosuppression and increased mastitis susceptibility [114]. Other mechanisms may be involved in the relative rapidity of leukocyte migratory responses, such as activation of the uroplasminogen cascade that is involved with breakdown of the basement membrane and extracellular matrix required for diapedesis and migration to the infection site. Ovine blood monocytes and neutrophils taken from healthy and mastitic ewes infected with *Strep. agalactiae* were analyzed for changes in genes associated with the plasminogen activation cascade [115]. Consistent with this theory, cells from mastitic ewes showed increased uroplasminogen and uroplasminogen receptor expression. Further research in this area is required to fully understand the mechanisms for impaired leukocyte migratory responses and increased susceptibility to mastitis.

Other factors contribute to the severity of mastitis and may involve complement components. High concentrations of complement component C5a are produced during sepsis [116]. In a recent study, C5a induced TLR4 signaling in bovine neutrophils and resulted in the production of IL8 in the absence of other stimulatory factors [117]. The ability of complement components to initiate TLR4 signaling may contribute to the severity of mastitis during prolonged inflammation. In contrast, C5a was not detected in milk following intramammary *S. aureus* infection [118]. This likely is secondary to a suboptimal immune response and decreased serum proteins in the mammary gland following infection. Human-specific *S. aureus* strains possess additional virulence factors, such as staphylococcal complement inhibitor that blocks C3b formation protecting it from neutrophil phagocytosis [119]. Future research in this area may reveal the significance of complement to mastitis pathology.

Conclusions

Mammary gland innate and adaptive immune responses are complex and highly interconnected. Optimal host defenses against mastitis-causing pathogens occur when mammary immune mechanisms are tightly regulated to effectively eliminate the injurious insults and return the immune system to homeostasis. Rapid resolution of intramammary infections will eliminate bystander tissue damage and prevent any noticeable changes to milk quantity or quality. Some mastitis-causing pathogens, however, have intrinsic properties that make the efficient elimination by the immune system difficult, and attempts by local mammary gland defenses to achieve control often results in significant tissue damage and reduced milk production. Whereas antibiotic therapy remains the mainstay for the treatment of mastitis in both human and veterinary medicine, there is a need for alternative and adjunct therapeutic options that target host immune responses. The challenge is to selectively down-modulate harmful host responses without diminishing beneficial responses that facilitate elimination of invading pathogens. In contrast to antimicrobial drugs used to treat mastitis, strategies that target host responses will minimize the risk that drug resistant bacteria will emerge.

Acknowledgements This work was supported in part by grants from the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number #2007-35204-18463, the Agriculture and Food Research Initiative Competitive Grants Program, grant number #2011-67015-30179, and by an endowment from the Matilda R. Wilson Fund (Detroit, MI).

References

1. Foxman B, D'Arcy H, Gillespie B, Bobo JK, Schwartz K. Lactation mastitis: occurrence and medical management among 946 breastfeeding women in the United States. Am J Epidemiol. 2002;155(2):103–14.
2. Inch S, von Xylander S. Mastitis: Causes and Management. Geneva, Switzerland: World Health Organization (WHO) Department of child and adolescent health and development; 2000.
3. Mavrogianni VS, Menzies PI, Fragkou IA, Fthenakis GC. Principles of mastitis treatment in sheep and goats. Vet Clin North Am Food Anim Pract. 2011;27(1):115–20.
4. Hogeveen H, Huijps K, Lam TJ. Economic aspects of mastitis: new developments. N Z Vet J. 2011;59(1):16–23.
5. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol. 2009;128(1–3):104–9.
6. Sordillo LM, Contreras GA, Aitken SL. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Health Res Rev. 2009;10(1):53–63.
7. Sordillo LM, Streicher KL. Mammary gland immunity and mastitis susceptibility. J Mammary Gland Biol Neoplasia. 2002;7(2):135–46.

8. Contreras GA, Sordillo LM. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp Immunol Microbiol Infect Dis. 2011;34(3):281–9.
9. Oliver SP, Sordillo LM. Udder health in the periparturient period. J Dairy Sci. 1988;71(9):2584–606.
10. Bannerman DD. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J Anim Sci. 2009;87(13 Suppl):10–25.
11. Capuco AV, Bright SA, Pankey JW, Wood DL, Miller RH, Bitman J. Increased susceptibility to intramammary infection following removal of teat canal keratin. J Dairy Sci. 1992;75 (8):2126–30.
12. Bramley AJ, Dodd FH. Reviews of the progress of dairy science: mastitis control–progress and prospects. J Dairy Res. 1984;51 (3):481–512.
13. Hogan JS, Duthie AH, Pankey JW. Fatty acid composition of bovine teat canal keratin. J Dairy Sci. 1986;69(9):2424–7.
14. Chaneton L, Tirante L, Maito J, Chaves J, Bussmann LE. Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. J Dairy Sci. 2008;91(5):1865–73.
15. Pecorini C, Sassera D, Rebucci R, Saccone F, Bandi C, Baldi A. Evaluation of the protective effect of bovine lactoferrin against lipopolysaccharides in a bovine mammary epithelial cell line. Vet Res Commun. 2010;34(3):267–76.
16. Patel D, Almeida RA, Dunlap JR, Oliver SP. Bovine lactoferrin serves as a molecular bridge for internalization of Streptococcus uberis into bovine mammary epithelial cells. Vet Microbiol. 2009;137(3–4):297–301.
17. Sordillo LM, Nickerson SC, Akers RM, Oliver SP. Secretion composition during bovine mammary involution and the relationship with mastitis. Int J Biochem. 1987;19(12):1165–72.
18. Chaneton L, Perez Saez JM, Bussmann LE. Antimicrobial activity of bovine beta-lactoglobulin against mastitis-causing bacteria. J Dairy Sci. 2011;94(1):138–45.
19. Riollet C, Rainard P, Poutrel B. Differential induction of complement fragment C5a and inflammatory cytokines during intramammary infections with Escherichia coli and Staphylococcus aureus. Clin Diagn Lab Immunol. 2000;7(2):161–7.
20. Rainard P. The complement in milk and defense of the bovine mammary gland against infections. Vet Res. 2003;34 (5):647–70.
21. Sordillo LM, Shafer-Weaver K, DeRosa D. Immunobiology of the mammary gland. J Dairy Sci. 1997;80(8):1851–65.
22. Alluwaimi AM. The cytokines of bovine mammary gland: prospects for diagnosis and therapy. Res Vet Sci. 2004;77 (3):211–22.
23. Riollet C, Rainard P, Poutrel B. Cells and cytokines in inflammatory secretions of bovine mammary gland. Adv Exp Med Biol. 2000;480:247–58.
24. Watson CJ, Oliver CH, Khaled WT. Cytokine signalling in mammary gland development. J Reprod Immunol. 2011;88 (2):124–9.
25. Babiuk LA, Sordillo LM, Campos M, Hughes HP, Rossi-Campos A, Harland R. Application of interferons in the control of infectious diseases of cattle. J Dairy Sci. 1991;74(12):4385–98.
26. Shafer-Weaver KA, Corl CM, Sordillo LM. Shifts in bovine CD4+ subpopulations increase T-helper-2 compared with T-helper-1 effector cells during the postpartum period. J Dairy Sci. 1999;82(8):1696–706.
27. Sordillo LM, Pighetti GM, Davis MR. Enhanced production of bovine tumor necrosis factor-alpha during the periparturient period. Vet Immunol Immunopathol. 1995;49(3):263–70.
28. Varela LM, Ip MM. Tumor necrosis factor-alpha: a multifunctional regulator of mammary gland development. Endocrinology. 1996;137(11):4915–24.
29. Plath A, Einspanier R, Peters F, Sinowatz F, Schams D. Expression of transforming growth factors alpha and beta-1 messenger RNA in the bovine mammary gland during different stages of development and lactation. J Endocrinol. 1997;155(3):501–11.
30. Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G, Smith GH. Ectopic TGF beta 1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. Dev Biol. 1995;168(1):47–61.
31. Paape M, Mehrzad J, Zhao X, Detilleux J, Burvenich C. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J Mammary Gland Biol Neoplasia. 2002;7(2):109–21.
32. De Vries LD, Dover H, Casey T, VandeHaar MJ, Plaut K. Characterization of mammary stromal remodeling during the dry period. J Dairy Sci. 2011;93(6):2433–43.
33. Levy L, Hill CS. Smad4 dependency defines two classes of transforming growth factor beta (TGF-{beta}) target genes and distinguishes TGF-{beta}-induced epithelial-mesenchymal transition from its antiproliferative and migratory responses. Mol Cell Biol. 2005;25(18):8108–25.
34. Sordillo LM, Nickerson SC. Quantification and immunoglobulin classification of plasma cells in nonlactating bovine mammary tissue. J Dairy Sci. 1988;71(1):84–91.
35. Zhang S, Mao Y, Huang J, Ma T, Zhang L, Zhu X, et al. Immunoglobulin gene locus events in epithelial cells of lactating mouse mammary glands. Cell Mol Life Sci. 2010;67(6):985–94.
36. Fetherston CM, Lai CT, Hartmann PE. Recurrent blocked duct(s) in a mother with immunoglobulin A deficiency. Breastfeed Med. 2008;3(4):261–5.
37. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34.
38. Jungi TW, Farhat K, Burgener IA, Werling D. Toll-like receptors in domestic animals. Cell Tissue Res. 2011;343(1):107–20.
39. Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol. 2007;7(3):179–90.
40. Bannerman DD, Goldblum SE. Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. Am J Physiol Lung Cell Mol Physiol. 2003;284(6):L899–914.
41. Vidal K, Donnet-Hughes A. CD14: a soluble pattern recognition receptor in milk. Adv Exp Med Biol. 2008;606:195–216.
42. Li C, Wang Y, Gao L, Zhang J, Shao J, Wang S, et al. Expression of toll-like receptors 2 and 4 and CD14 during differentiation of HL-60 cells induced by phorbol 12-myristate 13-acetate and 1 alpha, 25-dihydroxy-vitamin D(3). Cell Growth Differ. 2002;13 (1):27–38.
43. Wang Y, Zarlenga DS, Paape MJ, Dahl GE. Recombinant bovine soluble CD14 sensitizes the mammary gland to lipopolysaccharide. Vet Immunol Immunopathol. 2002;86(1–2):115–24.
44. Lee JW, Paape MJ, Elsasser TH, Zhao X. Recombinant soluble CD14 reduces severity of intramammary infection by Escherichia coli. Infect Immun. 2003;71(7):4034–9.
45. Wall R, Powell A, Sohn E, Foster-Frey J, Bannerman D, Paape M. Enhanced host immune recognition of mastitis causing Escherchia coli in CD-14 transgenic mice. Anim Biotechnol. 2009;20(1):1–14.
46. Nemchinov LG, Paape MJ, Sohn EJ, Bannerman DD, Zarlenga DS, Hammond RW. Bovine CD14 receptor produced in plants reduces severity of intramammary bacterial infection. FASEB J. 2006;20(9):1345–51.
47. Harizi H, Corcuff JB, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. Trends Mol Med. 2008;14(10):461–9.
48. Zia S, Giri SN, Cullor J, Emau P, Osburn BI, Bushnell RB. Role of eicosanoids, histamine, and serotonin in the pathogenesis of Klebsiella pneumoniae-induced bovine mastitis. Am J Vet Res. 1987;48(11):1617–25.

49. Maddox JF, Reddy CC, Eberhart RJ, Scholz RW. Dietary selenium effects on milk eicosanoid concentration in dairy cows during coliform mastitis. Prostaglandins. 1991;42(4):369–78.
50. Atroshi F, Parantainen J, Sankari S, Osterman T. Prostaglandins and glutathione peroxidase in bovine mastitis. Res Vet Sci. 1986;40(3):361–6.
51. Atroshi F, Rizzo A, Kangasniemi R, Sankari S, Tyopponen T, Osterman T, et al. Role of plasma fatty acids, prostaglandins and antioxidant balance in bovine mastitis. Zentralbl Veterinarmed A. 1989;36(9):702–11.
52. Boutet P, Bureau F, Degand G, Lekeux P. Imbalance between lipoxin A4 and leukotriene B4 in chronic mastitis-affected cows. J Dairy Sci. 2003;86(11):3430–9.
53. Peter AT, Clark PW, Van Roekel DE, Luker CW, Gaines JD, Bosu WT. Temporal changes in metabolites of prostanoids in milk of heifers after intramammary infusion of Escherichia coli organisms. Prostaglandins. 1990;39(4):451–7.
54. Pattanaik U, Prasad K. Oxygen Free Radicals and Endotoxic Shock: Effect of Flaxseed. J Cardiovasc Pharmacol Ther. 1998;3 (4):305–18.
55. Banting A, Banting S, Heinonen K, Mustonen K. Efficacy of oral and parenteral ketoprofen in lactating cows with endotoxin-induced acute mastitis. Vet Rec. 2008;163(17):506–9.
56. Vangroenweghe F, Duchateau L, Boutet P, Lekeux P, Rainard P, Paape MJ, et al. Effect of carprofen treatment following experimentally induced Escherichia coli mastitis in primiparous cows. J Dairy Sci. 2005;88(7):2361–76.
57. Wagner SA, Apley MD. Effects of two anti-inflammatory drugs on physiologic variables and milk production in cows with endotoxin-induced mastitis. Am J Vet Res. 2004;65(1):64–8.
58. McDougall S, Bryan MA, Tiddy RM. Effect of treatment with the nonsteroidal antiinflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis. J Dairy Sci. 2009;92 (9):4421–31.
59. Kühn H. Biosynthesis, metabolization and biological importance of the primary 15-lipoxygenase metabolites 15-hydro (pero)xy-5Z,8Z,11Z,13E-eicosatetraenoic acid and 13-hydro (pero)xy-9Z,11E-octadecadienoic acid. Progr Lipid Res. 1996;35(3):203–26.
60. Natarajan R, Nadler JL. Lipid inflammatory mediators in diabetic vascular disease. Arterioscler Thromb Vasc Biol. 2004;24(9):1542–8.
61. Wittwer J, Hersberger M. The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot Essent Fatty Acids. 2007;77(2):67–77.
62. Spector AA, Gordon JA, Moore SA. Hydroxyeicosatetraenoic acids (HETEs). Prog Lipid Res. 1988;27(4):271–323.
63. Reilly KB, Srinivasan S, Hatley ME, Patricia MK, Lannigan J, Bolick DT, et al. 12/15-Lipoxygenase activity mediates inflammatory monocyte/endothelial interactions and atherosclerosis in vivo. J Biol Chem. 2004;279(10):9440–50.
64. Cao YZ, Reddy CC, Sordillo LM. Altered eicosanoid biosynthesis in selenium-deficient endothelial cells. Free Radic Biol Med. 2000;28(3):381–9.
65. Sordillo LM, Streicher KL, Mullarky IK, Gandy JC, Trigona W, Corl CM. Selenium inhibits 15-hydroperoxyoctadecadienoic acid-induced intracellular adhesion molecule expression in aortic endothelial cells. Free Radic Biol Med. 2008;44(1):34–43.
66. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8(5):349–61.
67. Aitken SL, Karcher EL, Rezamand P, Gandy JC, VandeHaar MJ, Capuco AV, et al. Evaluation of antioxidant and proinflammatory gene expression in bovine mammary tissue during the periparturient period. J Dairy Sci. 2009;92(2):589–98.
68. Prosser CG, Davis SR, Farr VC, Lacasse P. Regulation of blood flow in the mammary microvasculature. J Dairy Sci. 1996;79 (7):1184–97.
69. Naccarato AG, Viacava P, Bocci G, Fanelli G, Aretini P, Lonobile A, et al. Definition of the microvascular pattern of the normal human adult mammary gland. J Anat. 2003;203 (6):599–603.
70. Stirling JW, Chandler JA. The fine structure of the normal, resting terminal ductal-lobular unit of the female breast. Virchows Arch A Pathol Anat Histol. 1976;372(3):205–26.
71. Matsumoto M, Kurohmaru M, Hayashi Y, Nishinakagawa H, Otsuka J. Permeability of mammary gland capillaries to ferritin in mice. J Vet Med Sci. 1994;56(1):65–70.
72. Matsumoto M, Nishinakagawa H, Kurohmaru M, Hayashi Y, Otsuka J. Pregnancy and lactation affect the microvasculature of the mammary gland in mice. J Vet Med Sci. 1992;54(5):937–43.
73. Ludewig T. Light and electron microscopic investigations of the blood-milk barrier in lactating cow udders. Anat Histol Embryol. 1996;25(2):121–6.
74. Abdul Awal M, Matsumoto M, Toyoshima Y, Nishinakagawa H. Ultrastructural and morphometrical studies on the endothelial cells of arteries supplying the abdomino-inguinal mammary gland of rats during the reproductive cycle. J Vet Med Sci. 1996;58(1):29–34.
75. Aitken SL, Corl CM, Sordillo LM. Pro-inflammatory and pro-apoptotic responses of TNF-alpha stimulated bovine mammary endothelial cells. Vet Immunol Immunopathol. 2011;140(3–4):282–90.
76. Corl CM, Contreras GA, Sordillo LM. Lipoxygenase metabolites modulate vascular-derived platelet activating factor production following endotoxin challenge. Vet Immunol Immunopathol. 2010;136(1–2):98–107.
77. Corl CM, Gandy JC, Sordillo LM. Platelet activating factor production and proinflammatory gene expression in endotoxin-challenged bovine mammary endothelial cells. J Dairy Sci. 2008;91(8):3067–78.
78. Maddox JF, Aherne KM, Reddy CC, Sordillo LM. Increased neutrophil adherence and adhesion molecule mRNA expression in endothelial cells during selenium deficiency. J Leukoc Biol. 1999;65(5):658–64.
79. Sordillo LM, Doymaz MZ, Oliver SP. Morphological study of chronic Staphylococcus aureus mastitis in the lactating bovine mammary gland. Res Vet Sci. 1989;47(2):247–52.
80. Sordillo LM, Nickerson SC, Akers RM. Pathology of Staphylococcus aureus mastitis during lactogenesis: relationships with bovine mammary structure and function. J Dairy Sci. 1989;72 (1):228–40.
81. Rinaldi M, Li RW, Bannerman DD, Daniels KM, Evock-Clover C, Silva MV, et al. A sentinel function for teat tissues in dairy cows: dominant innate immune response elements define early response to E. coli mastitis. Funct Integr Genomics. 2010;10(1):21–38.
82. Whelehan CJ, Meade KG, Eckersall PD, Young FJ, O'Farrelly C. Experimental Staphylococcus aureus infection of the mammary gland induces region-specific changes in innate immune gene expression. Vet Immunol Immunopathol. 2011;140(3–4):181–9.
83. Ibeagha-Awemu EM, Lee JW, Ibeagha AE, Bannerman DD, Paape MJ, Zhao X. Bacterial lipopolysaccharide induces increased expression of toll-like receptor (TLR) 4 and downstream TLR signaling molecules in bovine mammary epithelial cells. Vet Res. 2008;39(2):11.
84. Yang W, Zerbe H, Petzl W, Brunner RM, Gunther J, Draing C, et al. Bovine TLR2 and TLR4 properly transduce signals from Staphylococcus aureus and E. coli, but S. aureus fails to both activate NF-kappaB in mammary epithelial cells and to quickly induce TNFalpha and interleukin-8 (CXCL8) expression in the udder. Mol Immunol. 2008;45(5):1385–97.

85. Gunther J, Esch K, Poschadel N, Petzl W, Zerbe H, Mitterhuemer S, et al. Comparative kinetics of Escherichia coli- and Staphylococcus aureus-specific activation of key immune pathways in mammary epithelial cells demonstrates that S. aureus elicits a delayed response dominated by interleukin-6 (IL-6) but not by IL-1A or tumor necrosis factor alpha. Infect Immun. 2011;79(2):695–707.
86. Bougarn S, Cunha P, Harmache A, Fromageau A, Gilbert FB, Rainard P. Muramyl dipeptide synergizes with Staphylococcus aureus lipoteichoic acid to recruit neutrophils in the mammary gland and to stimulate mammary epithelial cells. Clin Vaccine Immunol. 2010;17(11):1797–809.
87. Petzl W, Zerbe H, Gunther J, Yang W, Seyfert HM, Nurnberg G, et al. Escherichia coli, but not Staphylococcus aureus triggers an early increased expression of factors contributing to the innate immune defense in the udder of the cow. Vet Res. 2008;39(2):18.
88. Goldammer T, Zerbe H, Molenaar A, Schuberth HJ, Brunner RM, Kata SR, et al. Mastitis increases mammary mRNA abundance of beta-defensin 5, toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clin Diagn Lab Immunol. 2004;11(1):174–85.
89. Mehrzad J, Duchateau L, Burvenich C. High milk neutrophil chemiluminescence limits the severity of bovine coliform mastitis. Vet Res. 2005;36(1):101–16.
90. Lippolis JD, Reinhardt TA, Goff JP, Horst RL. Neutrophil extracellular trap formation by bovine neutrophils is not inhibited by milk. Vet Immunol Immunopathol. 2006;113(1–2):248–55.
91. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532–5.
92. Gonen E, Vallon-Eberhard A, Elazar S, Harmelin A, Brenner O, Rosenshine I, et al. Toll-like receptor 4 is needed to restrict the invasion of Escherichia coli P4 into mammary gland epithelial cells in a murine model of acute mastitis. Cell Microbiol. 2007;9 (12):2826–38.
93. Sladek Z, Rysanek D, Ryznarova H, Faldyna M. Neutrophil apoptosis during experimentally induced Staphylococcus aureus mastitis. Vet Res. 2005;36(4):629–43.
94. Barrio MB, Rainard P, Prevost G. LukM/LukF'-PV is the most active Staphylococcus aureus leukotoxin on bovine neutrophils. Microbes Infect. 2006;8(8):2068–74.
95. Sordillo LM, Peel JE. Effect of interferon-gamma on the production of tumor necrosis factor during acute Escherichia coli mastitis. J Dairy Sci. 1992;75(8):2119–25.
96. Clarkson RW, Watson CJ. NF-kappaB and apoptosis in mammary epithelial cells. J Mammary Gland Biol Neoplasia. 1999;4 (2):165–75.
97. Zhao X, Lacasse P. Mammary tissue damage during bovine mastitis: causes and control. J Anim Sci. 2008;86(13 Suppl):57–65.
98. Long E, Capuco AV, Wood DL, Sonstegard T, Tomita G, Paape MJ, et al. Escherichia coli induces apoptosis and proliferation of mammary cells. Cell Death Differ. 2001;8(8):808–16.
99. Lauzon K, Zhao X, Bouetard A, Delbecchi L, Paquette B, Lacasse P. Antioxidants to prevent bovine neutrophil-induced mammary epithelial cell damage. J Dairy Sci. 2005;88(12):4295–303.
100. Blum JW, Dosogne H, Hoeben D, Vangroenweghe F, Hammon HM, Bruckmaier RM, et al. Tumor necrosis factor-alpha and nitrite/nitrate responses during acute mastitis induced by Escherichia coli infection and endotoxin in dairy cows. Domest Anim Endocrinol. 2000;19(4):223–35.
101. Wenz JR, Fox LK, Muller FJ, Rinaldi M, Zeng R, Bannerman DD. Factors associated with concentrations of select cytokine and acute phase proteins in dairy cows with naturally occurring clinical mastitis. J Dairy Sci. 2010;93(6):2458–70.
102. Madge LA, Pober JS. TNF signaling in vascular endothelial cells. Exp Mol Pathol. 2001;70(3):317–25.
103. Daniel S, Arvelo MB, Patel VI, Longo CR, Shrikhande G, Shukri T, et al. A20 protects endothelial cells from TNF-, Fas-, and NK-mediated cell death by inhibiting caspase 8 activation. Blood. 2004;104(8):2376–84.
104. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. Vet J. 2005;169(2):286–92.
105. Sordillo LM, O'Boyle N, Gandy JC, Corl CM, Hamilton E. Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J Dairy Sci. 2007;90(3):1186–92.
106. Erskine RJ, Eberhart RJ, Grasso PJ, Scholz RW. Induction of Escherichia coli mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am J Vet Res. 1989;50(12):2093–100.
107. Weiss WP, Hogan JS, Todhunter DA, Smith KL. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J Dairy Sci. 1997;80(8):1728–37.
108. Boueiz A, Hassoun PM. Regulation of endothelial barrier function by reactive oxygen and nitrogen species. Microvasc Res. 2009;77(1):26–34.
109. Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A, Duchateau L. Severity of E. coli mastitis is mainly determined by cow factors. Vet Res. 2003;34(5):521–64.
110. Diez-Fraile A, Meyer E, Burvenich C. Regulation of adhesion molecules on circulating neutrophils during coliform mastitis and their possible immunomodulation with drugs. Vet Immunol Immunopathol. 2002;86(1–2):1–10.
111. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histol Histopathol. 2008;23 (3):381–90.
112. Medina E. Neutrophil extracellular traps: a strategic tactic to defeat pathogens with potential consequences for the host. J Innate Immun. 2009;1(3):176–80.
113. Gupta AK, Joshi MB, Philippova M, Erne P, Hasler P, Hahn S, et al. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. FEBS Lett. 2010;584(14):3193–7.
114. Burton JL, Kehrli Jr ME, Kapil S, Horst RL. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J Leukoc Biol. 1995;57 (2):317–25.
115. Theodorou G, Daskalopoulou M, Chronopoulou R, Baldi A, Dell'orto V, Politis I. Acute mastitis induces upregulation of expression of plasminogen activator-related genes by blood monocytes and neutrophils in dairy ewes. Vet Immunol Immunopathol. 2010;138(1–2):124–8.
116. Guo RF, Riedemann NC, Ward PA. Role of C5a-C5aR interaction in sepsis. Shock. 2004;21(1):1–7.
117. Stevens MG, Van Poucke M, Peelman LJ, Rainard P, De Spiegeleer B, Rogiers C, et al. Anaphylatoxin C5a-induced toll-like receptor 4 signaling in bovine neutrophils. J Dairy Sci. 2011;94 (1):152–64.
118. Bannerman DD, Paape MJ, Lee JW, Zhao X, Hope JC, Rainard P. Escherichia coli and Staphylococcus aureus elicit differential innate immune responses following intramammary infection. Clin Diagn Lab Immunol. 2004;11(3):463–72.
119. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of Staphylococcus aureus are located on beta-hemolysin-converting bacteriophages. J Bacteriol. 2006;188(4):1310–5.
120. Lee JW, Paape MJ, Elsasser TH, Zhao X. Elevated milk soluble CD14 in bovine mammary glands challenged with Escherichia coli lipopolysaccharide. J Dairy Sci. 2003;86(7):2382–9.
121. Dziarski R, Gupta D. The peptidoglycan recognition proteins (PGRPs). Genome Biol. 2006;7(8):232.