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Pathophysiological Role of Cytokines in Bovine Mastitis

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Abstract

Cytokines are important agents in regulating immune response in many diseases, and knowledge of relevant cytokine immune networks is necessary for understanding processes encompassed within their pathophysiology. One such disease is mastitis, which is inflammation of the mammary gland and greatly impacts the quantity and quality of milk produced by dairy cows. Understanding the cytokine immune network will be helpful in developing effective immunotherapy for mastitis, which is particularly important in view of the increasing antibiotic resistance among the bacterial pathogens causing this disease.

Keywords: Cytokines, dairy cows, inflammation, mastitis.

Introduction

Cytokines are very important peptides or proteins affecting many processes within the body. They play pivotal roles in the development, homeostasis, activation, differentiation, regulation, and functions of innate and adaptive immunity. Identification and discovery of cytokines' pathophysiological roles are opening innovative frontiers in diagnosis and therapy (Alluwaimi, 2004).

Cytokines are involved, too, in the pathogenesis of inflammation. In particular, inflammation of the mammary gland (mastitis) is affected by numerous cytokines. Because mastitis is a costly disease in lactating cows and decreases the quality and quantity of milk produced, it is very important to study the pathophysiological roles of cytokines in mastitis (Sordillo, 1997). The use of cytokines as a tool in dairy herd health management is a prospective alternative to conventional methods of diagnosis and treatment. Their use in the diagnosis, immunotherapy, and prognosis of mastitis will grow with knowledge of the cytokine network in bovine mammary glands and development of effective cytokine diagnostic techniques (Alluwaimi, 2004).

Because mastitis is a multifactorial disease, the quality of feed for cattle is also very important. A prerequisite for high-quality fodder is clean and healthy phytomass. Epiphyte flora of plants comprise a number of microorganisms, including such undesirable microorganisms as clostridia and fungi. Development of microscopic fungi may lead to the formation of mycotoxins, which are secondary metabolites produced especially by fungi from the genera *Aspergillus*, *Penicillium*, and *Fusarium*. Mycotoxin production is caused by interactions and reactions of fungi to environmental conditions. Production of mycotoxins is associated with stress caused by extreme weather conditions or damage caused by insects or animals. Mycotoxins have a naturally negative impact on livestock. They cause alterations in hormonal functions, poor feed utilization, lower weight gain, and possibly death. Moreover, some mycotoxins may pass into the milk (Skladanka at al., 2011; Skladanka et al., 2012, Skladanka et al., 2013).

1. Functions of cytokines in the course of mastitis

1.1. Granulocyte-monocyte colony stimulating factor

Granulocyte-monocyte colony-stimulating factor (GM-CSF) is involved in the proliferation and differentiation of normal haematopoietic cells (Weisbart, 1985). The role of GM-CSF in udder health is not clear. Use of this cytokine for the diagnosis and prognosis of mastitis is not unequivocal due to the cytokine's minor participation in the mammary gland immune system (Alluwaimi, 2004). Bovine GM-CSF mRNA has been detected in bovine macrophages and monocytes (Ito and Kodama, 1996). GM-CSF has been discovered in healthy bovine mammary gland during the middle and late stages of lactation and with a significant increase in transcriptional activity during the late stage (Alluwaimi, 2002). The role of this cytokine in *Staphylococcus aureus* mastitis was observed to be miniscule (Alluwaimi, 2003). GM-CSF has a similar effect in coliform mastitis. Nonetheless, another ruminant (ewe) has displayed a significant increase in its expression (Persson and Colditz, 1999). Expression of GM-CSF is under the control of NF-κB, which has been confirmed by findings that the expression of GM-CSF is correlated with elevated NF-κB activity in neutrophils from cows with chronic mastitis (Boulanger, 2003). Recombinant bovine GM-CSF increases chemotactic and bactericidal activities and superoxide anion production by milk neutrophils (Sordillo et al., 1992). Stimulation of mammary glands by GM-CSF infected with *S. aureus* has shown that this cytokine activates the formation of oxygen radicals in the resident neutrophils (Daley et al., 1993). The effect on neutrophil influx into the mammary gland is not clear. Daley et al.1993 13 did not find any effect, but Kehrli et al. (1991) showed that GM-CSF increases the influx of neutrophils and enhances their antibacterial activity. Some authors (Sordillo et al., 1992; Hirai et al., 1999) have reported that recombinant bovine GM-CSF activates the bactericidal activities of blood neutrophils in both in vitro and in vivo experiments, thus suggesting that this recombinant cytokine could be a therapeutic agent in mastitic cows. Intra-mammary injection of recombinant bovine GM-CSF is effective in reducing *S. aureus* shedding at an early stage of subclinical mastitis, but it is less effective during late stages of *S. aureus* infection. Therefore, the extent to which an animal can be cured is related to establishing rates of fixation and survival of *S. aureus* in the mammary gland tissues and host cells. Another very important factor is a progressing inefficiency of host neutrophils in cases of long-lasting bacterial infection (Takahashi et al., 2004). Potential use of recombinant bovine GM-CSF for mastitis therapy has also been supported by other authors (Ozawa et al., 2012).

1.2. Interferon-γ

Interferon-γ (IFN-γ) plays an important role in the connection between innate and adaptive immunity. This cytokine is crucial for host immunity against intracellular pathogens (Bannerman, 2009). Sources of IFN-γ include lymphocytes, natural killer (NK) cells, as well as monocytes and/or macrophages (Schoenborn and Wilson, 2007). IFN-γ improves the microbicidal activity of neutrophils and macrophages by increasing phagocytosis, inducing respiratory bursts, and priming nitric oxide production (Ellis and Beaman, 2004; Shroder et al., 2004). IFN-γ also upregulates the expression of MHC I and MHC II (Shroder et al., 2004). Intramammary infusion of recombinant bovine IFN-γ has a large impact on the activation of T cells and IL-12 production. Moreover, there is increased expansion of clonal and antigen specific memory cells (Pighetti and Sordillo, 1996). Although experimentally induced *S. aureus* mastitis dampens the transcriptional activity of IFN-γ (Alluwaimi et al., 2003), in the case of coliform mastitis IFN-γ production is significantly elevated (Hisaeda et al., 2001). Intramammary infection with various strains of *S. aureus* causes different expression of IFN-γ in circulation and in casein-depleted milk, thereby demonstrating a unique host immune response (Kim et al., 2011). IFN-γ improves some facets of milk macrophage activation (Denis et al., 2006). Moreover, this cytokine decreases TNF-α production by bovine milk macrophages as a response to bacterial products (Pighetti and Sordillo, 1994). The weakness of IFN-γ in activating milk macrophages suggests that vigorous IFN-γ production in the infected mammary gland would be insufficient to fortify milk macrophages with stronger bactericidal activity against *S. aureus* (Denis et al., 2006).

1.3. Interleukin-1

Interleukin-1 (IL-1) exists in two isoforms – IL-1α and IL-1β – which have been detected in normal milk cells using real-time polymerase chain reaction (Okada et al., 1997). IL-1 is a very important agent in the inflammatory process of mammary glands infused with endotoxin or with coliform mastitis (Riollet et al., 2000; Persson et al., 2003). Rapid increase in IL-1 is associated with an influx of neutrophils during *Escherichia coli* infection (Riollet et al., 2000). In this infection, IL-1 is indirectly involved in the chemoattraction of neutrophils (Shuster et al., 1997). Release of IL-1 by monocytes has been detected to be more intensive than that by macrophages following stimulation with lipopolysaccharide (Politis et al., 1991). This cytokine plays a minor role in *S. aureus* mastitis, which is indicated by the slight contribution of the IL-1 response in this type of mastitis (Riollet et al., 2001). IL-1 is involved in mediating signs of acute septic shock (Ohtsuka et al., 2001). Monitoring IL-1 concentrations is useful in defining the stage of coliform mastitis and the effectively of the therapeutics (Alluwaimi, 2004). Infusion of IL-1β into a mammary gland chronically infected with *S. aureus* increases neutrophil influx and upregulates inducible oxygen radical formation, but intramammary infusion of this agent has no effect on phagocyte effectively (Daley et al., 1993). IL-1 receptor antagonist abrogates IL-1 activity in mastitis caused by an endotoxin, but it has little effect on mastitis (Shuster and Kehrli, 1995). IL-1 receptor antagonist fails to prevent endotoxin-induced neutrophil accumulation by itself but causes inhibition if administrated simultaneously with recombinant IL-1 (Persson, 1997). Efficient IL-1 receptor antagonist therapy depends on its being present prior to IL-1 accumulation (Shuster and Kehrli, 1995).

IL-1α generally remains intracellular and IL-1β is generally secreted. IL-1α predominantly regulates intracellular events and mediates local inflammation, whereas IL-1β mediates both local and systemic inflammatory responses (Bannerman, 2009; Bannerman et al., 2004). Induction of IL-1β is delayed following intramammary infusion of Gram-positive bacteria compared to infection with Gram-negative bacteria, but the relevance of the IL-1β response is comparable (Rambeaud et al., 2003; Kauf et al., 2007). IL-1β is generally known to be produced by dendritic cells, macrophages, and neutrophils (Petrilli and Martinon, 2007). Some authors have suggested that *S. aureus* is able to induce IL-1β mRNA expression in bovine mammary gland epithelial cells (Strandberg et al., 2005; Mazzilli and Zecconi, 2010). Nevertheless, it is still not known whether IL-1β is also produced and secreted due to stimulation with *S. aureus* (Kim et al., 2011).

1.4. Interleukin-2

Interleukin-2 (IL-2) is secreted by activated T cells which induce replication and clonal differentiation of other T helper and T cytotoxic cells (Rompato et al., 2006). IL-2 plays a pivotal role in immunoregulation and the adaptive immune response (Alluwaimi, 2004). Bovine IL-2 has been found in both normal and mastitic mammary gland cells, and its transcriptional activity in bovine mammary gland is increased late in lactation (Alluwaimi and Cullor, 2002). When comparing IL-2 levels pre- and postpartum, the prepartum level is lower (Sordillo et al., 1991). While low-dose IL-2 treatment in the supramammary lymph node after calving has no negative side effect on either cows or milk quality, this type of treatment can increase mammary gland immune defences and frequency of healthy quarters in the first 2 weeks after calving (Zecconi et al., 2009).

IL-2 has been detected in coliform as well as *S. aureus* mastitis (Alluwaimi et al., 2003; Riollet et al., 2000). IL-2-treated lymphocytes have elevated expression of MHC II (Sordillo et al., 1991). Neutrophils from IL-2-treated quarters exhibit active phagocytosis against *S. aureus* (Wedlock et al., 2000). Intramammary infusion of IL-2 induces significant increase in somatic cell count which is related to macrophages and plasma cells producing IgG1, IgG2, IgA, and IgM (Nickerson et al., 1992; Nickerson et al., 1993). The role of IL-2 in treatment of *S. aureus* has been investigated. Intra-mammary infusion of IL-2 into infected quarters causes immuno-potentiation manifested by recruitment of lymphocytes, neutrophils, macrophages, and eosinophils, upregulation of MHC II expression; and a high antibody titre in milk and serum (Quiroga et al., 1993; Quiroga et al., 1993; DeRosa and Sordillo, 1997). Although Daley et al. 1992, had reported that infusion of IL-2 into normal or mastitic mammary glands had no effect in either preventing or curing infection, other work has found otherwise. Intramammary infusion of recombinant bovine IL-2 into infected quarters cures only 38% of quarters due to clearing the bacteria and restoring the superoxide activity of phagocytic cells. The use of IL-2 immunotherapy alone for treatment of mastitis caused by *S. aureus* or together with adjunctive antibiotics can have a successful result if the cytokines are introduced when neutrophils are still intact. Moreover, IL-2 can be a promising alternative prophylactic against *S. aureus* mastitis (Alluwaimi, 2004).

1.5. Interleukin -4

IL-4 helps to activate lymphocyte formation and is therefore often referred to as a B-cell stimulating factor and B-cell growth factor. The ability of IL-4 is to stimulate the proliferation of B cells, and this is one of the most important factors that stimulates the production of IgE antibodies. Interleukin-4 also affects endothelial cells, enhances the expression of very late integrin antigen-4, which also participates in the migration of eosinophils, monocytes and T cells into the tissue (Fonseca et al., 2009).

1.6. Interleukin-6

Interleukin-6 (IL-6) is expressed by lymphocytes, monocytes, macrophages, neutrophils, epithelial cells, and fibroblasts (Biffl et al., 1996; Poll and Deventer, 1998). It is involved in both innate and adaptive immunity via its ability to induce fever, immunoglobulin production, and T cell activation, as well as to increase pro inflammatory responses of neutrophils (Biffl et al., 1996; Keller et al., 1996). IL-6 mRNA transcription increases in cows infected with *E. coli* as well as with Gram-positive bacteria (*S. aureus* and *Streptococcus* spp.) (Bannerman, 2009). Increased IL-6 concentrations are detected in the milk and blood of cows with naturally acquired (Ohtsuka et al., 2001; Hagiwara et al., 2001) and experimentally induced mastitis (Dernfalk et al., 2007). Three different studies have been concerned with measuring IL-6 concentrations in cows with naturally acquired mastitis. One suggests greater blood IL-6 concentrations in cows with severe mastitis (Hagiwara et al., 2001). The second study reported that cows which survived clinical mastitis had greater IL-6 concentrations than did those which did not survive, but this finding is not very definitive because of the very different number of animals in the mentioned groups of cows (Nakajima et al., 1997). The third study found no difference in blood IL-6 concentrations between cows with mild or severe mastitis (Ohtsuka et al., 2001). A significant difference in IL-6 levels in milk was observed between a group of cows with high individual somatic cell count (SCC) versus a group with low individual SCC. Those results suggest that the concentration of IL-6 in quarter milk might in future be used in predicting subclinical mastitis (Sakemi et al., 2011).

IL-6 is expressed by a variety of different cells, including lymphocytes, monocytes, macrophages, endothelial and epithelial cells, and fibroblasts, and is induced by bacteria. It also plays a role in activating T-cell antigen recognition and differentiation of cytotoxic T-cells and acts as a stimulant for B-cell differentiation into Ig-releasing cells of various classes. IL-6 acts mainly on lymphocytes, stimulating various cytokines, including IL-4, which was previously mentioned (Scheller et al., 2011; Ezzat et al., 2014). An important advantage of IL-6 is its ability to remain in the bloodstream for a longer period of time than other pro-inflammatory cytokines (Song and Kellum, 2005).

1.7. Interleukin-8

Interleukin-8 (IL-8) is an inflammatory cytokine belonging to the CXC chemokine family and is also known as CXCL8. It is produced by many types of cells, including lymphocytes, monocytes, macrophages, neutrophils, fibroblasts, and epithelial cells in response to such inflammatory stimuli as bacterial infections. This cytokine plays a crucial role in the recruitment of neutrophils (Moser eta al., 2004). It is known that bovine IL-8 is released by cattle suffering from mastitis (Kauf et al., 2007; Bannerman et al., 2005; Rainard et al., 2008; Simojoki et al., 2011). Neutrophils involved in bacterial clearance from the mammary gland (Paape et al., 2002; Burvenich et al., 2003) are recruited and activated by IL-8 (Mitchell et al., 2003). IL-8 gene expression level and polymorphisms in the IL-8 receptor-α gene are related to the incidence and severity of mastitis (Galvao et al., 2011; Stevens et al., 2011). Inflammatory lactoferrin-derived peptides induce IL-8 gene expression in bovine mammary epithelial cells (Komine et al., 2006). These cells are the principal sources of IL-8 production in mammary glands undergoing mastitis (Boudjellab et al., 1998; Barber et al., 1999). Infusion of recombinant bovine IL-8 into the mammary glands of dairy cows during the drying-off period induces inflammatory reactions related to mastitis symptoms, including the infiltration of neutrophils into mammary secretions, decline in casein concentration, and temporary increase in rectal temperature (Watanabe et al., 2008). Intramammary infusion of recombinant bovine IL-8 causes long-lasting neutrophil infiltration as well as extended secretion of leukocyte elastase, inflammatory lactoferrin-derived peptides, and IL-8 in dairy cows during the drying-off period (Watanabe et al., 2012). IL-8 also plays a crucial role in *E. coli* mastitis (Wang et al., 2002; Lee et al., 2003). CD14 induces a key signal in the activation of mammary epithelial cells to express IL-8 (Wang et al., 2002). Other Gram-negative bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens*), too, induce elevated IL-8 concentrations in milk within 20 h following intramammary infusion (Bannerman et al., 2004; Bannerman et al., 2005; Bannerman et al., 2004).

1.8. Interleukin-10

Interleukin-10 (IL-10) is produced by T helper 2 cells, B cells, eosinophils, mast cells, monocytes, and macrophages. This cytokine plays a critical role in limiting inflammation and influencing the nature of the adaptive immune response to infection (Assadullah et al., 2003). IL-10 has an anti-inflammatory effect on monocytes, macrophages, and neutrophils through inhibiting their production of pro inflammatory cytokines and chemokines (Moore et al., 2001). This cytokine is also involved in inducing regulation of IL-1 receptor antagonist and soluble TNF-α receptors. These impair the ability of the pro-inflammatory cytokines IL-1 and TNF-α to exert their effects while IL-10 impairs the ability of monocytes and macrophages to present the antigen to T cells (Moore et al., 2001; Conti et al., 2003; Mocellin et al., 2004). Intramammary infection by various bacterial pathogens (*E. coli*, *Str. uberis, Mycobacterium bovis, Ps. aeruginosa, Se. marcescens, K. pneumoniae*) increase IL-10 concentrations in milk (Bannerman et al., 2004; Bannerman et al., 2004; Kauf et al., 2007; Bannerman et al., 2005; Bannerman et al., 2004). In contrast, *S. aureus* intramammary infection does not increase IL-10 expression (Bannerman et al., 2004). It is not clear what effect bacterial cell wall type could have on the production of IL-10. *Str. uberis* induces IL-10 production in a similar concentration to that of *E. coli. M. bovis* induces a concentration similar to those observed during intramammary infection by *K. pneumoniae* or *Ps. aeruginosa*. Nevertheless, initial and maximum increases in IL-10 production are detected earlier in response to Gram-negative bacteria than to Gram-positive bacteria. On the other hand, induction of IL-10 is absent or delayed in cows with the greatest persistent concentrations of bacteria in milk. This may indicate that earlier induction of IL-10 production is beneficial for cows' ability to limit bacteria growth and eradicate the pathogens [Bannerman, 2009; Bannerman et al., 2004, Bannerman et al., 2004, Kauf et al., 2007). Lipopolysaccharide of *E. coli* and muramyl dipeptide (the smallest structural unit of peptidoglycan of Gram-positive bacteria) are also able to induce IL-10 production in bovine mammary gland leukocytes (Slama, 2011).

IL-10 reduces the production of cytokines, stimulates the cluster of differentiation CD4 + Th1 helper cells, suppresses the cytotoxic effects of monocytes and macrophages, as well as the synthesis of pro-inflammatory cytokines. L-10 is a major suppressor of the immune response and inflammatory activity. IL-10 is released later and regulates inflammation (Dąbrowski et al., 2015; Kjelgaard-Hansen et al., 2007).

1.9. Interleukin-12

Interleukin-12 (IL-12) plays a crucial role in modulating the host immune response to bacterial and parasitic intracellular pathogens (Trinchieri, 1998) and monocytes and dendritic cells are its major sources (Langrish et al., 2004). Neutrophils produce IL-12 in lower volumes, but their presence in large numbers at the site of inflammation affords a relevant source of this cytokine (Trinchieri, 1998). IL-12 production is induced by fungi, parasites, viruses, bacteria, and bacterial products such as lipopolysaccharide, lipoteichoic acid, and enterotoxins (Trinchieri, 1998). IL-12 contributes to the activation of macrophages Trinchieri, 2003). This cytokine upregulates other cytokines, including IFN-γ, IL-8, 1L-10, and TNF-α (Gately et al., 1998). IL-12 enhances the cytotoxic activity of TC lymphocytes and NK cells (Trinchieri, 1998). This cytokine also influences TH cells. Concretely, it plays an important role in altering the balance between TH1 and TH2 responses by promoting differentiation of T cells into TH1 cells which produce IFN-γ (Langrish et al., 2004). Cells isolated from cows experimentally infected with *E. coli* or *S. aureus* increase the expression of IL-12 mRNA (Alluwaimi et al., 2003; Lee et al., 2006). Expression of IL-12 mRNA is also increased in naturally occurring cases of *S. aureus* mastitis (Politis et al., 1991). The level of IL-12 is higher, too, within 32 h of experimental intramammary infection with *S. aureus, Str. uberis, E. coli, Ps. aeruginosa, or Se. marcescens* (Bannerman, Rainard et al., 2004; Bannerman, Hope at al., 2004; Bannerman et al., 2005; Bannerman, Hare et al., 2004) and within 96 h of intramammary infection with *M. bovis* (Kauf et al., 2007).

1.10. Transforming growth factor

The transforming growth factor (TGF) cytokine TGF-α is produced by many types of cells, including neutrophils, macrophages, eosinophils, epithelial cells, and fibroblasts (Calafat et al., 1997). TGF-α is a pro-inflammatory cytokine (Derynck, 1992). This cytokine upregulates IL-8 and prostaglandin E2 production and also boosts the effects of IL-1β and TNF-α (Bry, 1993; Subauste and Proud, 2001). TGF-β regulates cell growth and differentiation as well as inflammatory responses (Letterio and Roberts, 1998; Bonewald, 1999). TGF-β regulates ductal growth and alveolar development in the bovine mammary gland (Daniel et al., 2001). This cytokine suppresses immune and inflammatory responses (Letterio and Roberts, 1998; Flanders and Wakefield, 2009). TGF-β is associated with the presence of abundant collagen I in intralobular connective tissue in mammary glands chronically infected with *S. aureus* mastitis during active involution. This protein's greater expression in chronic *S. aureus* mastitis appears to be an essential response for limiting the extent of inflammation and injury to the host (Andreotti et al., 2014). Intramammary infection of *E. coli* induces the expression of TGF-α, TGF-β1, and TGF-β2 (Chockalingam et al., 2005). *S. aureus* is also able to induce increased production of TGF-α, TGF-β1, and TGF-β2 during intramammary infection (Bannerman et al., 2006). Lipopolysaccharide of *E. coli*, too, is able to induce TGF-β1 production in bovine mammary gland leukocytes (Slama et al., 2012).

1.11. Tumour necrosis factor-α

Tumour necrosis factor-α (TNF-α) is a proinflammatory cytokine with both positive and negative effects on body tissues (Poll and Lowry, 1995). It has been identified in normal and infected mammary glands (Alluwaimi and Cullor, 2002; Alluwaimi et al., 2003). Various cells have been found to produce TNF-α (Angelini et al., 2005), and this production is caused by fungal, viral, and parasitic pathogens, bacterial toxins, and bacterial wall products. It is also induced by such cytokines as IFN-γ and IL-1 (Bannerman, 2009). Increased TNF-α levels have been detected during lactation, involution, and also the periparturient period. These increased levels of TNF-α suggest its essential role in regulating immunological function of cells and factors involved in the physiological changes within the mammary gland (Alluwaimi, 2004). In coliform mastitis (experimental *E. coli* infection, LPS-stimulated mammary gland, natural coliform mastitis), TNF-α is elevated in serum and milk (Hisaeda et al., 2001; Hoeben et al., 2000; Perkins et al., 2002). TNF-α plays a very important role in coliform mastitis by recruiting and activating neutrophils, elevating intramammary nitrite and nitrate, and inducing production of plasma haptoglobin (Blum et al., 2000). Other Gram-negative bacteria (*K. pneumonia, Ps. aeruginosa, Se. marcescens*) induce TNF-α responses (Bannerman, Hope et al., 2004; Bannerman et al., 2005; Bannerman, Paape et al., 2004). Gram-positive bacteria (*S. aureus, M. bovis*) have been reported to induce minimal and delayed TNF-α responses (Bannerman, Rainard et al., 2004; Bannerman, Hope et al., 2004; Kauf et al., 2007; Bannerman et al., 2006). Yokomizu et al., (Watanabe et al., 2000) reported that *S. aureus* enterotoxins stimulate T cells to release TNF-α. This cytokine is involved in the chemotactic activity of neutrophils as the first wave of immunological responses to invading micro-organisms (Alluwaimi et al., 2003). Infusion of the mammary gland with recombinant bovine TNF-α causes an increase in the influx of neutrophils, which mainly is due to a weakening of the milk–blood barrier (Watanabe et al, 2000; Kushibiki et al., 2003). TNF-α increases the bactericidal activity of certain antibiotics. It can be used to monitor the severity of coliform mastitis and to detect *S. aureus* infection. It is also useful for determining prognosis for mastitis cases (Alluwaimi, 2004).

2. Conclusion

The cytokine immune network in bovine mastitis is very complicated. Deeper exploration of cytokines is needed in order to make use of cytokines for mastitis detection, immunotherapy, and reaching prognoses for recovery. Among other factors, this research should encompass cytokines' concurrence, competition, and effects on various cell types.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publishing of this paper.

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