Review

Effect of *Streptococcus uberis* infections on cell population of bovine mammary gland

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Streptococcal bacteria are one of the most important pathogens causing different type of bovine mastitis. In this respect, it is very important to study effect of streptococcal infections on cell populations of bovine mammary gland to improve knowledge how to prevent and treat streptococcal infections of bovine mammary gland. Streptococcal bacteria can affect different cell processes, for example, programmed cell death (apoptosis) and expression of some cell receptors on leukocytes such as neutrophils, lymphocytes and macrophages of mammary gland. This review summarizes information about effect of streptococcal infections on leukocytes of bovine mammary gland.

Key words: *Streptococcus uberis*, apoptosis, neutrophil, lymphocyte, macrophage, mammary gland and mastitis.

INTRODUCTION

Inflammation of bovine mammary gland (mastitis) is the most costly disease of lactating cows which decreases quality and quantity of produced milk. Therefore, it is very important to study the processes leading to this disease. The significance of bovine mammary gland pathogens as a factor increasing the bacterial count in herd bulk milk has been analysed before (Hayes et al., 2001; Jayarao et al., 2004; Zadoks et al., 2004; Rysanek et al., 2009a; Rysanek et al., 2009b). A significant correlation was found between the number of mastitis streptococci (*Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*) and the somatic cell count which is the most widely accepted standard for measuring udder health and milk quality (Fenlon et al., 1995; Bagnicka et al., 2011; Borne et al., 2011). *S. uberis* is one of the most important environmental pathogen which causes mastitis in dairy cows, particularly during the dry period, the period around calving and during early phase of lactation (Oliver et al., 2004; Prado et al., 2011; Smulski et al., 2011).

Resident and incoming leukocytes play an important role in the mammary gland defence system against invading pathogens. Lymphocytes and macrophages are the predominant resident cells in the healthy mammary gland. Intramammary infection induces the recruitment of leukocytes from blood into the mammary gland (Concha et al., 1986; Miller et al., 1991). The first step of the acute inflammatory response induced by *S. uberis* is the recruitment of neutrophils and accumulation of large numbers of these cells in the secretory acini (Thomas et al., 1994; Sladek et al., 2006). On the other hand, the influx of neutrophils into the mammary gland is not associated with a reduction in the number of bacteria present following infection with *S. uberis* (Grant and Finch, 1996; Rambeaud et al., 2003). Leukocytes accumulated at the site of infection have the potential to damage the neighbouring tissue through the release of inflammatory mediators by intact cells or through necrosis of leukocytes. Necrosis is the type of cell death with the

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releasing of toxic cellular contents (Weiss, 1989). Therefore, leukocytes in the mammary gland undergo apoptosis and subsequent phagocytosis by macrophages (Sladek and Rysanek, 2000a; Sladek and Rysanek, 2000b; Sladek and Rysanek, 2001). Apoptosis is the programmed cell death without spread of cellular contents. This process is critical for the resolution of inflammation during infection. Apoptosis of neutrophils reduces local tissue destruction, decreases recruitment of cells into the site of infection and supports the termination of the innate immune response by limiting pro-inflammatory capacity (Kobayashi et al., 2003).

In this review, we have briefly analysed the effect of streptococcal infections on cell population of bovine mammary gland, especially effect of *S. uberis* on apoptosis and expression of cell receptors of leukocytes such as neutrophils, lymphocytes and macrophages.

EFFECT ON LYMPHOCYTES

Lymphocytes are very important component of the mammary gland immune system (Hodgkinson et al., 2009; Denis et al., 2011). They recognize antigens through membrane receptors specific for invading bacteria (Sordillo et al., 1997). There are many bacterial pathogens that influence apoptosis of lymphocytes. Pathogens involved in lymphocyte apoptosis include Gram-positive or Gram-negative bacteria (Carrero and Unanue, 2006; Ullet and Anderson, 2006; Slama et al., 2009a; Slama and Kwak, 2011; Slama et al., 2011a) and their components, especially such bacterial toxins as lipopolysaccharide (Slama et al., 2009b) which can modulate leukocyte apoptosis through producing of cytokines (Paape et al., 2003; Slama et al., 2011b). Lymphocyte apoptosis can be modulated by streptococcal infection, for example by *S. uberis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Carrero and Unanue, 2006; Ullet and Anderson, 2006).

S. uberis is a facultatively anaerobic coccus causing subclinical and clinical mastitis (Pedersen et al., 2003). During bouts of acute mastitis caused by streptococcal infections, an increase number of lymphocytes are detected in the mammary gland (Soltys and Quinn, 1999). Infection of the mammary gland with *S. uberis* leads to gradual increase in lymphocyte apoptosis. It suggests that these bacteria can delay apoptosis of lymphocytes. This result of *in vivo* experiment was confirmed by *in vitro* course with initial ratio of lymphocytes to bacteria 1:1 (Slama et al., 2009a). In another experiment, we investigated an effect of different lymphocyte-bacteria ratios (1:10, 1:50, 1:100) on lymphocyte apoptosis. We found out that lymphocyte apoptosis is significantly increased following 6 hours of co-cultivation with ratios mentioned above, contrary to the ratio 1:1 (Slama et al., 2011a). These results correspond with experimental work focused on co-cultivation of

lymphocytes with *Borrelia burgdorferi* (Perticarari et al., 2003). In this article, authors referred that increasing lymphocyte apoptosis corresponds with increasing cell-bacteria ratio (1:10 → 1:100).

Many authors described changes in the distribution of the lymphocyte subpopulation in mammary gland secretion. Milk from healthy cows contains predominantly T cells (approximately 60% of lymphocytes) and relatively few B cells (approximately 20% of lymphocytes). The majority of T cells represent CD8$^+$ (cytotoxic T cells). CD4$^+$ cells (T helper cells) are in a lower proportion (Taylor et al., 1994; Soltys and Quinn, 1999). These cells represent a greater percentage in infected mammary glands (Taylor et al., 1997). During inflammatory response to *S. uberis*, approximately 75% of lymphocytes were found to be CD2$^+$ (Slama et al., 2009a). Faldyna et al. (2006) reported that bovine peripheral blood contained 93% of CD2$^-$ cells. They also observed that almost 90% of γδ-TCR was CD2$^+$ before stimulation, and about 60% of γδ-TCR was CD2$^+$ following experimental infection of mammary gland. Lymphocytes play very important role during inflammation of the mammary gland. CD4$^+$ and CD8$^+$ T cells participate in the production of pro-inflammatory cytokines that promote recruitment and phagocytosis of neutrophils in the infected mammary gland (Riollet et al., 2000). Following experimental infection with *S. uberis*, there are about 50% of CD4$^+$ and about 25% of CD8$^+$ lymphocytes (Slama et al., 2009a). A relative increase in the proportion of CD8$^+$ lymphocytes is associated with chronic processes (Park et al., 1992; Park et al., 1993). Reversal ratio CD4$^+$:CD8$^+$ T cells suggest an inability of the immune system to produce a protective response to staphylococcal infection (Park et al., 2006). Proportion of T lymphocytes depends on the stage of lactation (Sordillo et al., 1997) and the dominant lymphocyte population depends on the type of bacteria that are present in the mammary gland (Soltys and Quinn, 1999; Denis et al., 2011).

EFFECT ON NEUTROPHILS

Neutrophils are the first line of immune defence of the mammary gland against bacterial pathogens. They are produced in the bone marrow, enter the peripheral blood and migrate into the lumen of mammary gland where phagocytose invading bacteria (Paape et al., 2003). The granules of neutrophils contain enzymes that kill pathogens (Bainton, 1975; Paape et al., 2003). When neutrophils undergo necrosis they release histotoxic content to the extracellular space and damage the mammary gland tissue (Capuco et al., 1986; Jaeschke et al., 2012). Therefore it is very important that neutrophils undergo apoptosis. Apoptotic neutrophils are removed from the mammary gland by macrophages. Neutrophil apoptosis is an effective protective mechanism that prevents mammary gland tissue damage (Sladek and

Rysanek, 2001). Accumulation of persistent neutrophils in the mammary gland is typical for subclinical and chronic mastitis in dairy cows and may be caused by delayed apoptosis (Boutet et al., 2004). Intramammary infection caused by *S. uberis* predominantly subclinical and can persist in a chronic state (Phuektes et al., 2001).

During experimental infection with *S. uberis*, the apoptotic neutrophils were found in both the early and late stages of apoptosis. Low relative number and high total count of apoptotic neutrophils was observed 24 h following infection. These apoptotic neutrophils represent freshly infiltrated cells as the mammary gland was rinsed before *S. uberis* administration (Sladek et al., 2006). This results show that during the early stage of the inflammatory response the longevity of neutrophils is decreased primarily by migration (Oostveldt et al., 2002) and less by the environment of the mammary gland. Higher proportion of neutrophils in the early phase of apoptosis represented by phosphatidylserine translocation was found 3 and 7 days following experimental infection with *S. uberis*. Lower number of karyopyknotic neutrophils was detected 2 and 7 days following infection. These results suggest delaying of neutrophil apoptosis during *S. uberis* infection (Sladek et al., 2006). The co-cultivation of neutrophils with *S. uberis* decreased the proportion of karyopyknotic and zeiotic neutrophils (Sladek et al., 2005). These results are in the contrast to the effect of Gram-negative bacteria on blood neutrophils (Stevens and Czuprynski, 1996; Watson et al., 1996; Yang et al., 1998). The proportion of apoptotic neutrophils present during the resolution of the inflammatory response of the mammary gland does not directly reflect the actual dynamics of apoptosis as apoptotic neutrophils are immediately phagocytosed by macrophages (Sladek and Rysanek, 2001). It is obvious that although apoptosis of neutrophils is initiated by *S. uberis*, the completion of the program is delayed. Neutrophils can stay functioning and capacity of pathogens to induce such stage of apoptosis does not contribute to their virulence (Sladek et al., 2005).

Initiation and resolution of inflammation during infection with *S. uberis* is also accompanied with changes in count and proportion of CD14$^+$ neutrophils. There was found low proportion of CD14$^+$ neutrophils in blood before experimental infection, while neutrophils from intact mammary gland had higher expression of CD14 (Sladek and Rysanek, 2006). Bovine blood neutrophils have lower expression of CD14 than mammary gland neutrophils from lactating cows (Paape et al., 1996) or from heifers (Sladek et al., 2002). This difference is caused by the microenvironment of the mammary gland sinuses, because neutrophils dispose of an intracytoplasmatic CD14 pool in secretion vesicles (Dentener et al., 1993). Translocation of CD14 from the intracytoplasmatic pool to the cell surface is influenced by microenvironmental factors (Paape et al., 1996). Low proportion of CD14$^+$ neutrophils during the initial phase of

inflammation caused by *S. uberis* (Sladek and Rysanek, 2006) could be explained by two possibly reasons. First: The migrated blood neutorphils with the lower CD14 expression dilute current mammary gland population of neutrophils with the higher CD14 expression. Second: The decrease in the proportion of CD14$^+$ neutrophils is due to a release of CD14 from the cell surface (Paape et al., 1996; Sladek and Rysanek, 2006). Sladek and Rysanek (2006) observed the decrease of CD14$^+$ neutrophils count together with the mild increase in their portion during resolution of *S. uberis* infection. Decrease of CD14$^+$ neutrophils is correlated with clearance of apoptotic neutrophils just as in lipopolysaccharide mediated injury (Sladek and Rysanek, 2001). These authors (Sladek and Rysanek, 2006) expect a higher portion of CD14$^+$ neutrophils in the population of neutrophils accumulated in chronic mastitis.

EFFECT ON MACROPHAGES

Monocytes migrate from blood into the mammary gland during the initial phase of an inflammatory reaction. These inflammatory macrophages are called non-vacuolized macrophages (Jensen and Eberhart, 1975; Sladek and Rysanek, 2008). Structural changes occur during the maturation to fully immunocompetent cells. Such cells increase in size and show abundant cytoplasm containing many granules (Wardley et al., 1976). These cells are called as vacuolized macrophages (Jensen and Eberhart, 1975; Sladek and Rysanek, 2008). These macrophages represent a dominant population of macrophages in the resolution of the inflammatory response (Wardley et al., 1976; Sladek and Rysanek, 2008).

Macrophages play a prominent role in the defence of the mammary gland against *S. uberis* mastitis (Hill et al., 1994; Grant and Finch, 1996). Apoptosis of neutrophils induced by *S. uberis* and subsequent phagocytosis of apoptotic neutrophils by macrophages is delayed. It may cause the transition of the acute inflammation to a chronic state (Sladek et al., 2006).

Apoptosis of bovine mammary gland macrophages was studied only in course of co-cultivation with *E. coli* lipopolysaccharide but not with Gram-positive bacteria. Lipopolysaccharide delays apoptosis of non-vakuolized macrophages, but not in vacuolized macrophages. It may have an important role in inflammatory response to bacteria (Slama et al., 2009c).

Non-vacuolized macrophages of mammary gland are migrated blood monocytes with their morphological features (Sladek et al., 2002). Functional maturation of macrophages is process of conversion from monocytes to the activated cells. Bacteria and their products play an important role in activation of macrophages. Peptidoglycan and muramyl dipeptide of Gram-positive bacteria increase expression of CD14 on monocytes

(Wang et al., 2001; Takada et al., 2002). Higher total count and proportion of CD14$^+$ non-vacuolized macrophages was found in the initial stage of infections as a result of macrophage activation following interaction with bacteria. The peak of CD14$^+$ non-vacuolized macrophages total count was observed 24 h following infection with *S. uberis*. Contrary to that, infection with *S. uberis* down-regulates CD14 surface expression on vacuolized macrophages during the initial phase of infection. Inflammation of bovine mammary gland caused by *S. uberis* induces a local immune response characterised by the increase in total counts of CD14$^+$ neutrophils and CD14$^+$ macrophages particularly. Resolution of inflammation is accompanied by an increase in relative counts of CD14$^+$ neturophils, CD14$^+$ vacuolized macrophages and apoptotic neutrophils. It suggests the possibility of involving the CD14 in recognition of apoptotic neutrophils by macrophages (Sladek and Rysanek, 2006).

CONCLUSION

Considering what is known from previous studies, it is evident that much more experimental work is necessary in order to understand the mechanisms of leukocytes apoptosis and related processes in streptococcal infection of bovine mammary gland. Many bacterial pathogens influence the programmed cell death of leukocytes. It is clear that apoptosis is an important component of both beneficial and detrimental host responses to bacterial infection. Advantages or disadvantages of apoptosis must be identified at the consequences of each disease and bacterial pathogen. Future investigations may lead to developing novel treatment therapies for mammary gland inflammations.

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