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Review Article

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Abstract

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DEFENCE MECHANISMS AND THE MODULATION OF IMMUNE RESPONSES IN THE BOVINE UDDER

Mastitis is one of the most costly problems affecting dairy cows and is in most cases caused by a bacterial infection. The immune response of the mammary gland is crucial for it's defence against invading pathogens. The defence of the mammary gland is a combination of anatomical, cellular and soluble mechanisms. The teat canal and the flushing effect of milking can limit bacterial penetration. Polymorphonuclear neutrophils (PMN) and Macrophages (M) phagocytose pathogens. Furthermore, M releases inflammatory mediators and are also antigen-presenting cells. Lymphocytes consisting of B-cells and different T-cells are important for humoral and cell-mediated immunity. Soluble factors associated with the defence of the mammary gland are immunoglobulins, lactoferrin, lysozyme, the complement system and the lactoperoxidase/SCN^- /H_2O_2 system. The defence mechanisms change substantially during early involution and around calving. The immune functions are also depressed during the periparturient period. During these periods the udder is highly susceptible to new intramammary infections. Current measures for control of mastitis have not been able to deal with the problem. Therefore, new approaches in the prevention of udder infections, such as stimulation of the immune responses are warranted. Vaccines against major pathogens have been tried as well as immunostimulation, using cytokines, Ginseng and glucan.

Key words : Bovine udder, defence mechanisms, modulation of immune responses

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กลไกป้องกันตนเองและการกระตุ้นภูมิคุ้มกันในเต้านมโค

เต้านมอักเสบในโคเป็นปัญหาที่ก่อให้เกิดความสูญเสียทางเศรษฐกิจ โดยสาเหตุส่วนใหญ่เกิดจากการติดเชื้อแบคทีเรีย การตอบสนองของภูมิคุ้มกันเป็นขบวนการในการป้องกันตนเองที่สำคัญต่อการรุกรานของจุลินทรีย์ที่เข้ามาในเต้านม โดยทั่วไปกลไกการป้องกันตนเองของเต้านม ประกอบด้วย ลักษณะทางกายวิภาคของเต้านม ระบบภูมิคุ้มกันชนิดเซลล์ และระบบภูมิคุ้มกันที่ไม่เกี่ยวข้องกับเซลล์โดยตรง ลักษณะรูหัวนม และการรีดนมออกเป็นขบวนการป้องกันที่สามารถลดการติดเชื้อที่เต้านม เซลล์แมคโครเฟจ และ นิวโทรฟิลล์สามารถเก็บกินเชื้อที่ก่อโรคในเต้านม นอกจากนั้น ในระบบภูมิคุ้มกันเซลล์แมคโครเฟจยังสามารถหลั่งสารสื่ออักเสบ และ ถ่ายทอดสัญญาณที่เกี่ยวข้องกับแอนติเจนให้กับเซลล์อื่นๆ ลิมโฟไซต์ ทั้ง บีเซลล์ และ ทีเซลล์ มีบทบาทสำคัญในระบบภูมิคุ้มกันในเต้านมเช่นเดียวกัน ระบบภูมิคุ้มกันที่ไม่เกี่ยวข้องกับเซลล์โดยตรง ได้แก่ แอนติบอดี แลคโตเฟอริน ไลโซไซม์ ระบบคอมพลิเมนท์ และระบบเอนไซม์แลคโตเปอร์ออกซิเดส/ไธโอไซยาเนท/ไฮโดรเจนเปอร์ออกไซด์ เป็นต้น ระบบป้องกันตนเองของเต้านมมีการเปลี่ยนแปลงอย่างมาก ในช่วงแรกหลังระยะพักนม และในช่วงคลอดที่มีการกดของระบบภูมิคุ้มกัน ในช่วงเหล่านี้จึงเป็นช่วงที่มีความไวต่อการติดเชื้อเป็นอย่างมาก ในปัจจุบันการป้องกันเต้านมอักเสบยังไม่มีวิธีใดที่สามารถลดปัญหานี้ได้ การกระตุ้นระบบภูมิคุ้มกันในเต้านม เช่น โดยการใช้วัคซีน ไซโตคีนส์ โสม หรือ กลูแคน อาจลดปัญหาเหล่านี้

คำสำคัญ : เต้านมโค ระบบป้องกันตนเอง การกระตุ้นภูมิคุ้มกัน

Introduction

Mastitis means inflammation of the mammary gland caused by alien substances (e.g. bacteria and toxins) or tissue injury, but the main cause of mastitis is bacterial infection. Some of the most important udder pathogens are *Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae.* Inflammation may lead to clinical mastitis with obvious signs, such as clotting or discoloration of the milk and swelling and/or hardness of the mammary gland or it may lead to a subclinical mastitis that produces no visible signs but can be detected by various laboratory tests. The severity of the inflammation depends on the pathogen, host factors and environmental and genetic influences (Bramley, 1991).

Generally, udder diseases are considered to be one of the most costly problems of dairy cows. Big economic losses result from decreased milk production, discarded milk, increased costs for replacement animals, medicines, veterinary fees and labour. Furthermore, mastitis reduces the quality and quantity of both the milk and manufactured milk products derived from it (DeGraves and Fetrow, 1993). On average 11% of milk production is lost when clinical mastitis occurs before peak milk production and 6.4% is lost on average over all stages of lactation (Lucey and Rowlands, 1984). Total milk losses due to subclinical mastitis are estimated to be 10-26% of production (Fetrow, 1980). In the USA, the economic losses due to mastitis were approximately $ 200 per cow per year and the total loss to USA dairy industry was on average

$ 2 billion per year (Philpot, 1984). In Sweden, the total loss was estimated to be on average 800 million Swedish crowns per year (Nilsson and Holmberg, 1996). Therefore, it is essential to find good management methods and good monitoring systems, in order to prevent and control mastitis.

Normally, a mastitis control program consists of good husbandry and good milking practice, regular maintenance and annual testing of the milking machine, dry cow therapy, treatment of all clinical cases of mastitis and culling of cows with recurrent mastitis. Antibiotics have been used as dry cow therapy to eliminate bacterial infections in the udder at drying off and to reduce new intramammary infections (IMI). Dry cow therapy is recommended especially, for the control of contagious mastitis *(S. aureus, Str. agalactiae, Str. dysgalactiae)*. The cure rates in cases of *Str. agalactiae, Str. dysgalactiae* and *Str. uberis* mastitis treated by antibiotics during both lactation and the drying off period are good, but the cure rate for *S. aureus* is low (Pyörälä, 1995). *S. aureus* has many virulence factors which make it possible for this bacteria to survive and resist the hosts defence system as well as antibiotic therapy. Toxins produced by *S. aureus* can damage polymorphonuclear neutrophil (PMN) membranes and make them nonphagocytic (Fox and Gray, 1993). *S. aureus* can form a capsule and can survive intracellulary which also interferes with phagocytosis and with the activity of antibiotics (Fox and Gray, 1993). Furthermore, *S. aureus* can produce so-called L-forms which lack a cell wall and which therefore are resistant to some antibiotics (Pyörälä, 1995).

As antibiotics have limited efficacy, the need to test alternative methods that enhance mammary resistance to bacterial infection is important. Several mastitis vaccines have been developed and tested, but few have been successful and even fewer are available commercially. Intramammary polyethylene devices (IMD) inserted into the udder cistern to increase somatic cell counts (SCC) during lactation have been tested (Paape et al., 1981a). Nickerson et al. (1991) reported that IMD can induce milk leukocytosis but that the quarters with IMD had prominent histological changes and decreased milk production. Various cytokines such as interleukin-1, interleukin-2 and interferon-γ and some products like ginseng and glucan, have also been tested for their ability to improve the defence mechanisms in the udder (Buddle et al., 1988; Hu et al., 1995; Concha et al., 1996; Sordillo et al., 1997; Persson Waller and Colditz, 1998a). In addition, the importance of different micronutrients and the genetics of the immune response are also research areas of major interest and should be considered in control programs against mastitis. Deficiencies in selenium and vitamins E and A result in decreased immune resistance against infections in the udder (Erskine, 1993). New ways to enhance the immune response of the udder are needed for the future in order to reduce the incidence of mastitis on dairy farms and thus reduce the costs associated with mastitis. However, the process of inflammation and the regulation of defence mechanisms have to be comprehensively understood before immune stimulation can be performed successfully.

1. Regulation of Inflammation

Inflammation is the tissues response to an injury and can be divided into acute and chronic inflammation. The cardinal signs of acute inflammation are heat, redness, swelling, pain and loss of function (Tizard, 1995). The response may persist if the causal material or organisms are not destroyed and the inflammation becomes chronic. Tissue degeneration and regeneration are characteristic of the chronic proliferative phase.

Inflammatory mediators such as histamine, serotonin, kinin, eicosanoids (e.g. prostaglandins, leukotrienes), platelet-activating

factor, complement factors (e.g. C3a, C5a) and cytokines (e.g. interleukin-1, interleukin-2, tumor necrosis factor-α), are released from various cells during inflammation and produce vasodilation, increased vascular permeability, smooth muscle contraction, pain and leukocyte emigration (Lee, 1991). Bacterial products, such as lipopolysaccharides or substances released by damaged tissues, such as thrombin, histamine, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), can trigger the expression of adhesion proteins on both endothelial cells and leukocytes which enables leukocyte migration from the blood to the udder during an inflammatory response (Sandholm, 1995; Tizard, 1995).

When pathogens invade the udder, the body responds by initiating the inflammatory process which leads to the classical symptoms of mastitis. The composition of milk will change, for example, the concentrations of sodium and chlorine ions, somatic cells, and serum proteins increase, while the production of casein, fat and lactose decrease (Sandholm, 1995). The inflammatory response can often eliminate the pathogens but if the pathogens persist in the udder, the infection can become chronic which may be difficult to treat and control.

2. Defence Mechanisms of the Mammary Gland

The defence mechanisms against udder pathogens may be divided into anatomical, cellular and soluble defence mechanisms.

2.1. Anatomical Defence Mechanisms

The teat end is considered to be the first line of defence against invading pathogens. Occlusion of the teat canal limits bacterial penetration while trauma or wounds at the teat end and teat skin predispose to mastitis (Persson, 1992). The smooth muscle sphincter surrounding the duct remains tightly closed between milkings and limits bacterial penetration (Nickerson, 1989a). The flushing effect of milking can also eliminate bacteria from the udder (Craven and Williams, 1985). It has been reported that the diameter of the teat duct influences the susceptibility to infection (Hibbitt et al., 1992). The teat canal is lined with keratin which acts as a physical barrier to infection by the inhibiting the proximal progression of the bacteria. Removal of the keratin diminishes resistance to infections. Keratin also contains antimicrobial long chain fatty acids and basic proteins, which can be inhibitory to some bacteria (Craven and Williams, 1985).

2.2. Cellular Defence Mechanisms

Macrophages (M), polymorphonuclear neutrophils (PMN), lymphocytes (L) and epithelial cells are all found in milk. The total numbers of cells and proportions of different cell populations vary between and within cows, depending on the stage of lactation and on the health status of the gland (Burvenich et al., 1995). The somatic cell counts (SCC) increase substantially in the early dry period and decline gradually 2 weeks before calving decreasing rapidly post partum (Jensen and Eberhart, 1981). M is the predominant cell type during lactation and the dry period, while PMN is the predominant cell type in colostrum and during the first week after drying off (Lee et al., 1980; Concha, 1986; Outteridge and Lee, 1988). Jensen and Eberhart (1981) reported however, that the predominant cell type in colostrum is M. The numbers of L tend to increase during involution until just before parturition (Jensen and Eberhart, 1981; McDonald and Anderson, 1981a, b) and L is the predominant cell type during the first two weeks before parturition (Paape et al., 1991). The proportion of epithelial cells is less than 2 % during mid-lactation, but can be as high as 15 % during the first four weeks of lactation (Burvenich et al., 1995). The different cell types have varying functions in the defence of the mammary gland.

2.2.1. Macrophages (M) and Polymorphonuclear Neutrophils (PMN)

M and PMN play a crucial role when microorganisms invade the host. M phagocytose mastitis pathogens and release inflammatory mediators, e.g. cytokines, which initiates the inflammatory reaction including the attraction of PMN to the site of infection (Verhoef, 1991). M are antigen-presenting cells, they ingest and process antigens, and present them together with their own major histocompatibility complex (MHC) class II protein, on the cell membrane (Janeway et al., 1997; Sordillo et al., 1997). Helper - T - lymphocytes are then able to recognise and respond to foreign antigens (Verhoef, 1991; Janeway et al., 1997; Sordillo et al., 1997). Mammary M were less efficient in stimulating T-cell proliferation, expression of MHC class II molecules and production of IL-1 than was blood M (Politis et al., 1992). M also have the ability to kill microorganisms in both oxygen-dependent and independent systems (Verhoef, 1991) and the phagocytic capacity of M can be increased substantially in the presence of opsonic antibodies for specific pathogens (Sordillo et al., 1997).

The main functions of PMN are phagocytosis and intracellular killing of microorganisms. Their rapid migration from the blood to the mammary gland is induced by a cascade of inflammatory mediators, e.g. cytokines and complement making these cells of the greatest importance during acute inflammation (Craven and William, 1985; Sordillo et al., 1997). Neutrophils exert their bactericidal effect through a respiratory burst and bacteria are killed by the action of superoxide ions, hypochlorite and hydrogen peroxide. During phagocytosis, bacteria may also be exposed to several oxygen-independent reactants, such as peroxidase, lysozyme, various hydrolytic enzymes and lactoferrin (Sordillo et al., 1997). The phagocytic and bactericidal activity of PMN and M decrease during the dry and periparturient periods and during infection (Paape et al., 1981b; Nagahata et al., 1988; Nickerson, 1989b). Mammary gland PMN and M are less effective at phagocytosis than blood leukocytes because of their ingestion of fat, casein, and other milk components (Sordillo et al., 1997). Inefficient intracellular killing of bacteria can result in re-establishment of the infection upon the cells death (Daley et al., 1991a).

2.2.2. Lymphocytes

Lymphocytes consist of B and T cells that are important in humoral and cell-mediated immunity. T lymphocytes are further subdivided into T-helper (CD4+), T - cytotoxic or T-suppressor (CD8+), and γα T (WC1+) lymphocytes. The induction of an immune response involves the activation of T-helper cells and requires the interaction of antigen presenting cells (Unanue and Allen, 1987). T-helper lymphocytes recognise antigen-MHC complexes on B lymphocytes and M and release cytokines which can activate B-lymphocytes, T-lymphocytes, M and other cells that participate in the immune response (Janeway, et al., 1997; Sordillo et al.,1997). Guiguen et al. (1996) found that some 61% of milk lymphocytes in goat's milk are CD8+ T cells, 17% are CD4+ and about 20% of these express class II antigens; less than 4% are B cells. In ewe's milk 51% of lymphocytes are CD8+ T cells, 7% are CD4+, 3 % are WC1+, 9% are B cell+ and 2% of the lymphocytes express IL2-R while15 % express MHC class II (Persson Waller and Colditz, 1998b). The proportions of IL2-R+ and MHC class II+ lymphocytes and CD14+ leukocytes increase substantially in the dry period and the increased proportion of these subpopulations might lead to increased immunological responsiveness of the udder during the dry period (Persson Waller and Colditz, 1998b). Taylor et al. (1994) showed that B lymphocytes were a minor population in bovine milk, when compared to peripheral blood and that CD4+ T cells were present in

relatively low numbers in the milk of cows during the first 50 days of lactation but increased as lactation progressed. The proportion of CD4+ cells was smaller than the proportion of CD8+ cells in milk, few cells in milk expressed MHC class II and no cells expressed IL-2 receptors (CD25) (Taylor et al., 1994). The depletion in T-helper and the enrichment of T-suppressor cells in mammary secretions might contribute to the risk of mastitis (Taylor et al., 1994). However, in a study of bovine dry udder secretion the ratio of CD4:CD8+ cells was 2-3:1 and during the week prior to calving, the percentage of B cells increased until it was similar to that of T cells (Hurley et al., 1990). The increase in B cells during the period of colostrogenesis may be related to the greatly increased level of immunoglobulin seen in the gland at calving.

The activated CD8+ T - lymphocyte subpopulation is present during all stages of the lactation cycle (Park et al., 1991) but Shafer-Weaver and Sordillo (1997) reported that cells obtained from mid lactation cows exhibited cytotoxicity but no cytotoxic activity was observed for CD8+ cells that were isolated from postpartum cows. Oliver and Sordillo (1988) reported that CD8+ T - lymphocytes represent a high proportion of lymphocytes at times when the incidence of IMI is increased, which may be important in the prevention of mastitis. However, Park et al. (1993) have shown that CD4+ cells were suppressed by activated CD8+ lymphocytes and that the suppression was clearly enhanced in cows with *S. aureus* IMI. Few studies have been done on the presence of γα T lymphocytes in bovine milk. Their functions may be associated with the protection of epithelial surfaces. However, Shafer- Weaver et al. (1996) found that the percentage of γα T lymphocytes decreases significantly in mammary parenchyma during times of increased susceptibility to disease, which suggests that these lymphocytes may constitute an essential line of defence against the bacteria causing mastitis.

2.2.3. Epithelial cells

Epithelial cells are normally found in small amounts in milk. They are however, able to phagocytose milk fat globules and casein micelles which may indicate that epithelial cells play a role in protecting the mammary gland from invading bacteria (Brooker, 1983).

2.3. Soluble Defence Mechanisms

Soluble factors associated with the defence of the mammary gland are immunoglobulins, lactoferrin, lysozyme, the complement system and the lactoperoxidase/ SCN^- /HO (LP) system. They act in concert with cellular defence mechanisms and each system modifies the effector functions of the other.

2.3.1. Immunoglobulins

The immunoglobulins are produced by antigen activated B lymphocytes that subsequently proliferate into antibody-secretory plasma cells (Tizard, 1995; Janeway et al., 1997). Antibodies in bovine mammary secretions are of the isotypes IgG_1, IgG_2, IgA and IgM and are known to influence the mammary gland's defence against the bacteria which cause mastitis (Lascelles et al., 1981; Sordillo et al., 1997). They may be locally synthesised or transported selectively or passively into the gland. The study of Watson and Lascelles (1973) suggested that IgA and IgM can be produced by plasma cells located in close association with the glandular epithelium of the mammary gland. However, IgG specific cells may also be present (Larson et al., 1980). Guidry et al. (1980a, b) reported that all isotypes were selectively accumulated during colostrum formation and suggested that all isotypes of immunoglobulin can be passively transferred during inflammation.

The concentrations of immunoglobulins in bovine mammary secretions vary depending on the cow, the quarter of the udder,

age, parity, breed and the nutritional status (McFadden et al., 1997). Moreover, the concentration of immunoglobulin isotypes varies depending on the stage of lactation. It is low during most of the lactation, increases slowly during late lactation and involution, and reaches the highest levels in colostrum before decreasing rapidly post partum (Guidry et al., 1980a; Oliver and Bushe, 1987).

It is possible that IgG_1 or IgG_2 may promote opsonization and phagocytosis by M or PMN. IgG_1 receptors were detected on bovine alveolar macrophages and IgG_2 receptors on bovine PMN from both peripheral blood and milk (Howard et al., 1980). Guidry et al. (1993, 1994) reported that IgG_1 may serve as an effective opsonin for bovine M, while IgG_2 and IgM are opsonic for bovine PMN but unfortunately, IgG_1 inhibits the activity of both. An opposing view is that IgM, rather than IgG_1 or IgG_2, is the best opsonin for *S. aureus* and that subsequent phagocytosis by neutrophils and intracellular killing is most efficient with IgM (Williams and Hill, 1982). IgA does not bind complement or opsonize bacteria. Instead, IgA appears to contribute to the agglutination of bacteria, prevents bacterial colonization and neutralizes toxins (Musoke et al., 1987).

2.3.2. Lactoferrin

Lactoferrin is an iron binding protein produced by epithelial cells and leukocytes. Lactoferrin limits the growth of bacteria, especially coliforms, that require iron as a nutrient (Sandholm and Korhonen, 1995). Moreover, it can enhance killing by phagocytes by withholding iron from the bacteria, which prevents the production of dismutase, a bacterial enzyme that inactivates superoxide radicals. It may also be active in modulation and regulation of macrophage, lymphocyte and neutrophil functions and it also has an opsonizing effect (Sordillo et al., 1997). Citrate can form complexes with iron making the iron available for bacteria. The lactoferrin content in milk is low and the citrate content is high in the lactation period. The citrate content decreases during the dry period while lactoferrin increases. Therefore, the activity of lactoferrin reaches the optimal period for bacteriostatic activities during this period (Persson , 1992).

2.3.3. Lysozyme

The origin of lysozyme is not known. It may come from the blood or be liberated from leukocytes or epithelial cells (Persson, 1992). Lysozyme hydrolyses the peptidoglycan structure of bacterial cell walls, intensifies antibody- and complement-mediated bacteriolysis, stimulates opsonization by IgM and the binding of the IgM-bacterium complex to phagocytes (Sandholm and Korhornen, 1995). However, the concentration of lysozyme in milk is low and its direct bacteriolytic effect in milk is weak (Sandholm and Korhornen, 1995), although the concentration increases during mastitis.

2.3.4. The complement system

Various complement factors leak from the blood into milk in response to inflammation and have important defence functions , e.g. opsonization of microorganisms and enhancement of phagocytosis (C3b), chemoattraction of neutrophils (C5a), and direct lysis of the cell membranes of bacteria (C5b-9) (Persson, 1992). Concentrations of complement are highest in colostrum, inflamed mammary glands and during involution (Sordillo et al., 1997). However, complement is inactivated by the fat in milk, and is therefore, not presumed to be an important defence mechanism in milk (Sandholm and Korhonen, 1995).

2.3.5. The lactoperoxidase/SCN^-/H_2O_2 (LP) system

The LP-system consists of the enzyme lactoperoxidase, thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2) (Persson, 1992). The LP system exerts its antibacterial properties

through the production of hypothiocyanate, which is a reactive metabolite from the oxidation of thiocyanate (Sandholm and Korhonen, 1995). Bovine milk has a high concentration of lactoperoxidase compared with milk from other mammals. However, the oxygen tension is low in mammary glands, which influences the production of hydrogen peroxide (Sordillo et al., 1997). The content of thiocyanate in milk depends on the feeding regime of the cow. The LP-system is considered to have a limited function as an antimicrobial system against udder pathogens (Sandholm and Korhonen, 1995; Sordillo et al., 1997).

3. Modulation of Immune Responses

Different methods have been tested in order to stimulate the immune response of the udder. Immunostimulation may be directed towards specific and/or non-specific immune responses. The specific, or acquired immune system is directed towards specific antigens, such as on bacteria and consist of antibodies, lymphocytes and macrophages (Tizard, 1995; Sordillo et al., 1997). The non-specific, or innate immune system is effective against a variety of pathogens, is mediated by neutrophils, macrophages and certain soluble factors and participates in the early stages of the inflammatory response to infection (Verhoef, 1991; Sordillo et al., 1997). Most research has been done using vaccines (specific response) and cytokines (non-specific response) but some studies using other immunomodulators, like glucan, have also been conducted.

3.1. Stimulation of the Immune Response Using Vaccines

Mastitis vaccines have been researched and developed for a long time in an attempt to enhance the immune defence against a unique specific antigen (Yancey, 1992). Mastitis vaccines are expected to eliminate existing infections, prevent new intramammary infections (IMI), and reduce the frequency and severity of clinical mastitis (Yancey, 1992; Sordillo et al., 1997). However, vaccines that are currently available apparently do not consistently reduce the incidence of new IMI or eliminate chronic mastitis. Most vaccine work has dealt with vaccines against *S. aureus* and *E. coli* infections and these will be described below. In addition, some studies have been done with vaccines against streptococcal mastitis, *Clostridium perfringens* mastitis and mycoplasma mastitis (Tyler et al., 1993).

3.1.1. Vaccines against *S. aureus*

Many kinds of vaccines against *S. aureus* infections in the udder have been used in both laboratory and field studies. A *S. aureus* bacterin could elevate the level of both serum and local antibodies and was effective in both reducing the severity of disease and increasing the spontaneous cure rate in *S. aureus* infections (Nickerson et al., 1993) but did not reduce the incidence of *S. aureus* IMI (Pankey et al., 1985). A vaccine against protein A also decreased the number of clinical mastitis cases and the spontaneous cure rate of cows treated with this vaccine was better than in cows treated with the bacterin although again, it did not reduce new IMI (Pankey et al., 1985; Yancey, 1992; Nickerson et al., 1993). The latter vaccine may increase the opsonic activity by allowing the binding of Ig to the bacterial surface (Sordillo et al., 1997). An attenuated live staphylococcal vaccine was developed and was used in heifers in late pregnancy. This vaccine increased the concentration of IgG_1 and IgG_2, and the opsonizing capacity for *S. aureus* (Watson, 1984) and was effective in reducing the incidence of clinical mastitis and subclinical mastitis in a herd that had a serious staphylococcal mastitis problem (Watson et al., 1996). Nordhaug et al. (1994a) tested a *S. aureus* vaccine containing inactivated bacteria with pseudocapsule mixed with α and β toxoids with a mineral adjuvant. The serum IgG_1 and IgG_2 antibody response to pseudocapsule and

α toxin increased moderately as well as did the concentration of IgG_1 in the milk. The vaccine had a positive effect in decreasing the incidence of *S. aureus* mastitis and on individual SCC but the differences were not statistically significant (Nordhaug et al., 1994b). The efficiency of vaccines against *S. aureus* in field use is still questionable, especially for the reduction of IMI.

3.1.2. Vaccines against *E. coli*

An *Escherichia coli* J5 bacterin vaccine against coliform udder infections is commercially available in California (Yancey, 1992). Hogan et al. (1992a) reported that the vaccine was able to produce antibodies that were cross-reactive with a variety of heterologous Gram-negative bacteria but the vaccine could not prevent IMI. In a field trial, immunization with *E. coli* J5 bacterin did not reduce the level of subclinical mammary infections with Gram-negative bacteria at calving but reduced the incidence of clinical mastitis (Hogan et al., 1992b). Later, an *Escherichia coli* J5 lipopolysaccharide (LPS) conjugated vaccine was produced which increased the magnitude of cross reactivity with heterologous Gram-negative bacteria and increased the specific antibody titre to LPS (Tomita et al., 1995). The *E. coli* J5 LPS conjugated vaccine elicited a specific immune response to LPS and may further reduce the severity and frequency of episodes of clinical symptoms that are generally associated with Gram-negative IMI by inhibiting pathogenesis induced by LPS.

3.2. Stimulation of the Immune Response Using Cytokines

Cytokines are hormone-like soluble proteins and glucoproteins synthesised naturally by both immune and non-immune cells. They are involved in the regulation of most aspects of the host defence and inflammatory responses to infection. Many cytokines have been identified and some of them e.g. interleukins, colony stimulating factors (CSF) and interferons have been used in research to treat or prevent, mastitis in cows.

3.2.1. Interleukin-1β

Interleukin-1β (IL-1β) is a cytokine synthesised by macrophages and it has been shown to activate B cells, T cells and macrophages. Shuster et al. (1993) reported that the concentration of IL-1 increased before the influx of leukocytes into endotoxin-infused glands. These results suggested that IL-1 may have a role in mammary gland defences. IL-1β induced the accumulation of leukocytes, mainly neutrophils, in the ovine udder after intramammary infusion (Persson Waller et al., 1996). IL-1β has also been used in order to treat *S. aureus* mastitis and 83% of the glands treated with IL-1β responded to therapy (Daley et al., 1991b). However, 50% of the treated quarters relapsed and only a total of 42% of the quarters treated with IL-1β were cured (Daley et al., 1991b). It is possible that IL-1β may become a suitable alternative to, or may be used in combination with, antibiotics in mastitis therapy.

3.2.2. Interleukin-2

Interleukin-2 (IL-2) is a cytokine synthesised by helper T cells. It stimulates T- and B-cell proliferation and modulation, as well as antibody production (Nickerson, 1994). Sordillo et al. (1991a) reported that the levels of IL-2 in mammary gland secretions decreases before calving, which might contribute to an increased susceptibility to infections during the periparturient period. Several studies have been performed in an attempt to improve the defence mechanisms of the mammary gland using IL-2. Nickerson et al. (1992) showed that intramammary infusion of IL-2 can enhance the SCC in bovine milk. Leukocytes infiltrated the connective tissue stroma after IL-2 infusion and the concentrations of Ig-producing plasma cells increased in the tissue; IgG_1 plasma cells

predominated, followed by IgG_2, IgA and IgM (Nickerson et al., 1992). IL-2 is an effective immuno-enhancer of mononuclear cells during the non-lactating and pre partum period (Torre et al., 1992). IL-2 can increase cytotoxic activity and bactericidal activity of mammary gland lymphocytes and also enhance the expression of MHC class II molecules on lymphocytes (Sordillo et al., 1991b). In addition, IL-2 can activate phagocytosis of PMN (Daley et al., 1991b) and increase their bactericidal activity *in vitro* (Shafer - Weaver and Sordillo, 1996). IL-2 has been used not only in mastitis prevention but also in order to treat *S. aureus* infected mammary glands (Daley et al., 1991b). It has been used as adjuvant (Pighetti and Sordillo, 1995) and as adjunct therapy with antibiotics to improve their therapeutic efficiency (Daley et al., 1992). The range however between therapeutic and toxic doses in cases of mastitis of IL-2 may be narrow (Sordillo et al., 1991c).

3.2.3. Colony stimulation factors

Colony stimulating factors (CSF) are a group of cytokines required for the proliferation and differentiation of a variety of hematopoetic stem cells and CSF can also activate cell function (Nickerson, 1994; Sordillo et al., 1997). Each CSF has a special cell line as a target, e.g. G-CSF (granulocytes) and GM-CSF (granulocytes and macrophages). Subcutaneous injection of G-CSF in dairy cows resulted in leukocytosis in the blood and the mammary gland, an increase in neutrophils and mononuclear cells in the milk and it reduced new intramammary infections (IMI) (Nickerson et al., 1989; Kehrli et al., 1991a). In addition, G-CSF administration increased the bactericidal and cytotoxic ability of neutrophils (Kehrli et al., 1991b). Cows receiving rBoG-CSF during the periparturient period did not show any adverse reactions attributed to the injections and their milk production and milk quality were unaffected (Kehrli et al., 1991b).

Neutrophils from both blood and milk treated with rBoGM-CSF *in vitro*, exhibited significantly more chemotactic and bactericidal activity, tended to produce more superoxide anion than control cells (Sordillo et al., 1992a) and enhanced phagocytosis of neutrophils (Daley et al., 1993). The effects of rBoGM-CSF on bovine neutrophil populations appeared to be dose-dependent (Sordillo et al., 1992a). GM-CSF could increase both the total number and the function of mammary gland neutrophils after parenteral administration in goats during the early dry period (Sordillo et al., 1997).

3.2.4. Interferon-γ

Interferon-γ (IFN-γ) is a T-lymphocyte derived cytokine which is often produced in response to antigen or mitogen stimulation. IFN-γ can increase the bacterial phagocytosis and the bactericidal activity of mammary neutrophils during the periparturient period (Sordillo and Babiuk, 1991a). Intramammary infusion of rBoIFN-γ enhances phagocytosis of *E. coli* and *S. aureus* and increases the production of oxygen dependent bactericidal components during the early phase of the dry period (Fox et al., 1990). Administration of rBoIFN-γ can enhance the activity of T-lymphocytes that express IL-2 receptors in the mammary gland indicating that rBoIFN-γ can stimulate specific immunity in the bovine mammary gland and may be an effective adjuvant in mastitis immunization protocols (Pighetti and Sordillo, 1996). Intramammary infusion of a single treatment dose of rBoIFN-γ as high as 105 U/quarter can be given without altering the composition normal mammary gland secretion in the treated quarters. However, the range between therapeutic and toxic doses of rBoIFN-γ may be narrow (Sordillo et al., 1992b). IFN-γ infused into mammary glands before challenge with *E. coli* after calving, resulted in fewer infected quarters, a shorter duration of mastitis, less severe clinical symptoms and lower mortality than in untreated

controls (Sordillo and Babiuk, 1991b).

3.3. Stimulation of the Immune Response Using Other Immunostimulants

A number of different substances are reported to be able to stimulate the immune response in various species (Raa, 1996). So far, only a few, glucan and ginseng, have been evaluated for their effect as immunostimulants of the udder of ruminants. Ginseng can enhance the activity of phagocytic cells and lymphocytes in bovine blood and milk *in vitro*, but *in vivo* studies evaluating its effect have not been performed (Hu et al., 1995; Concha et al., 1996). More studies have been done using glucans as immunostimulants. β1,3-glucan has been used to stimulate the ovine mammary gland defence and to modify the response to staphylococcal mammary challenge during the dry and lactation periods of ewes (Buddle et al., 1988). Glucan treatment reduced the number of staphylococcal infections compared with the controls and the SCC reached peak levels earlier in the glucan-treated group. Moreover, the activity of macrophages from the ovine mammary gland was substantially enhanced by glucan (Buddle et al., 1988). Persson Waller and Colditz (1998a) reported that intramammary infusion of β1,3-glucan can induce increased numbers of leukocytes, mainly monocytes and macrophages, and a selection for CD4+, B-cell, WC1+, and L-selectin+ lymphocytes and for CD14+ leukocytes in ovine mammary secretions. β1,3-glucan was also used as supportive treatment to dry cow therapy (Oxaclen foam) 10-20 days before calving by subcutaneous injection into the supramammary lymph node region and was considered to improve udder health and reduce clinical signs of mastitis (Vasil, 1994).

4. Conclusion

Udder diseases are one of the most common problems among dairy cows. Different herd health programs, including the use of antibiotics at drying off time to treat subclinical infections or to prevent new infections, have only had limited success in controlling the disease. Antibacterial components in mammary secretions increase substantially after drying off but the rate of change during early involution is apparently not sufficient to prevent new intramammary infections (IMI). The cellular and humoral immune status changes substantially during involution. Immunity functions are depressed during the periparturient period. Therefore, new approaches in the prevention of udder infections are warranted, such as the stimulation of inflammatory reactions and immune responses during the early involution and the periparturient periods. Stimulation of immune and inflammatory reactions may reduce IMI. An increasing interest has lately been directed towards different ways of stimulating the immune defence of the udder in order to enhance udder health and prevent mastitis. Although there have many successful researches on the use of immunostimulants to modulate inflammatory and immune responses in the mammary gland. there is limited research in field trials. The complexity of the defences mechanisms of the udder may influence the success of studies. More studies are needed to provide additional information before we can apply immunostimulants for the prevention of bovine mastitis.

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