Introduction

Over the past thirty-five years, milk production has dramatically increased in Western Europe and North-America. Producers attempt to increase milk yield as a means of increasing profitability. However, several studies have demonstrated that high-producing cows are at increased risk of infectious diseases (Grohn et al., 1994). Among those diseases, clinical mastitis is the most costly disease of the dairy industry (Esslemont and Peeler, 1993). Costs associated with clinical mastitis include decreased production, cost of treatment, extra labor, and an increased rate of cow replacement (Bartlett et al., 1990). The immune response to an incoming infection into the mammary gland is of utmost importance for the health of the dairy cow. Understanding the basic principles of this immune response is important to be able to guide dairy farmers with regard to management of risk factors and determining reasonable goals with regard to somatic cell counts and clinical mastitis. The goal of this paper is to explain the basic components of the immune response to intramammary infection.

Physical barriers

The teat canal provides the first effective barrier to invasion of the mammary gland by potential pathogens. The teat or streak canal is lined with keratin; a scleroprotein derived from the teat canal epithelium. It is a mesh-like substance that partially occludes the opening of the teat canal and inhibits the penetration of bacteria into the teat cistern. Keratin contains a high concentration of fatty acids. The composition of those fatty acids does affect the ability of the keratin to resist infection. Those animals whose keratin contains high concentrations of lauric, myristic and palmitoeic acid are much less...
susceptible to infection than those that have higher concentrations of stearic, linoleic and oleic acids. The heritability of fatty acid content of keratin I high and may offer a means of breeding nonspecific resistance to infection in the future. The keratin of animals found to be more susceptible to infection is often thinner, less dense and detached from the epithelium than cows that are less susceptible to infection. Keratin also contains cationic proteins (ubiquitin) which have been show to bind to the cell wall of *Streptococcus agalactiae* and *Staphylococcus aureus* disrupting the osmoregulatory mechanism of the bacteria causing their destruction.

The smooth muscle surrounding the sphincter also provides a physical barrier to invasion of the teat cistern by bacteria. Leaking teats are a recognized risk factor for new infection by environmental pathogens particularly coliforms. The teat canal can take up to two hours after milking to resume its normal confirmation after milking. This the reason for providing feed and water immediately after milking to encourage cows to remain standing and the reason for having freshly cleaned and bedded stalls when the cows do lie down. Injury to the teat muscle and /or keratin lining caused by crushing, inappropriate treatment or manipulation of the teat canal, or from the development of teat end lesions associated with faulty milking equipment or chronic over milking can cause an increase in new infections.

The flushing action of daily milking is also a protective mechanism.

**Mammary gland immune response**

Similar to most infectious diseases, mastitis incidence depends on three components: exposure to microbes, udder defense mechanisms, and environmental risk factors. Innate udder defense plays a role to protect the healthy gland (Kerhli and Shuster, 1994). In a normal non-infected and non-inflamed quarter, the somatic cell population consists of polymorphnuclear cells, macrophages, lymphocytes and epithelial cells (Concha 1986). The majority of cells are macrophages (approximately 60% of cells). The remaining cells include lymphocytes (approximately 30%), polymorphnuclear cells (approximately 10%)
and epithelial cells (about 2%) (Burvenich et al. 1995, Paape and Contreras 1997, Paape et al. 1991). Somatic cell counts are relatively low, usually at or below 50,000 cells per ml. In Figure 1, the upper 95% confidence limit is shown, indicating that 95% of uninfected cows are below approximately 100,000 cells per ml., except in the last month of lactation. In long term uninfected cows the number of cells in milk follow an approximate inverse lactation curve (Laevens et al. 1997, Schepers et al. 1997, Harmon 1994, Brolund 1985).

Figure 1. The upper 95% confidence limit of somatic cell counts in uninfected cows, the numbers in the legend indicate three parity groups: first parity, second parity and third and higher parities (Adapted from Schepers et al. 1997).

The macrophages, monocytes and PMNs constitute part of the innate immune defense mechanism and act as a first line of defense once microorganisms have penetrated into the teat cistern. They use primitive nonspecific recognition systems to bind, ingest and destroy microorganisms. Once infection is established PMNs are recruited to the site of infection by the chemoattractant effects of cytokines released by lymphocytes. The PMNs make up 90% of the leukocytes present during infection and they kill by phagocytosis. Their function can however be hindered by their presence in milk. The milk environment limits their sources of energy, dilutes the concentration of cytokines
which mediate immune function and milk fat and protein (casein) can interfere with their phagocytic function. Concurrent disease that is often present during the periparturient period also disrupts PMN and lymphocyte function and recruitment.

Several researchers have reviewed the normal physiological processes of udder defense mechanisms (Burvenich et al, 1994, Craven and Williams, 1985, Kremer et al., 1990, Sordillo et al., 1997). Macrophages play a crucial role in the innate defence mechanisms of the mammary gland. The macrophages have the important function of ingesting of foreign materials such as micro organisms and cellular debris, but also have a key function in antigen processing and presentation in association with the major histocompatibility complex (Sordillo et al. 1997). Finally, macrophages produce cytokines, small inflammatory modulators which are important in initiating the inflammatory response to incoming microorganisms (Zecconi et al. 2000).

Lymphocytes maintain a steady state of induction versus suppression of the immune response (Burvenich et al. 1995, Sordillo et al. 1997). In milk from normal cows, the lymphocyte population is comprised predominantly of T-cells (approximately 60% of lymphocytes) with relatively few B-cells (approximately 20% of lymphocytes). The majority of T-cells are CD8+ (cytotoxic T-cells) with a lower proportion of CD4+ cells (T-helper cells) (Taylor et al. 1997, Soltys and Quinn 1999). These lymphocytes produce a host of cytokines that are important to maintain a steady state and a balanced immune response to invading micro organisms. The cytokines form a chemical gradient towards which the polymorphnuclear cells show a chemotactic movement. This process is depicted in figure 2. Lymphocytes also function as scavengers, removing old or damaged cells. The T-cell distribution in the mammary gland is similar to populations found on other mucosal surfaces (Roitt et al. 1998, Asai et al. 2000).

Polymorph-nuclear cells are the primary warriors in the mammary gland, functioning specifically in phagocytosis and killing of invading bacteria (Sordillo et al. 1997).

During the inflammatory response, all these resident cells act in an organized manner to eliminate the inflammatory noxe. During the early inflammatory response the resident
cells attempt to phagocytose and kill the incoming bacteria, and they produce cytokines to attract immune cells toward the site of inflammation. Consequently, there is a massive influx of polymorphnuclear cells, and a somewhat pathogen dependent preferential influx of CD4+ T-cells (Taylor et al. 1997, Sordillo et al. 1997, van Werven et al. 1997).

**Figure 2. The process of PMN chemo-attraction into the mammary gland (From Suriyasathaporn et al. 2000b).**

Antibody concentration in the normal mammary gland is low (1mg/ml) although concentration in colostrum can range from 30 to 50 mg/ml. And is dependant upon vascular permeability of udder tissue. Active infection disrupts vascular barrier causing an increase in immunoglobulin levels. Immunoglobulin is selectively transferred to mammary secretions and are present as IgG, IgM and IgA. The main function of IgG is opsonization to promote phagocytosis, the antibody binds to the offending pathogen enhancing its destruction by phagocytosis or ingestion by the PMNs. IgA does not promote opsonization but will prevent bacterial adherence to the epithelial membrane of mammary tissue, inhibit bacterial multiplication neutralize toxin and agglutinate bacteria.

**Response to infection**

After bacteria invasion, leukocytes in milk recognize the pathogens and attempt to remove this intramammary infection (IMI) immediately (Figure 2A). In case the initial
response does not resolve the infection, the udder defense shows an early induced response at about 4-12 h after infection (Figures 2B and 2C). Regardless of the type of bacteria that cause the initial infection, both quantity and quality of polymorphonuclear leukocytes (PMN) in the udder are important components of udder defence. (Burvenich et al., 1994, Daley et al, 1991). Both bacteria and leukocytes in the infected quarters release products (such as cytokines), many of which are chemoattractants for leukocytes circulating in the blood stream (Figure 2B). Neutrophils move rapidly from the blood stream into the infected quarters (Figure 2C). If the bacteria are destroyed, recruitment of neutrophils into the gland is ceased, and only a mild inflammatory episode will be required to restore health in the gland (Kehrli et al., 1994). The cell count response of a cow with a successful response is shown in Figure 3.

Figure 3. Somatic cell count pattern in the successful response of the mammary immune system to an incoming bacterial infection.

Occasionally, the defense mechanisms of the infected mammary gland looses the battle with bacteria, and bacteria multiply. A more serious response with systemic clinical signs may be due to an abundant production of soluble factors, such as cytokines, that cause clinical signs of mastitis (Kremer et al.1990). Cytokines such as interleukin-1 (II-1) and tumor necrosis factor (TNF) trigger a more systemic response. Body temperature increases, cells are mobilized, blood flow increases, and in some extreme situations a massive response occurs that leads to systemic shock-like symptoms. In most situations
these symptoms are caused by endotoxic mediations, and not necessarily by the microorganisms.

In other cases, a more chronic response may occur, bacteria multiply and the immune system is continuously activated. Chronic infections result in long term high somatic cell counts in milk (Daley et al. 1991). It has been suggested that intra-cellular survival of bacteria is essential in the pathogenesis of such chronic infections (Daley et al. 1991). In Figure 4, such a chronic increased somatic cell count pattern is shown.

**Figure 4. Typical somatic cell count pattern of a chronically infected cow**

Chemotaxix, phagocytosis and killing

The capacity of blood leukocytes to migrate into infected udder (so-called chemotaxis) has been shown to be an essential parameter for the protection of the mammary gland to overcome a bacterial infection (Hill, 1981; Shuster et al., 1996, Van Werven, 1999). The in vitro PMN chemotaxis was related to the severity of infection indicated by repeated bacterial counts during the course of disease (Kremer et al., 1993a,b, Van Werven et al., 1997). Mechanism of the migration of leukocytes out of blood vessels, or extravasation, depends on adhesive interactions activated by the chemoattractant (Figure 2). The adhesive molecule P- and E-selectin appears on epithelial cell after exposure to chemoattractant, like (Figure 2A). Then integrins, like CD11a:CD18 and CD11b:CD18, on leukocytes acts to form ICAM-1. In consequence, the leukocyte attaches firmly to the
endothelium and the rolling is arrested (Figure 2B). In the last step, leukocyte extravasates, and then migration through the tissues under the influence of chemoattractant (Figure 2C). This step is related to CD31 in leukocytes and endothelial cells. However, preinfection expression of CD11a and CD11b were not related to severity of mastitis (Dosogne et al., 1997; Van Werven et al., 1997). The lack of this relation may be due to the expression of integrin was increased after infection (Paape et al., 1996; Smits et al., 1998). The lack of appearance of chemotactic and CD18- upregulating activities until 12 h after challenge indicated that delays in neutrophil recruitment resulted from an initial lack of bacterial recognition and inflammatory mediator production (Shuster et al., 1997). Therefore, the upregulating of integrin expression after infection is more closely related to capacity of udder defence (Shuster et al., 1996). Some substances, for example cortisol, induce a decrease in the number of the enhancement of CD18 receptors after experimental IMI, probably modulating the acute inflammatory response in mammary glands of lactating cows (Roets et al., 1999).

Udder phagocytes, usually PMN, act to remove the invaded pathogen by the specific process which includes opsonization, ingestion, and killing mechanism (Figure 2A, B, C, respectively). Efficiency of removal of pathogens is related to either quantity or quality of phagocytes in the udder (Daley et al., 1991). After ingestion by PMN, microorganisms are subjected to destructive mechanisms. Two mechanisms have been described: an oxygen-dependent mechanism and an oxygen-independent mechanism. Although the oxidizing agents form a potent antibacterial system against the common udder pathogens, it looses bactericidal activity in milk-containing cultures (Cooray and Bjorck, 1995). A deficiency in the luminol-enhanced chemiluminescence in destructive mechanism was shown in PMN of cows developing mastitis compared to healthy controls (Cooray and Bjorck, 1995; Zecconi et al., 1994). The generation of reactive oxygen species is also related to the severity of experimental IMI (Lohuis et al., 1990; Heyneman et al., 1990).

Other soluble factors

The lactoperoxidase/thiocyanate/H2O2 system in milk will inhibit the growth of or kill *Staph aureus*, many *Streptococci sp.* and coliform bacteria. H2O2 produced by many
Streptococci bacteria stimulates lactoperoxidase to oxidize thiocyanate, which in turn will damage bacterial cell walls causing their death. Lysozyme present in the udder will also destroy bacteria by disrupting their cell walls.

Lactoferrin is a product present in milk and colostrum which is bacteriostatic. Its iron chelating properties inhibit the growth of many potential pathogens including coliform and Staphylococcus sp. Lactoferrin may also be involved in the modulation of the activity of macrophages, lymphocytes and PMNs.

**Mastitis and the periparturient period**

The incidence of both clinical and subclinical mastitis is greatest in most herds during early lactation and is most often caused by opportunistic environmental pathogens. (Strep sp and coliforms). This is depicted in figure 5. However those infections most frequently occur during the dry period. New infections by environmental organisms are highest during the first two weeks after dry-off and the last two weeks prior to calving.

**Figure 5. Incidence of clinical mastitis in the periparturient period (Barkema et al. 1998).**
The intramammary infection rate is 2 to 12 times higher during the dry period than at any other time during the lactation cycle of the cow. These infections usually persist through the dry period and are still present at parturition. They usually result in clinical mastitis at or within 60 days of calving. The proportion of all cases of clinical mastitis due to coliform infections at 2, 4 and 8 weeks postpartum are 25%, 45%, and 60% respectively. Poor hygiene and frequent inattention to the dry cow nutrition causes these animals to be more readily infected by opportunistic bacteria.

Increased infection rate is not limited to the mammary system. Infectious diseases of the GI, respiratory, and reproductive system and multiple organ systems are more common at this stage of the gestation cycle of dairy cattle.

It has become clear that opportunistic infections are associated with severe compromises of host defense mechanisms and are specifically related to immuno-suppression of cattle during the periparturient period. Research has demonstrated critical PMN dysfunction affecting chemotaxis and phagocytosis. Lymphocyte function at this time is depressed as indicated by depression of immunoglobulin production and decreased cytosine production. Causes for these phenomenon’s are unknown but are believed to be associated with basic endocrine changes associated with pregnancy and impending parturition. There is a specific and broad range of suppression of antigen specific immunity in the periparturient animal. The purpose of this innate phenomenon is to prevent development of immunity to self, paternal and offspring antigens that could ordinarily occur with pregnancy and parturition.

**Immunosuppresion and pathogenesis of mastitis**

Recruitment of leukocytes into tissues is crucial for surveillance of infectious agents and is an integral component of cellular mediated immunity. This allows for rapid conscription of neutrophils (PMNs) at the infection of site of injury. Lymphocytes (B type) sensitized at the site of infection will then migrate to secondary lymphoid tissues (lymph nodes) for responses against antigens presented in germinal centers. The
interaction of leukocytes of various types with endothelial cells of the circulatory system is essential for effective leukocyte egress and migration. Leukocyte egress and migration is a complicated multistep process which involves a variety of cytokines and other modulators which allow leukocyte adhesion to and migration through endothelial cells to the site of infection. This process is altered by many stress-related factors including parturition.

Phagocytosis and bacterial killing mechanisms of neutrophils in conjunction with humoral factors (antibodies) are critical defense mechanisms of the mammary gland. During the periparturient period neutrophil function is changed or compromised. At this time there are fewer circulating neutrophils and their ability to respond to an inflammatory response is delayed. Neutrophil microbiocidal function is impaired by the stress associated with impending parturition. Lymphocyte function is also suppressed or altered at this time. Their role in the production of cytokines responsible for activation and recruitment of macrophages and neutrophils is depressed.

There are likely a number of physiological reasons for altered immune function. Neurohormones likely contribute to immune dysfunction. Increased plasma levels of endogenous opioids during this period will reduce immune function. Generally pregnancy suppresses cell-mediated immunity and enhances humoral immunity as pregnancy progresses. Both estrogen and progesterone have this capability. Progesterone levels associated with pregnancy are known to depress lymphocyte function; γ interferon and interleukin-2 production is dramatically reduced. The greatly increased levels of estrogen associated with parturition not only depress neutrophil function but may also make immune cells even more sensitive to the immune suppressive effects of progesterone. Lymphocyte function may also be altered by the normal increase in circulating levels of other hormones such as prolactin, somatotropin and insulin. Hormone sensitivities of immune cells during gestation are normal and result in functional changes that are protective of the host and her fetus.

Metabolic shifts associated with impending parturition and lactation may also contribute to impaired immune function and immunosuppression. Recent research has demonstrated
conclusive evidence that immune suppressive effects of lactation are real and contribute to postpartum immune suppression. Researchers at NADC showed that mastectomized animals recover their normal immune function within one week of calving (Kimura et al. 1999). Intact lactating cows required at least two to three weeks to resume normal immune function. Severe negative energy and protein balance during the transition period of cows will exacerbate immune suppression of already immune-compromised animals. Concurrent disease such as metritis, respiratory disease, milk fever etc. will also contribute to further immune dysfunction. Release of endogenous corticosteroids associated with any inflammatory response will have negative effects on immunity. They will suppress leukocyte function as well as depress humoral immunity.

**Herd cell count levels and clinical mastitis**

There appears to be somewhat of a negative correlation at herd level between the types of responses that was discussed above (figure 3 and 4). When the number of chronic infections in a herd is low, the bulk milk somatic cell count is usually also low. Herds that have very few cows with chronic infections appear to have more acute short-term infections (Erskine 1988, Green et al. 1996).

**Figure 6. Relationship between bulk milk SCC and clinical mastitis incidence**
Recently, it was reported by Miltenburg et al. (1996) that they observed an increase in herd level incidence of clinical mastitis with a decreasing bulk milk SCC. Barkema et al. (1988) reported a slight but non-significant increase in clinical mastitis in herds with a low bulk milk SCC (Figure 6), but they observed a greater proportion of clinical cases with systemic signs of illness in herds with a low bulk milk SCC compared to herds with a higher bulk milk SCC.

Discussion and Summary

The immunological response to intramammary infection is a normal function of the cow’s immune system. The biological features of this response have been extensively studies and reported (see for a review Harmon 1994). When the response is successful, the mammary gland returns to a normal uninfected state. Unsuccessful attempts to eliminate infection from the mammary gland require attention of the producer and the veterinarian. It is especially important to understand the normal immune response, to be able to diagnose an abnormal response.

The immune mechanisms of the mammary gland of the dairy cow are complex and affected by a multitude of internal and external factors including the stage of gestation, lactation, and nutritional status the presence of concurrent disease. External factors include environmental and housing factors, health management factors such as immunization, feeding and nutrition management, and existing level of disease in the herd. The immune response of the normal healthy dairy cow is capable of resisting and clearing many infection challenges very effectively.

It has become obvious however; that the immunosuppresion associated with the peri-parturient period of the dairy cow is a major contributing factor the overall incidence of mastitis in a herd and is particularly associated with the level of opportunistic environmental infections that take place during the dry period. These infections are often expressed as clinical mastitis or elevated somatic cell counts during early lactation (first
100 days). Since the changes in immune function associated with the peri-parturient period of the cow are innate and serve a greater purpose it is more appropriate to take other more obvious steps to protect her from infection during this period. The dry period of the cow and peri-parturient period for the soon to calve heifer represent that period of the gestation cycle that is most often neglected by management. Prevention of mastitis and other diseases commonly associated with the early postpartum period are dependent upon a superior level of hygiene and the need to provide appropriate and adequate nutrition. Herd managers must develop specific management protocols for animals during this period.

REFERENCES


Miltenburg JD, DeLange D, Crauwels APP et al. (1996) Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands Vet Rec 139:204-207


