Role of neutrophils in the pathogenesis of bovine coliform mastitis

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Summary

Around parturition dairy cows are at increased risk for intramammary coliform infections. A high proportion of these infections may develop disease characterized by inflammatory signs and sepsis during the first 60-70 days of lactation. The clinical picture is reputed as toxic mastitis. There is a remarkable variability in the clinical expression and complications of coliform mastitis around parturition, ranging from clinical severe to moderate and mild. The increased incidence of intramammary infections with coliforms around parturition can be explained by decreased innate immunity and dysregulation of the inflammatory reaction (immunocompromised condition) that is related to parturition and rapid induction of lactation. Neutrophils are one of the most important components of the efferent innate arm. Since the beginning of the 90ies, research on bovine mammary innate defense mechanisms, especially neutrophil functions, increased significantly. Most efforts have been focused on diapedesis, phagocytosis and killing by these phagocytes. Viability and programmed cell death (apoptosis) in neutrophils, was also studied. How neutrophil functions and viability/apoptosis modulate the clinical outcome of coliform mastitis have also been the subject of intensive research. The metabolic demands of increasing milk secretion (protein and energy) seem to impact the ability of the periparturient cow to manage its metabolism and its ability to recover from its immunocompromised condition. Cows with low glucose, ketosis, fatty liver and elevated NEFAs have poorer immune, especially neutrophil function. A new active named Phibrate promise to work efficient in mobilization of fat acids from liver and reduce cholesterol and triglycerides from liver. The study of the afferent (sensing) arm that recognizes of a diverse array of pathogens, is a new area of interest of the last years. The mechanism of endotoxin sensing may have immense practical importance. The easiest prophylaxis today to prevent toxic mastitis is to provide periparturient cows not only with optimal hygiene conditions but also with appropriate diets during the transition period and as few additional stress events as possible around calving. Vaccine strategies have had limited success in reducing clinical symptoms associated with Gram-negative mastitis. Anti-microbial treatment of Gram-negative intramammary infections and therapeutic treatment to counteract the excessive inflammatory response elicited by LPS and Gram-negative pathogens remain often sub-optimal.

Innate immunity in the bovine mammary gland

Innate immunity is the most universal and the most rapidly acting type of immunity. Invertebrates survive through innate immune mechanisms alone. Vertebrates developed alternative systems for pathogen recognition and elimination, called adaptive immunity. The innate immune system is an evolutionary old system that has been refined for a longer period of time than the adaptive immune system. It is more perfect in almost every way (Beutler, 2004). In the lactating bovine mammary gland it is the most important defense system. Vaccinating cows against mastitis generally is not very successful (Nickerson, 1985). The elimination of even one subset of innate immune effector cells (for example, neutrophils) may be sufficient to cause a profound immunodeficiency state (Jain et al, 1971).

Cells are not required for all innate immune reactions. Proteins and other molecules are sufficient to kill microbes that have not yet been engulfed by cells (Beutler, 2004). Complement, lysozyme, lactoferrin, and antimicrobial peptides are among the proteins most commonly cited in this regard (Grün, 1985 ; Malinowski, 2002 ; Schmitz et al., 2004). There is no doubt that several innate immune proteins provide a measure of protection in the mammary gland cisterns. However, in the bovine udder the protective role of some of them seem to be unimportant (e.g. lysozyme is not a significant defense protein in the bovine mammary gland). Furthermore some of them have actions that depend on the stage of the lactation cycle (e.g. lactoferrin, only effective during steady state involution in absence of citrate; Smith and Schanbacher 1977; Rejman et al. 1989). At the end of this review special attention will be drawn on the protective effect of CD14 and LPS binding protein (LBP).

Innate immunity is an enormously broad study object and one effector, the bovine neutrophil, has been extensively studied over the last 20 years (see reviews of Paape et al., 2000, 2002 & 2003). Three aspects of innate immunity are important in the defense against intramammary *E.coli* infections : 1) the afferent (sensing) arm that recognizes a diverse array of pathogens, 2) the efferent (effector) arm that kills these pathogens. Each arm is divided into cellular and humoral components. The molecules that sense microbes are not necessarily the same as those that kill them, and 3) sparing tissues of the host ; i.e. there must be self-tolerance (Beutler, 2004).

The innate immune system of the bovine mammary gland seems to have a battle plan against intramammary infections that is dependent on the stage of lactation ; i.e. dry period, colostrogenesis and established lactation (Burvenich et al., 2003 & 2004) and parity (Vangroenweghe et al., 2004a & 2004b).

Intramammary infections and early mammary involution

Susceptibility of the mammary gland to new intramammary infections is markedly increased during early involution (drying off ; active involution, first week or two, highest incidence of new infections) and during the periparturient period (colostrogenesis) (Nickerson 1989; Oliver and Sordillo, 1988). These periods coincide with unique local and systemic physiological phenomena that interrupt or induce lactation. Considerable changes in mammary tissue remodeling and nutritional demands occur.

A number of local phenomena contribute to the high incidence of new intramammary infections during the early dry period. Milk, an excellent growth medium for bacteria, is no longer periodically removed from the gland and leakage from the teats occurs because of the increase in intramammary pressure during the first days. Leukocytes, mainly phagocytes, start entering the gland by day +6 after dry-off (McDonald and Anderson, 1981). The cell counts do not reach protective levels until day 8, about 1 million. One of us just finished an experiment where the cell count was increased to a million over the first 8 days by injecting an irritant into the gland and we got better protection than with antibiotics (Paape, 2005, USDA Patent Application S.N. 10/895, 797, November 12, 2004, Methods for Prevention and Treatment of Mastitis). An immunocompromised condition of these phagocytes is established through local factors. Indeed, after diapedesis, phagocytes start ingesting milk fat, casein and cell debris. Ingestion decreases the phagocytic function and induces apoptosis. The citrate : lactoferrin ratio is very high which makes any bacteriostatic action of lactoferrin ineffective. Citrate chelates iron and make it available to bacteria.

Intramammary infections and mammary steady state involution

In the cow, active involution is completed by 3 to 4 weeks after drying off and followed by a phase of steady state involution. This is the period of greatest resistance to intramammary infection ; especially to Gram-negatives. If an infection occurs, it usually is spontaneously eliminated. This mid-dry period shows the lowest incidence of new infections because teats have become sealed, the small fluid volume in the udder cisterns and the unfavorable composition of medium to bacterial growth. Very high concentrations of phagocytes are reached. The immunocompromised condition is suspended because there is practically no milk fat or casein that could eventually inhibit phagocyte function. Defensins are relatively small, cationic peptides with an amphiphilic charge distribution that enables them to interact with, and disrupt, bacterial cell membranes. Their antibacterial action appears to result from their ability to form pores in target membranes leading to cell lysis. Defensins are produced in neutrophils and macrophages (Kaiser and Diamond, 2000). Lactoferrin, the major protein found in mammary secretions during steady state involution reaches very high concentrations (Smith and Schanbacher 1977 ; Rejman et al. 1989). The mechanism of lactoferrin activity has not been clearly defined but appears to originate primarily through iron sequestration and/or through direct interaction of its cationic *N*-terminal region with bacterial components. Bacteria which have high iron requirements are susceptible to lactoferrin. Among mastitis pathogens, *E. coli* are the most susceptible, followed by *S. aureus*. Streptococci seem to be resistant (Rainard, 1986a). The citrate : lactoferrin ratio is lowered (because milk synthesis has stopped) and immunoglobulin concentrations are elevated. The bacteriostatic activity of lactoferrin can be enhanced by antibodies specific to mastitis-causing bacteria, possibly by interfering with the bacterial iron-acquisition systems (Oliver and Bushe, 1987; Rainard, 1986b).

Intramammary infections and end of dry period

Transition cows are referred to as cows in their second half of the dry period through 2 to 4 weeks postpartum. Any physiological maladaptation from a pregnant, non-lactating to a non-pregnant, lactating status represents a challenging period in the production cycle of a modern dairy cow. Most metabolic diseases of dairy cows such as milk fever and ketosis, and abomasal displacement occur within the first 2 weeks of lactation.

From the second half of the transition period and especially during colostrogenesis, cows become more susceptible to new intramammary bacterial infections (Smith et al., 1985a & 1985b) . The contribution of lactoferrin to protection of the mammary gland is compromised by its low concentration in milk and the presence of citrate. Milk citrate can effectively compete with lactoferrin for iron binding and the resulting iron-citrate complex can be utilized by bacteria (Schanbacher et al., 1993). The phagocyte numbers not only decrease but their phagocytic capacity is reduced again because of the appearance of fat and casein (immunocompromised condition induced by local factors). However, during colostrogenesis systemic factors also affect the immune condition of the cow (immunocompromised condition induced by systemic factors). In addition the majority of infectious diseases experienced by the dairy cow also become clinically apparent during early lactation. This is especially the case for mastitis, but also includes diseases such as Johne's disease and salmonellosis.

Toxic mastitis during the periparturient period

A high proportion of intramammary coliform infections present at parturition, may develop disease characterized by severe inflammatory signs and sepsis during the first 60-70 days of lactation (Malinowski et al., 1983, Smith et al., 1985b, Smith et al., 1985a, Hogan et al., 1989) . The clinical picture is reputed as toxic mastitis and is very well known by veterinarians. Of those cows with severe Gram-negative infections, nearly 25% will either die or be culled. There is remarkable variability in the clinical expression and complications of coliform mastitis around parturition, ranging from clinical severe to moderate and mild (Hill et al., 1979, Hill, 1981, Heyneman et al., 1990, Vandeputte-Van Messom et al., 1993, Burvenich et al., 1994, Shuster et al., 1996, Hirvonen et al., 1999, Burvenich et al., 2003) . This is in sharp contrast with the more moderate clinical expression of coliform mastitis during established lactation (Burvenich et al., 1994, Shuster et al., 1996) . Delays in the inflammatory response in cows with peracute coliform mastitis have been reported in certain cows shortly after calving (Hill et al., 1979, Hill, 1981, Heyneman et al., 1990, Vandeputte-Van Messom et al., 1993) . The autotoxic character of the severe inflammatory response to infection falls in the category of sepsis research. The major point to be made about sepsis is that the same mechanisms that contain a small infection can, if generalized, threaten the survival of the host. Nonetheless, evolution has calculated that it is best that these mechanisms be maintained (Beutler, 2004).

Cow parity and severity of clinical mastitis

There is a tendency to see more severe clinical coliform mastitis cases in multiparous cows (Van Werven et al., 1997, Mehrzad et al., 2001a & 2001b, Mehrzad et al., 2002, Burvenich et al., 2003, Vangroenweghe et al., 2004a) . Blood neutrophil function was higher in younger animals than in cows after their 4th parturition. The drop in neutrophil ROS production around parturition is more pronounced in multiparous cows (Mehrzad et al., 2002) . The pronounced reduction in neutrophil oxidative burst capacity and viability in milk neutrophils of multiparous cows may be involved in the underlying mechanisms that make these animals more susceptible to periparturient infectious diseases. Moreover, white blood cell viability and oxidative burst have been found to be significantly different between primiparous cows and multiparous cows during the periparturient period. Using relative high *E. coli* inoculum doses, all primiparous cows reacted as moderate responders based on their quarter milk production in the non-infected quarter on day +2 post-infection. Based on clinical severity scoring, all animals were scored as mild to moderate in their clinical response throughout the entire experimental challenge period (Vangroenweghe et al., 2004a & 2004b).

Lymphocyte function in the periparturient cow

For nearly 30 years it has been recognized that the periparturient bovine immune system is less capable of battling pathogens and exhibits a wide range of immunological dysfunction (Guidry et al., 1976, Newbould, 1976, Wells et al., 1977, Paape et al., 2000) , manifested by impaired neutrophil and lymphocyte capabilities. The adaptive defense consists of a network of memory cells and immunoglobulins (Paape et al., 2000; Burvenich et al., 2000). Circulating lymphocytes have reduced capacity to produce interferon-gamma (IFN-gamma) (Ishikawa et al., 1994) and there are reduced IFN-gamma and interleukin-2 levels in lacteal secretions of periparturient cows (Sordillo et al., 1991) . This immunosuppression is most evident in the Th1 branch of lymphocyte activity, and may be essential in preventing unwanted immune reactions against self and paternal antigens exposed to the mother's immune system as a result of tissue damage in the reproductive tract during parturition. Lymphocytes exposed to 1alfa, 25-dihydroxyvitamin D_3