Immune Dysfunction in Periparturient Dairy Cows: Insight into Pharmacologic and Dietary Immune Treatments

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INTRODUCTION

With a $40.5 billion gross domestic value for milk produced in the U.S. during 2013, the dairy industry was the third largest sector of the 2013 U.S. animal agriculture economic engine. The value of milk produced in 2013 represented 24 % of the total value of animal agriculture production; this figure had grown from $21-23 billion/y over a decade ago. The 2007 NAHMS Dairy Study reported that during 2006, 23.6 % of cows were culled from operations, 26.3 % and 23 % were removed for reproductive and udder health problems (USDA, 2007). In addition, 16.5 % of cow mortalities were due to mastitis. Clearly, the economic value of controlling mastitis pathogens is immense. Most economic analyses of the cost of mastitis cite a 10 % production loss as only one part of the overall cost of the disease. A majority (65 to 70 %) of losses are associated with decreased milk yield resulting in lower production efficiency; the remaining costs are attributed to treatment. In addition to these direct losses, mastitis causes significant problems in milk quality control; dairy manufacturing practices; quality and yield of cheese; nutritional quality of milk; antibiotic residue problems in milk, meat and the environment; and genetic losses due to premature culling. These additional costs are very significant and are not always included in economic analyses of mastitis costs.

Because of the need for a safe, economical, and stable supply of food, those of us serving the livestock health industry must be prepared to provide the best quality advice and care in managing our nation's dairy herd. For dairy producers, the critical factor in providing a low somatic cell count milk supply is keeping cows free from mastitis. Mastitis is anything causing inflammation of the mammary gland, and infectious mastitis is caused by a plethora of microbial agents (Watts, 1988). Nearly half of the nation's herd of dairy cows will experience at least 1 episode of mastitis during each lactation. Research has already resulted in genetic selection for cows with lower somatic cell counts by the incorporation of this trait into the A.I. sire summary ranking indices. This approach mainly serves to reduce the normal increase in mastitis incidence that occurs as milk production goes up. Coliforms and environmental streptococci are the most common etiologic agents isolated from clinically severe mastitis cases on well-managed dairy farms (Anderson et al., 1982; Hogan et al., 1989). Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in cattle with clinical or subclinical coliform mastitis (Erskine et al., 1991; Jones and Ward, 1990; Kirk and Barlett, 1984). Hence, the advent of the *Escherichia coli* J-5 and other endotoxin core mutant vaccines in veterinary medicine many years ago provided us a tool to reduce the incidence and severity of clinical coliform mastitis (Gonzalez et el., 1989; Hogan et al., 1992a,b, 1995). However, there remains an unmet veterinary medical need of new ways to prevent or treat mastitis caused by environmental pathogens. For several years, research at the USDA's National Animal Disease Center in Ames, IA undertook a 2-fold approach for improving the dairy cow's resistance to mastitis - immunomodulation and genetic selection for superior immune systems. In this paper, we will focus on:

- The evidence for immune suppression in periparturient dairy cows,
- How this sets the cow up for infectious diseases such as mastitis, metritis and retained placental membranes, and
- Some of the early research on immune modulation of the transition dairy cow and how that impacted resistance to mastitis.

[1] No endorsements are herein implied. USDA is an equal opportunity provider and employer.

ROLE OF THE IMMUNE SYSTEM IN MASTITIS

Immunity against infectious diseases of cattle is mediated by diverse, yet interdependent, cellular and humoral mechanisms. Many environmental and genetic factors influence the ability of livestock to mount effective defense strategies against the various pathogens and normal flora that they are exposed to throughout their lifetime. Innate resistance to infectious diseases reflects the inherent physiological attributes of an animal that make it more or less susceptible to disease development by a particular pathogen. There are several cell lineages that comprise the immune system (e.g., B-cells, T-cells, neutrophils, eosinophils, basophils, macrophages, and mast cells). Each of these cell types has distinct responsibilities in providing host defense. Innate immunity represents the various immune components that are not intrinsically affected by prior contact with an infectious agent (Roitt, 1994). Lymphocytes provide the adaptive immune reactions that are antigen specific in nature and possess memory for future encounters with the same pathogen. In this paper we will present a novel approach of immune modulation of the innate immune system as a potential means to reduce antibiotic usage in veterinary medicine.

Our first understanding of cellular immunity is more than a century old and it actually involves research into the causes of bovine mastitis and the immune response. In his 1908 Nobel Lecture the Russian zoologist, Elie Metchnikoff, described disease as consisting "of a battle between a morbid agent, the external microorganism, and the mobile cells of the organism itself. A cure would represent the victory of the cells, and immunity would be the sign of an activity on their part sufficiently great to prevent an invasion of microorganisms (Metchnikoff, 1908)." Metchnikoff cited the work of a Swiss veterinary expert, Zschokke, who found that "plentiful phagocytosis of streptococci in the battle against infectious mastitis in cows, was a good sign. When phagocytosis was insignificant or not present, the cows were written off as no longer capable of producing good milk." This was later extended to include the idea that not only must the phagocytes engulf the microorganisms, but that these devouring cells must utterly destroy the microorganisms. In some cases, the streptococci of mastitis were found to "destroy the phagocytes after being engulfed by them thus liberating themselves to carry on their deadly work."

Today we have a far more detailed knowledge of the cow's immune response to pathogens in the mammary gland (and elsewhere). Neutrophils are one of the most important cell types of native defense mechanisms because they respond quickly (within minutes) and do not require previous exposure to a pathogen to effectively eradicate the microbe. A major function of neutrophils is the phagocytosis and destruction of microorganisms that invade the body. Phagocytosis is probably the most widely distributed defense reaction, occurring in virtually all phyla of the animal world.

NEUTROPHILS ARE CRITICAL AGAINST MASTITIS

Native defenses of cattle are continually challenged by exposure to pathogens (bacteria, fungi, and viruses) and many factors affect the outcome of this interaction. Establishment of an infection in any organ or tissue is dependent upon a delicate balance between defense mechanisms of the body and the abilities of pathogens to resist unfavorable survival conditions. The neutrophil is one of the most important cells of the innate defense mechanisms because it can act quickly (within minutes) in large numbers, and in most cases, does not require previous exposure to a pathogen to effectively eradicate the microbe. Studies have shown that it takes approximately 1-2 h for neutrophils to accumulate in response to *E. coli* infection in tissues (Persson et al., 1988, 1992, 1993; Persson and Sandgren, 1992). What this means is that microorganisms will have a 2-h head start on the host immune response and any further delay in the inflammatory response will result in significantly more pathogens for the host to deal with. Unfortunately, delays in inflammatory responses in stressed animals are well documented (Shuster et al., 1996; Hill et al., 1979; Hill, 1981), and some of the mechanisms responsible for delayed inflammation have been identified (Lee and Kehrli, 1998; Burton and Kehrli, 1995; Burton et al., 1995). The importance of the neutrophil in protecting virtually all body tissues (especially against bacteria) has been repeatedly demonstrated experimentally and in nature (Schalm et al., 1964a,b; Jain et al., 1968, 1978; Ackermann et al., 1993, 1996; Gilbert et al., 1993a). Early and rapid accumulation of sufficient numbers of neutrophils is paramount in the ability of the host to effect a cure of invading pathogens (Anderson, 1983). Neutrophils can also release cytokines that in turn result in additional recruitment signals for more neutrophils (Canning and Neill, 1989; Cicco et al.,

1990; Goh et al., 1989; Ohkawara et al., 1989). Circulating *neutrophils represent the major recruitable host defense against acute tissue infection*, such as mastitis (Hill, 1979, 1981; Jain, 1968; Schalm et al., 1976).

IMMUNOSUPPRESSION IN THE PATHOGENESIS OF MASTITIS

A literal definition of immunosuppression is diminished immune responsiveness. This simplistic definition impacts a highly diverse system that affords protection against disease. Periparturient immunosuppression research was initiated by the observation that most clinical mastitis occurs in dairy cows in early lactation and the view that most bovine mastitis is caused by opportunistic pathogens and; therefore, these cows must be immunosuppressed. What evidence supported the hypothesis of periparturient immunosuppression? Practical experience teaches us that opportunistic infections are associated with severe compromises of host defense mechanisms. Over the past couple decades, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle (Figure 1) and sows has been generated in research institutes around the world (Shuster et al., 1996; Lee and Kehrli, 1998; Burvenich et al., 1994, 2007; Cai et al., 1994; Detilleux et al., 1994, 1995a,b; Dosogne et al., 1998, 1999; Guidry et al., 1976; Harp et al., 1991; Heyneman and Burvenich, 1989; Hoeben et al., 1997, 2000a,b; Ishikawa and Shimizu, 1983; Ishikawa, 1987; Ishikawa et al., 1994; Kehrli and Goff, 1989; Kehrli et al., 1989a,b; Kelm et al., 1997; Kimura et al., 1999a,b, 2002a,b; Lippolis et al., 2006; Löfstedt et al., 1983; Mehrzad et al., 2001, 2002; Monfardini et al., 2002; Nagahata et al., 1988, 1992; Nonnecke et al., 2003; Pelan-Mattocks et al., 2000; Shafer-Weaver and Sordillo, 1997; Sordillo et al., 1991, 1992, 1995; Stabel et al., 1991; Van Werven et al., 1997; Vandeputte-Van Messom et al., 1993). Periparturient immune dysregulation impacts the occurrence of infectious diseases of virtually any organ system of livestock (e.g., gastrointestinal, respiratory, and reproductive tracts all have increased disease incidence in postpartum animals).

First of all, there is an extremely high incidence of clinical disease in postpartum cows with nearly 25 % of all clinical mastitis occurring during the first 2 wk after calving. Clinical mastitis caused by virtually all pathogens (but especially coliform bacteria and streptococci other than *Streptococcus agalactiae*) has a very high incidence in early lactation. Cows must first become infected and then develop clinical mastitis. The rates of new intramammary infections (**IMI**) caused by environmental pathogens are highest during the first and last 2 wk of a 60-d, nonlactating period of dairy cows (Hogan et al., 1989; Smith et al., 1985a,b; Oliver and Mitchell, 1983). The rate of new IMI during these periods of peak susceptibility is 2 to 12 X higher than any other time in the production cycle of the cow. Most coliform and environmental streptococcal infections, established in the nonlactating period and that are present at parturition, result in clinical mastitis soon afterward (Smith et al., 1985a; McDonald and Anderson, 1981). The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 wk of lactation has been reported to be 25, 45 and 60 %, respectively (Malinowski et al., 1983; Jackson and Bramley, 1983).

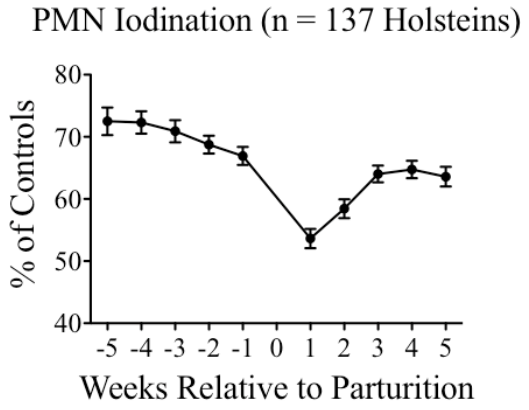

Figure 1. Neutrophil (PMN) iodination measures the myeloperoxidase-catalyzed halogenation of proteins, a phenomenon that takes place in phagolysosomes of neutrophils that have phagocytosed bacteria. *In vivo*, this halogenation disrupts the function of critical bacterial membrane proteins and results in the oxidative killing of the bacteria by the neutrophil. This bactericidal activity depends on a series of events to occur in the process of phagocytosis: successful opsonization and uptake of the bacteria by the β2-integrins into a phagosome, the generation of superoxide anion and its dismutation into hydrogen peroxide (H_2O_2), the fusion of the phagosome with a primary granule to produce a phagolysosome in which myeloperoxidase utilizes the H_2O_2 and cellular halides to halogenate the bacterial surface proteins. (Data from Detilluex, et al., 1995b.)

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that we are traditionally taught that opportunistic infections are associated with severe

compromises of host defense mechanisms. Most mastitis pathogens are considered opportunistic pathogens. These 2 points led to experiments evaluating how functional a cow's immune system is around calving time. Today the data tells us the immune system becomes progressively more compromised at the end of gestation, cows become more readily infected in the mammary gland, then as the immune system *bottoms out* the first week or two after calving, these subclinical infections begin to win the battle with the cow's immune system and clinical mastitis results.

WHAT CAUSES PERIPARTURIENT IMMUNOSUPPRESSION?

Many neuroendocrine changes develop in cows during the periparturient period. Periparturient hormone fluxes may adversely affect immune cell function. Surprisingly, there is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers (Roth et al., 1982, 1983). However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function (Bodel et al., 1972; Klebanoff, 1979). These high concentrations of estrogens may be germane to immunosuppression and the high new IMI rates prior to calving. Before calving, total plasma estrogen concentrations increase in the cow (at least 10 X greater than during estrus) (Comline et al., 1974). Moreover, during normal pregnancy, the progesterone binding capacity of human lymphocytes is increased (perhaps as a result of increasing estrogen levels) and the concentration of progesterone in serum during pregnancy combine as sufficient to reduce lymphocyte functions (Szekeres-Bartho et al., 1983, 1985). This raises the possibility that hormone sensitivities of immune cells during late gestation may be altered and result in functional changes in immune cells due to rising estrogen levels. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows (Comline et al., 1974). This may be germane to the onset of impaired lymphocyte function in the prepartum cow whose lymphocyte hormone binding capacity may be higher than that in barren cows.

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 wk preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin (Comline et al., 1974; Houdebine et al., 1985; Convey, 1974; Akers, 1985). During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for energy and protein. Negative energy and protein balances that exist during early lactation may also contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression observed. The nutritional demands of lactation contribute to the duration of immune suppression (Kimura et al., 1999b; Nonnecke et al., 2003; Stabel et al., 2003) and postpartum neutrophil glycogen stores have been associated with postpartum uterine diseases (Galvão et al., 2010).

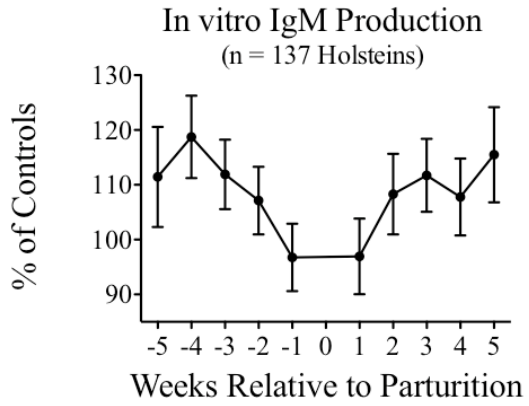

Figure 2. *In vitro* production of IgM by lymphocytes is reduced in the immediate week around calving time. (Data from Detilleux et al., 1995b.)

The specific physiological factors contributing to periparturient immunosuppression and increased incidence of clinical disease have not been fully elucidated. We do know, however, that there is a very broad-based suppression of immune function in cows the 1st wk or 2 after calving. Wide variation in leukocyte functional activities has been documented between dairy cows and between different production stages (e.g., around calving time) (Ishikawa, 1987, 1994; Nagahata et al., 1988, 1992; Guidry et al., 1976; Newbould, 1976; Manak, 1982; Gunnink, 1984a,b,c; Saad et al., 1989; Gilbert et al., 1993b). Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported (Kelm et al., 1997; Kimura et al., 2002a; Cai et al., 1994). Periparturient immunosuppression is not limited to cattle. Investigations of immunosuppression and coliform mastitis in sows revealed depressed neutrophil function to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli*

(Löfstedt et al., 1983). Defects in lymphocyte function also contribute to immune suppression during the periparturient period (Figures 2 and 3). In addition to reduced antibody production, other impacted roles of lymphocytes in periparturient cows include reduced production of cytokines that activate and direct both innate and adaptive immunity (Detilleux et al., 1995; Ishikawa, 1987; Ishikawa et al., 1994; Manak, 1982; Wells et al., 1977; Kashiwazaki, 1984; Kashiwazaki et al., 1985).

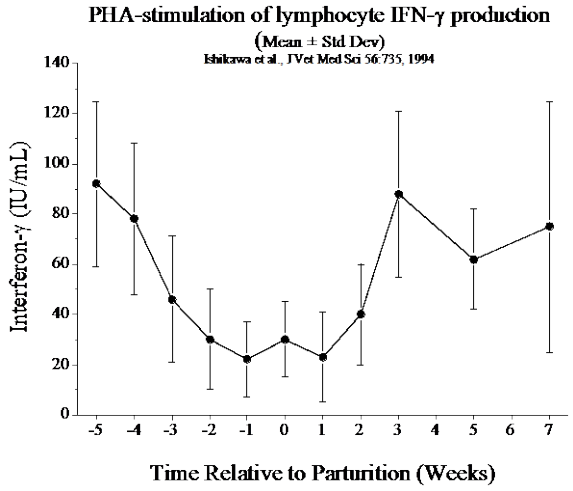

Figure 3. *In vitro* production of interferon- γ (INF- γ) by lymphocytes is reduced in the week around calving time. (Data from Ishikawa et al., 1994.)

Today it is well recognized that the bovine immune system is less capable of battling pathogens during the periparturient period. The periparturient cow has suppressed immune competence, manifest as reduced capacity for nearly all types of immune cells that have been studied. Interestingly, there may be a teleological reason for immunosuppression in the Th1 branch of the immune system that may be essential in preventing unwanted immune reactions against self and fetal antigens exposed to the mother's immune system as a result of normal tissue damage in the reproductive tract during parturition (Kehrli and Harp, 2001). However, an inadvertent and perhaps unintended consequence of this suppression of the Th1 branch of the immune system is that many of the cytokines normally produced by these cells are critical to fully activate neutrophils that are absolutely critical to the defense of the mammary gland. Without a fully functional cellular immune system, both adaptive and innate branches of the cellular immune system operate at diminished capacity for immune surveillance and pathogen clearance. This is the very circumstance that periparturient cows find themselves in and why it is so critical to manage transition cows to minimize their exposure to pathogens in the environment and to avoid metabolic disorders that might further stress their immune system.

The take-home message here is a multitude of factors of the immune system of a dairy cow become impaired as early as 2 - 3 wk before she actually gives birth (long before the elevation of endogenous cortisol which occurs from 36 h before to 36 h after calving). The cow's immune system then bottoms out and is seriously impaired for 1 - 2 wk after calving. This effect is known as periparturient immunosuppression. Regardless of its causation, periparturient immunosuppression makes the dairy cow highly susceptible to the establishment of new infections (particularly in the mammary gland) and the subsequent progression of these new subclinical infections into clinical disease (mastitis, metritis, and postpartum outbreaks of intestinal diseases such as salmonellosis, just to name a few).

WHAT ARE THE PROSPECTS FOR IMMUNOMODULATION TO PREVENT DISEASE?

Pharmacologic treatments that serve as immune modulators in cattle and other species have been under investigation for many years. Biotherapeutic immune modulators can be given to prevent or lessen disease symptoms caused by various pathogens (viral and bacterial). A general goal of such a biotherapeutic compound is to provide the desired effect on host immunity for a sufficient period of time to sustain immunity through a period of immune dysfunction the host is experiencing. In the past couple years 2 products have received approval by regulatory agencies that fall under this category but that work through very different innate immunity mechanisms.

According to the manufacturer, Zelnate™ (Bayer Healthcare LLC, Animal Health, Shawnee Mission, KS) was approved in 2015 as a USDA-Center for Veterinary Biologics approved immune modulator based on technology developed by Juvaris BioTherapeutics (Pleasanton, CA). As such, it represented a new class of drug for bovine respiratory disease (**BRD**) as an immune modulator; it is not an antibiotic nor a vaccine. Zelnate DNA Immunostimulant is a bacterial-produced plasmid DNA with a liposome carrier that stimulates the

innate immune system in cattle. Per the label claim, Zelnate is indicated for use as an aid in the treatment of BRD due to *Mannheimia haemolytica* in cattle 4 mo of age or older, when administered at the time of, or within 24 h after, a perceived stressful event. Although no peer-reviewed publications are available at this time, a summary of the technical studies conducted for regulatory approval is available: http://www.zelnate.com/static/documents/Zelnate-ChallengeStudy_Detailer.pdf.

In 2016, Imrestor™ (pegbovigrastim) (Elanco Animal Health, Indianapolis, IN) was approved by the Food and Drug Administration as the first and only immune restorative for periparturient dairy cows and heifers. Per the label claim usage, Imrestor reduces the incidence of clinical mastitis by 28 % in the first 30 d of lactation in dairy cows and heifers. Recent peer-reviewed studies describe the mechanism of action of pegbovigrastim and report an even greater reduction in clinical mastitis incidence in 4 studies conducted in the United States (Kimura et al., 2014; Hassfurther et al., 2015; Canning et al., 2017; McDougall et al., 2017).

Pegbovigrastim is a cytokine that is naturally part of a cow's immune system that works to turn on the innate immune response provided by neutrophils. Cytokines are a class of compounds that have been investigated for many years for potential biotherapeutic value. Administration of recombinant cytokines to modulate immunity in immunocompromised hosts is thought to prevent bacterial infections (Broxmeyer and Vadhan-Raj, 1989). In an effort to study methods to ameliorate the effects of periparturient immunosuppression, several scientists have evaluated various cytokines that are part of the cow's normal immune system (Sordillo et al., 1991b,1992; Zecconi et al., 1999, 2009; Sordillo and Babiuk, 1991; Campos et al., 1992; Sordillo and Peel, 1992). Granulocyte-colony stimulatory factor (**G-CSF**) is a cytokine that triggers the bone marrow to produce leukocytes – neutrophils in particular, which in turn, fight infectious disease. Human G-CSF has been successfully used for many years as an adjunct therapy for cancer patients undergoing chemotherapy. In a series of studies, G-CSF has been evaluated for its effects on bovine immunity and as a prophylactic against mastitis (Stabel et al., 1991; Kehrli et al., 1991a; Cullor et al., 1990a,b, 1992; Nickerson et al., 1989). Our research findings indicate no adverse effects and that it can reduce the incidence and severity of clinical coliform mastitis by 50 % during the 1st wk of lactation following experimental challenge (Kehrli, 1998). G-CSF has also been shown to be beneficial against *Staphylococcus aureus* and *Klebsiella pneumoniae* mastitis (Nickerson et al., 1989; Kehrli et al., 1991b). It is crucial to understand that immunomodulators work best in immunocompromised hosts; hence the periparturient period is an excellent time for such compounds to be given to cows as they will work to restore the immune system. Acceptable alternatives to the use of antibiotics in food animal practice need to be explored and the use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic approaches to prevent and combat infectious disease during periods of peak disease incidence. Research in the area of biotherapeutic immune modulation continues today (Kimura et al., 2014).

Dietary immune treatments are also an area of intense investigation. While not a major focus of this paper, considerable research has been done and managing optimal nutrition levels, with ingredients such as vitamin E and selenium, is well recognized to avoid immune impairment associated with nutrient deficiencies (Weiss et al., 1990, 1992, 1997; Hogan et al., 1990, 1992c, 1993, 1994; Smith et al., 1997). However, there is little evidence to support hyper-supplementation of nutrients such as these, as a means to enhance immune function.

Immunomodulatory feed ingredients have also received considerable research interest investigating possible beneficial effects on immunity and health in dairy cows. One such product, Omnigen-AF (Phibro Animal Health Corp., Teaneck, NJ), is perhaps the best studied product reported to enhance innate immunity parameters and increase milk production in dairy cows (Brandao et al., 2016; Leiva et al., 2017; Fabris et al., 2017; Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014).

WHAT DOES THIS ALL MEAN FOR YOU?

Bovine mastitis is one of the most economically important diseases to the beef and dairy cattle industries. The pathogenesis is highly complex and involves many factors including various microbial etiologies, stress, management and environmental hygiene. Bovine mastitis has not been adequately controlled by vaccination or antibiotics. In many diseases, immunosuppression due to various stressors is responsible for increased susceptibility to bacterial colonization or growth. Over the past 50 y a considerable body of evidence of impaired neutrophil

and lymphocyte function in periparturient dairy cows has emerged that coincides with the high incidence of new intramammary infections 2 wk prepartum and clinical mastitis in early lactation. To overcome this immunosuppression, immunomodulatory agents have been and are being evaluated for their ability to prevent economic losses associated with periparturient diseases such as mastitis. Researchers have investigated immunomodulation as an approach to provide dairy farmers with a new tool to prevent infectious disease in their herds, although biotherapeutic products have not yet made it to the market place. The consequences of immune suppression are increases in infectious disease and premature loss from the herd, both of which add significantly to the cost of production and decrease the profitability of dairy farming. Simple solutions will not likely be found for something as complex as immune suppression; however, without additional significant research into this topic we can be assured that no progress will be made.

Production of milk from mastitis-free cows is quite simple, right? Keep your cows in clean, dry, and unstressful environments and feed them what they need, when they need it – far easier said than done! For years we have emphasized feeding cows optimal rations because the production and functional activities of leukocytes in combating microbial infection are complex and all involve expenditure of cellular energy, protein and other nutrients. The average cow has ~3500 neutrophils/µL of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1800 lb Holstein cow. The circulating half-life of neutrophils is about 6 h, so the cow is replacing half of those cells every 6 h from bone marrow stores. Clearly, a significant component of the dietary energy and protein consumption for maintenance is spent on replenishment of immune cells. The negative energy and protein balance of dairy cows during the periparturient period and up to peak lactation undoubtedly influences immune function. We know that cows without the stress of lactation recover from periparturient immunosuppression within 1 wk after calving, whereas lactating cows remain immunosuppressed for 2 - 3 wk postpartum (Kimura et al., 1999a,b, 2002b). Today we have a new immune restorative to give transition cows. In combination with the best possible hygienic conditions and the best possible dietary management, we can further reduce the incidence of disease in early lactation and better enable cows to reach their full genetic potential.

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