Immunological Dysfunction in Periparturient Cows: Evidence, Causes and Ramifications

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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 to 23 billion/year over a decade ago. The 2007 National Animal Health Monitoring System (**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 respectively. 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. The majority (65 to 70%) of losses is 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 [1]. Nearly half of the Nation's herd of dairy cows will experience at least one 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 artificial insemination (**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 [2, 3]. Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in

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cattle with clinical or subclinical coliform mastitis [4-6]. 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 [7-10]. 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 two-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.

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 [11]. 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, Élie 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 [12]." 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 to 2 hours for neutrophils to accumulate in response to *E. coli* infection in tissues [13-16]. What this means is that microorganisms will have a 2-hour 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 [17-19], and some of the mechanisms responsible for delayed inflammation have been identified [20-22]. The importance of the neutrophil in protecting virtually all body tissues, especially against bacteria, has been repeatedly demonstrated experimentally and in nature [23-29]. Early and rapid accumulation of sufficient numbers of neutrophils is paramount in the ability of the host to effect a cure of invading pathogens [30]. Neutrophils can also release cytokines that in turn result in additional recruitment signals for more neutrophils [31-34]. Circulating ***neutrophils represent the major recruitable host defense against acute tissue infection***, such as mastitis [18, 19, 25, 35].

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 and sows has been generated in research institutes around the world [17, 20, 36-76]. 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 periparturient cows with nearly 25% of all clinical mastitis occurring during the first 2 weeks 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 weeks of a 60-day, nonlactating period of dairy cows [3, 77-79]. The rate of new IMI during these periods of peak susceptibility is 2 to 12 times 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 [77, 80]. The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 weeks of lactation has been reported to be 25, 45 and 60%, respectively [81, 82].

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. These two points led to experiments evaluating how functional a cow's immune system is around calving time. Over the past several years, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle has been generated in several research institutes around the world [17, 20, 36-75]. 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. This can also be extended to infectious diseases of virtually any system of the postpartum cow (gastrointestinal, respiratory and reproductive tracts all have increased disease incidence in postpartum cows).

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 [83, 84]. However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function [85, 86]. 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-fold greater than during estrus [87]. 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 [88, 89]. 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 concentrations. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows [87]. 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 weeks preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin [87, 90-92]. During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for nutrients. Negative energy and amino acid 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 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 in the first week or two after calving. Wide variation in leukocyte functional activities has been documented between dairy cows and between different production stages (e.g., around calving time) [54, 56-58, 60, 93-99]. Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported [45, 51, 59]. 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* [76]. Defects in lymphocyte function also contribute to immune suppression during the periparturient period. 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 [44, 54, 56, 94, 100-102].

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 [103]. 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 to 3 weeks before she actually gives birth, and long before the elevation of endogenous cortisol which occurs from 36 hours before to 36 hours after calving. The cow's immune system then bottoms out and is seriously impaired for 1 to 2 weeks 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 such as 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?

Biotherapeutic immune modulators can be given to prevent or lessen disease symptoms caused by various viral and bacterial pathogens. 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. Cytokines are one class of compounds that have been investigated for potential biotherapeutic value. Administration of recombinant cytokines to modulate immunity in immunocompromised hosts is thought to prevent bacterial infections [104]. 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 [41, 105-110]. 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 [40, 111-115]. Our research findings indicate no adverse effects and that it can reduce the incidence and severity of clinical coliform mastitis by 50% during the first week of lactation following experimental challenge [116]. Granulocyte-colony stimulatory factor has also been shown beneficial against *Staphylococcus aureus* and *Klebsiella pneumoniae* mastitis [115, 117]. 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.

What Does This All Mean for You?

Bovine mastitis is one of the most economically important diseases to dairy cattle industry. 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 years 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 IMI 2 weeks 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 ~3,500 neutrophils per microliter of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1,800 lb Holstein cow. The circulating half-life of neutrophils is about 6 hours, so the cow is replacing half of those cells every 6 hours 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 influence immune function. We know that cows without the stress of lactation recover from periparturient immunosuppression within 1 week after calving, whereas lactating cows remain immunosuppressed for 2 to 3 weeks postpartum [47, 48, 50]. The most we can do today is to give transition cows the best possible hygienic conditions and appropriate diets.

References

1. Watts JL: Etiological agents of bovine mastitis. *Vet Microbiol* 1988, 16:41-66.
2. Anderson KL, Smith AR, Gustaffson BK, Spahr SL, Whitmore HL: Diagnosis and treatment of acute mastitis in a large dairy herd. *J Am Vet Med Assoc* 1982, 181(7):690-693.

3. Hogan JS, Smith KL, Hoblet KH, Schoenberger PS, Todhunter DA, Hueston WD, Pritchard DE, Bowman GL, Heider LE, Brockett BL *et al*: Field survey of clinical mastitis in low somatic cell count herds. *J Dairy Sci* 1989, 72(6):1547-1556.

4. Erskine RJ, Tyler JW, Riddell MG, Jr., Wilson RC: Theory, use, and realities of efficacy and food safety of antimicrobial treatment of acute coliform mastitis. *J Am Vet Med Assoc* 1991, 198(6):980-984.

5. Jones GF, Ward GE: Evaluation of systemic administration of gentamicin for treatment of coliform mastitis in cows. *J Am Vet Med Assoc* 1990, 197(6):731-735.

6. Kirk JH, Barlett PC: Nonclinical *Pseudomonas aeruginosa* mastitis in a dairy herd. *J Am Vet Med Assoc* 1984, 184:671-673.

7. Gonzalez RN, Cullor JS, Jasper DE, Farver TB, Bushnell RB, Oliver MN: Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. *Can J Vet Res* 1989, 53(3):301-305.

8. Hogan JS, Smith KL, Todhunter DA, Schoenberger PS: Field trial to determine efficacy of an *Escherichia coli* J5 mastitis vaccine. *J Dairy Sci* 1992, 75(1):78-84.

9. Hogan JS, Weiss WP, Smith KL, Todhunter DA, Schoenberger PS, Sordillo LM: Effects of an *Escherichia coli* J5 vaccine on mild clinical coliform mastitis. *J Dairy Sci* 1995, 78(2):285-290.

10. Hogan JS, Weiss WP, Todhunter DA, Smith KL, Schoenberger PS: Efficacy of an *Escherichia coli* J5 mastitis vaccine in an experimental challenge trial. *J Dairy Sci* 1992, 75(2):415-422.

11. Roitt IM: Essential Immunolgy, 8th edn. Boston: Blackwell Scientific Publications; 1994.

12. Metchnikoff E: On the present state of the question of immunity in infectious diseases. *Scand J Immunol* 1908, 30(4):383-398.

13. Persson K, Holmberg O, Astrom G: Studies of defence mechanisms and inflammatory reactions in the bovine teat using a new experimental method. *Acta Vet Scand* 1988, 29(3-4):519-520.

14. Persson K, Larrson I, Sandgren CH: Effects of certain inflammatory mediators on bovine neutrophil migration in vivo and in vitro. *Vet Immunol Immunopathol* 1993, 37(2):99-112.

15. Persson K, Sandgren CH: A study of the development of endotoxin-induced inflammation in the bovine teat. *Acta Vet Scand* 1992, 33(4):283-295.

16. Persson K, Sandgren CH, Rodriguez-Martinez H: Studies of endotoxin-induced neutrophil migration in bovine teat tissues, using indium-111-labeled neutrophils and biopsies. *Am J Vet Res* 1992, 53(12):2235-2240.

17. Shuster DE, Lee E-K, Kehrli ME, Jr.: Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in periparturient versus midlactation cows. *Am J Vet Res* 1996, 57(11):1569-1575.

18. Hill AW: Factors influencing the outcome of Escherichia coli mastitis in the dairy cow. *Res Vet Sci* 1981, 31(1):107-112.

19. Hill AW, Shears AL, Hibbitt KG: The pathogenesis of experimental *Escherichia coli* mastitis in newly calved dairy cows. *Res Vet Sci* 1979, 26(1):97-101.

20. Lee E-K, Kehrli ME, Jr.: Expression of adhesion molecules on neutrophils of periparturient cows and neonatal calves. *Am J Vet Res* 1998, 59(1):37-43.

21. Burton JL, Kehrli ME, Jr.: Regulation of neutrophil adhesion molecules and shedding of *Staphylococcus aureus* in milk of cortisol- and dexamethasone-treated cows. *Am J Vet Res* 1995, 56(8):997-1006.

22. Burton JL, Kehrli ME, Jr., Kapil S, Horst RL: Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. *J Leukocyte Biol* 1995, 57(2):317-325.

23. Schalm OW, Carroll EJ, Lasmanis J: The leukocyte barrier and serologic investigations of experimental coliform (Aerobacter aerogenes) mastitis in cattle. *Am J Vet Res* 1964, 25(104):90-96.

24. Schalm OW, Lasmanis J, Carroll EJ: Effects of pre-existing leukocytosis on experimental coliform (Aerobacter aerogenes) mastitis in cattle. *Am J Vet Res* 1964, 25(104):83-96.

25. Jain NC, Schalm OW, Carroll EJ, Lasmanis J: Experimental mastitis in leukopenic cows: Immunologically induced neutropenia and response to intramammary inoculation of Aerobacter aerogenes. *Am J Vet Res* 1968, 29:2089-2097.

26. Jain NC, Schalm OW, Lasmanis J: Neutrophil kinetics in endotoxin-induced mastitis. *Am J Vet Res* 1978, 39:1662-1667.

27. Ackermann MR, Kehrli ME, Jr., Morfitt DC: Ventral dermatitis and vasculitis in a calf with bovine leukocyte adhesion deficiency. *J Am Vet Med Assoc* 1993, 202(3):413-415.

28. Ackermann MR, Kehrli ME, Jr., Laufer JA, Nusz LT: Alimentary and respiratory tract lesions in eight medically fragile Holstein cattle with Bovine Leukocyte Adhesion Deficiency (BLAD). *Vet Pathol* 1996, 33(3):273-281.

29. Gilbert RO, Rebhun WC, Kim CA, Kehrli ME, Jr., Shuster DE, Ackermann MR: Clinical manifestations of leukocyte adhesion deficiency in cattle: 14 cases (1977-1991). *J Am Vet Med Assoc* 1993, 202(3):445-449.

30. Anderson JC: The mouse mastitis model: Observations relevant to the treatment and control of coliform mastitis. *Vet Res Commun* 1983, 7:223-227.

31. Canning PC, Neill JD: Isolation and characterization of interleukin-1 from bovine polymorphonuclear leukocytes. *J Leukocyte Biol* 1989, 45(1):21-28.

32. Cicco NA, Lindemann A, Content J, Vandenbussche P, Lübbert M, Gauss J: Inducible production of interleukin-6 by human polymorphonuclear neutrophils:

role of granulocyte-macrophage colony-stimulation factor and tumor necrosis factor-alpha. *Blood* 1990, 75(10):2049-2052.

33. Goh K, Furusawa S, Kawa Y, Negishi-Okitsu S, Mizoguchi M: Production of interleukin-1-alpha and -beta by human peripheral polymorphonuclear neutrophils. *Int Arch Allergy Appl Immunol* 1989, 88:297-303.

34. Ohkawara S, Goto K, Mori S, Goto F, Saita N, Sagara T, Yoshinaga M: Interleukin-1 production by polymorphonuclear leukocytes during the course of acute inflammation on rabbits. *Dermatologica* 1989, 179:84-90.

35. Schalm OW, Lasmanis J, Jain NC: Conversion of chronic staphylococcal mastitis to acute gangrenous mastitis after neutropenia in blood and bone marrow produced by an equine anti-bovine leukocyte serum. *Am J Vet Res* 1976, 37:885-890.

36. Kehrli ME, Jr., Goff JP: Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. *J Dairy Sci* 1989, 72:1188-1196.

37. Kehrli ME, Jr., Nonnecke BJ, Roth JA: Alterations in bovine lymphocyte function during the periparturient period. *Am J Vet Res* 1989, 50:215-220.

38. Kehrli ME, Jr., Nonnecke BJ, Roth JA: Alterations in bovine neutrophil function during the periparturient period. *Am J Vet Res* 1989, 50:207-214.

39. Harp JA, Kehrli ME, Jr., Hurley DJ, Wilson RA, Boone TC: Numbers and percent of T lymphocytes in bovine peripheral blood during the periparturient period. *Vet Immunol Immunopathol* 1991, 28(1):29-35.

40. Stabel JR, Kehrli ME, Jr., Thurston JR, Goff JP, Boone TC: Granulocyte colony-stimulating factor effects on lymphocytes and immunoglobulin concentrations in periparturient cows. *J Dairy Sci* 1991, 74(11):3755-3762.

41. Sordillo LM, Afseth G, Davies G, Babiuk LA: Effects of recombinant granulocyte-macrophage colony-stimulating factor on bovine peripheral blood and mammary gland neutrophil function in vitro. *Can J Vet Res* 1992, 56:16-21.

42. Detilleux JC, Koehler KJ, Freeman AE, Kehrli ME, Jr., Kelley DH: Immunological parameters of periparturient Holstein cattle: genetic variation. *J Dairy Sci* 1994, 77(9):2640-2650.

43. Detilleux JC, Kehrli ME, Jr., Freeman AE, Fox LK, Kelley DH: Mastitis of periparturient Holstein cattle: A phenotypic and genetic study. *J Dairy Sci* 1995, 78(10):2285-2293.

44. Detilleux JC, Kehrli ME, Jr., Stabel JR, Freeman AE, Kelley DH: Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Vet Immunol Immunopathol* 1995, 44(3):251-267.

45. Kelm SC, Detilleux JC, Freeman AE, Kehrli ME, Jr., Dietz AB, Fox LK, Butler JE, Kasckovics I, Kelley DH: Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. *J Dairy Sci* 1997, 80(8):1767-1775.

46. Dosogne H, Burvenich C, Freeman AE, Kehrli ME, Jr., Detilleux JC, Sulon J, Beckers JF, Hoeben D: Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. *Vet Immunol Immunopathol* 1999, 67(1):47-54.

47. Kimura K, Goff JP, Kehrli ME, Jr.: Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. *J Dairy Sci* 1999, 82(11):2385-2392.

48. Kimura K, Goff JP, Kehrli ME, Jr., Harp JA: Phenotype analysis of peripheral blood mononuclear cells in periparturient dairy cows. *J Dairy Sci* 1999, 82(2):315-319.

49. Pelan-Mattocks LS, Kehrli ME, Jr., Casey TA, Goff JP: Fecal shedding of coliform bacteria during the periparturient period in dairy cows. *Am J Vet Res* 2000, 61(12):1636-1638.

50. Kimura K, Goff JP, Kehrli ME, Jr., Harp JA, Nonnecke BJ: Effects of mastectomy on composition of peripheral blood mononuclear cell populations in periparturient dairy cows. *J Dairy Sci* 2002, 85(6):1437-1444.

51. Kimura K, Goff JP, Kehrli ME, Jr., Reinhardt TA: Decreased neutrophil function as a cause of retained placenta in dairy cattle. *J Dairy Sci* 2002, 85(3):544-550.

52. Nonnecke BJ, Kimura K, Goff JP, Kehrli ME, Jr.: Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. *J Dairy Sci* 2003, 86(7):2359-2368.

53. Burvenich C, Bannerman DD, Lippolis JD, Peelman L, Nonnecke BJ, Kehrli ME, Jr., Paape MJ: Cumulative physiological events influence the inflammatory response of the bovine udder to *Escherichia coli* infections during the transition period. *J Dairy Sci* 2007, 90 Suppl 1:E39-54.

54. Ishikawa H: Observation of lymphocyte function in perinatal cows and neonatal calves. *Jpn J Vet Sci* 1987, 49:469-475.

55. Ishikawa H, Shimizu T: Depression of B-lymphocytes by mastitis and treatment with levamisole. *J Dairy Sci* 1983, 66(3):556-561.

56. Ishikawa H, Shirahata T, Hasegawa K: Interferon-g production of mitogen stimulated peripheral lymphocytes in perinatal cows. *J Vet Med Sci* 1994, 56(4):735-738.

57. Nagahata H, Makino S, Takeda S, Takahashi H, Noda H: Assessment of neutrophil function in the dairy cow during the perinatal period. *J Vet Med B* 1988, 35:747-751.

58. Nagahata H, Ogawa A, Sanada Y, Noda H, Yamamoto S: Peripartum changes in antibody producing capability of lymphocytes from dairy cows. *Vet Q* 1992, 14:39-40.

59. Cai T-Q, Weston PG, Lund LA, Brodie B, McKenna DJ, Wagner WC: Association between neutrophil functions and periparturient disorders in cows. *Am J Vet Res* 1994, 55(7):934-943.

60. Guidry AJ, Paape MJ, Pearson RE: Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids and neutrophil phagocytosis in the cow. *Am J Vet Res* 1976, 37(10):1195-1200.

61. Burvenich C, Paape MJ, Hill AW, Guidry AJ, Miller RH, Heyneman R, Kremer WDJ, Brand A: Role of the neutrophil leukocyte in the local and systemic reactions during experimentally induced E. coli mastitis in cows immediately after calving. *Vet Q* 1994, 16(1):45-50.

62. Heyneman R, Burvenich C: The respiratory burst activity of blood neutrophils during hyperacute experimentally induced Escherichia coli mastitis in cattle immediately after parturition. In: *7th Int Conf Prod Dis Farm Anim: 1989; Cornell Univ., Ithaca, NY*; 1989.

63. Vandeputte-Van Messom G, Burvenich C, Roets E, Massart-Leen AM, Heyneman R, Kremer WD, Brand A: Classification of newly calved cows into moderate and severe responders to experimentally induced Escherichia coli mastitis. *J Dairy Res* 1993, 60(1):19-29.

64. Hoeben D, Heyneman R, Burvenich C: Elevated levels of beta-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. *Vet Immunol Immunopathol* 1997, 58(2):165-170.

65. Van Werven T, Noordhuizen-Stassen EN, Daemen AJ, Schukken YH, Brand A, Burvenich C: Preinfection in vitro chemotaxis, phagocytosis, oxidative burst, and expression of CD11/CD18 receptors and their predictive capacity on the outcome of mastitis induced in dairy cows with Escherichia coli. *J Dairy Sci* 1997, 80(1):67-74.

66. Dosogne H, Capuco AV, Paape MJ, Roets E, Burvenich C, Fenwick B: Reduction of acyloxyacyl hydrolase activity in circulating neutrophils from cows after parturition. *J Dairy Sci* 1998, 81(3):672-677.

67. Hoeben D, Burvenich C, Trevisi E, Bertoni G, Hamann J, Bruckmaier RM, Blum JW: Role of endotoxin and TNF-a in the pathogenesis of experimentally induced coliform mastitis in periparturient cows. *J Dairy Res* 2000, 67(4):503-514.

68. Hoeben D, Monfardini E, Opsomer G, Burvenich C, Dosogne H, De Kruif A, Beckers JF: Chemiluminescence of bovine polymorphonuclear leucocytes during the periparturient period and relation with metabolic markers and bovine pregnancy-associated glycoprotein. *J Dairy Res* 2000, 67(2):249-259.

69. Mehrzad J, Dosogne H, Meyer E, Heyneman R, Burvenich C: Respiratory burst activity of blood and milk neutrophils in dairy cows during different stages of lactation. *J Dairy Res* 2001, 68(3):399-415.

70. Mehrzad J, Duchateau L, Pyorala S, Burvenich C: Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition and early lactation. *J Dairy Sci* 2002, 85(12):3268-3276.

71. Monfardini E, Paape MJ, Wang Y, Capuco AV, Husheem M, Wood L, Burvenich C: Evaluation of L-selectin expression and assessment of protein tyrosine

phosphorylation in bovine polymorphonuclear neutrophil leukocytes around parturition. *Vet Res* 2002, 33(3):271-281.

72. Lippolis JD, Peterson-Burch BD, Reinhardt TA: Differential expression analysis of proteins from neutrophils in the periparturient period and neutrophils from dexamethasone-treated dairy cows. *Vet Immunol Immunopathol* 2006, 111(3-4):149-164.

73. Sordillo LM, Redmond MJ, Campos M, Warren L, Babiuk LA: Cytokine activity in bovine mammary gland secretions during the periparturient period. *Can J Vet Res* 1991, 55(3):298-301.

74. Sordillo LM, Pighetti GM, Davis MR: Enhanced production of bovine tumor necrosis factor-α during the periparturient period. *Vet Immunol Immunopathol* 1995, 49(3):263-270.

75. Shafer-Weaver KA, Sordillo LM: Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the postpartum period. *Vet Immunol Immunopathol* 1997, 56(1-2):53-64.

76. Löfstedt J, Roth JA, Ross RF, Wagner WC: Depression of polymorphonuclear leukocyte function associated with experimentally induced Escherichia coli mastitis in sows. *Am J Vet Res* 1983, 44(7):1224-1228.

77. Smith KL, Todhunter DA, Schoenberger PS: Environmental pathogens and intramammary infection during the dry period. *J Dairy Sci* 1985, 68(2):402-417.

78. Smith KL, Todhunter DA, Schoenberger PS: Environmental mastitis: cause, prevalence, prevention. *J Dairy Sci* 1985, 68(6):1531-1553.

79. Oliver SP, Mitchell BA: Susceptibility of bovine mammary gland to infections during the dry period. *J Dairy Sci* 1983, 66:1162-1166.

80. McDonald JS, Anderson AJ: Experimental intramammary infection of the dairy cow with Escherichia coli during the nonlactating period. *Am J Vet Res* 1981, 42:229-231.

81. Malinowski E, Krzyzanowski J, Wawron W, Slawomirski J, Gluszak J: Analysis of cases of Escherichia coli mastitis in cows. *Med Weter* 1983, 39:608-610.

82. Jackson E, Bramley J: Coliform mastitis. *In Practice* 1983, 5(4):135-146.

83. Roth JA, Kaeberle ML, Appell LH, Nachreiner RF: Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. *Am J Vet Res* 1983, 44:247-253.

84. Roth JA, Kaeberle ML, Hsu WH: Effect of estradiol and progesterone on lymphocyte and neutrophil functions in steers. *Infect Immun* 1982, 35(3):997-1002.

85. Bodel P, Dillard GM, Jr., Kaplan SS, Malawista SE: Anti-inflammatory effects of estradiol on human blood leukocytes. *J Lab Clin Med* 1972, 80(3):373-384.

86. Klebanoff SJ: Effect of estrogens on the myeloperoxidase-mediated antimicrobial system. *Infect Immun* 1979, 25(1):153-156.

87. Comline RS, Hall LW, Lavelle RB, Nathanielsz PW, Silver M: Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. *J Endocrinol* 1974, 63:451-472.

88. Szekeres-Bartho J, Csernus V, Hadnagy J, Pacsa AS: Progesterone-prostaglandin balance influences lymphocyte function in relation to pregnancy. *Am J Reprod Immun* 1983, 4:139-141.

89. Szekeres-Bartho J, Hadnagy J, Pacsa AS: The suppressive effect of progesterone on lymphocyte cytotoxicity; unique progesterone sensitivity of pregnancy lymphocytes. *J Reprod Immunol* 1985, 7:121-128.

90. Houdebine L-M, Djiane J, Dusanter-Fourt I, Martel P, Kelly PA, Devinoy E, Servely J-L: Hormonal action controlling mammary activity. *J Dairy Sci* 1985, 68:489-500.

91. Convey EM: Serum hormone concentraitons in ruminants during mammary growth, lactogenesis, and lactation: a review. *J Dairy Sci* 1974, 57:905-917.

92. Akers RM: Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. *J Dairy Sci* 1985, 68:501-519.

93. Newbould FHS: Phagocytic activity of bovine leukocytes during pregnancy. *Can J Comp Med* 1976, 40(1):111-116.

94. Manak RC: Mitogenic responses of peripheral blood lymphocytes from pregnant and ovariectomized heifers and their modulation by serum. *J Reprod Immunol* 1982, 4:263-276.

95. Gunnink JW: Pre-partum leukocyte activity and retained placenta. *Vet Q* 1984, 6:52-55.

96. Gunnink JW: Retained placenta and leukocytic activity. *Vet Q* 1984, 6(2):49-51.

97. Gunnink JW: Post-partum leucocytic activity and its relationship to caesarian section and retained placenta. *Vet Q* 1984, 6:55-57.

98. Saad AM, Concha C, Åström G: Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. *J Vet Med B* 1989, 36:337-345.

99. Gilbert RO, Gröhn YT, Miller PM, Hoffman DJ: Effect of parity on periparturient neutrophil function in dairy cows. *Vet Immunol Immunopathol* 1993, 36:75-82.

100. Wells PW, Burrells C, Martin WB: Reduced mitogenic responses in cultures of lymphocytes from newly calved cows. *Clin Exp Immunol* 1977, 29(1):159-161.

101. Kashiwazaki Y: Lymphocyte activities in dairy cows with special reference to outbreaks of mastitis in pre- and post-partus. *Jpn J Vet Res* 1984, 32:101.

102. Kashiwazaki Y, Maede Y, Namioka S: Transformation of bovine peripheral blood lymphocytes in the perinatal period. *Jpn J Vet Sci* 1985, 47:337-339.

103. Kehrli ME, Jr., Harp JA: Immunity in the Mammary Gland. In: *Vet Clin North Am [Food Anim Pract]. Volume 17*, edn. Edited by Roth JA. Philadelphia, PA: W. B. Saunders Company; 2001: 495-516.

104. Broxmeyer HE, Vadhan-Raj S: Preclinical and clinical studies with the hematopoietic colony-stimulating factors and related interleukins. *Immunol Res* 1989, 8:185-201.

105. Zecconi A, Bronzo V, Casula A, Luzzago C, Moroni P, Piccinini R, Spreafico G: Efficacy of a biological response modifier in preventing *Staphylococcus aureus* intramammary infections after calving. *J Dairy Sci* 1999, 82(10):2101-2107.

106. Zecconi A, Piccinini R, Fiorina S, Cabrini L, Dapra V, Amadori M: Evaluation of interleukin-2 treatment for prevention of intramammary infections in cows after calving. *Comparative immunology, microbiology and infectious diseases* 2009, 32(5):439-451.

107. Sordillo LM, Babiuk LA: Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon gamma. *Vet Microbiol* 1991, 28(2):189-198.

108. Sordillo LM, Snider M, Hughes H, Afseth G, Campos M, Babiuk LA: Pathological changes in bovine mammary glands following intramammary infusion of recombinant interleukin-2. *J Dairy Sci* 1991, 74(12):4164-4174.

109. Campos M, Hughes HPA, Godson DL, Sordillo LM, Rossi-Campos A, Babiuk LA: Clinical and immunological effects of single bolus administration of recombinant interleukin-2 in cattle. *Can J Vet Res* 1992, 56:10-15.

110. Sordillo LM, Peel JE: Effect of interferon-γ on the production of tumor necrosis factor during acute *Escherichia coli* mastitis. *J Dairy Sci* 1992, 75(8):2119-2125.

111. Kehrli ME, Jr., Goff JP, Stevens MG, Boone TC: Effects of granulocyte colony-stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. *J Dairy Sci* 1991, 74(8):2448-2458.

112. Cullor JS, Fairley N, Smith WL, Wood SL, Dellinger JD, Inokuma M, Souza LM: Hemogram changes in lactating dairy cows given human recombinant granulocyte colony-stimulating factor (r-MethuG-CSF). *Vet Pathol* 1990, 27(5):311-316.

113. Cullor JS, Smith W, Fairley N, Wood SL, Dellinger JD, Souza L: Effects of human recombinant granulocyte colony stimulating factor (HR-GCSF) on the hemogram of lactating dairy cattle. *Vet Clin Pathol* 1990, 19(1):9-12.

114. Cullor JS, Smith W, Zinkl JG, Dellinger JD, Boone T: Hematologic and bone marrow changes after short- and long-term administration of two recombinant bovine granulocyte colony-stimulating factors. *Vet Pathol* 1992, 29(6):521-527.

115. Nickerson SC, Owens WE, Watts JL: Effects of recombinant granulocyte colony-stimulating factor on *Staphylococcus aureus* mastitis in lactating dairy cows. *J Dairy Sci* 1989, 72(12):3286-3294.

116. Kehrli ME, Jr.: Efficacy of granulocyte-colony stimulatory factor as an immunomodulator to prevent *Escherichia coli* mastitis during early lactation. In: *37th Annual Meeting National Mastitis Council, Inc: Jan 27, 1998 1998; St. Louis, MO*: National Mastitis Council, Inc.; 1998: 336-338.

117. Kehrli ME, Jr., Cullor J, Nickerson SC: Immunobiology of hematopoietic colony-stimulatory factors: potential application to disease prevention in the bovine. *J Dairy Sci* 1991, 74(12):4399-4412.

SESSION NOTES