Causes and Effects of Periparturient Immunosuppression

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Take Home Messages

- Dairy cows experience reduced immune function from about 3 weeks before calving until about 3 weeks after calving
- This immunosuppression results in an increased incidence and severity of infections around the time of calving
- The cause of periparturient immunosuppression is unknown, but many factors seem to be involved
- Among the factors studied, aspects of metabolism including negative energy balance, nonesterified fatty acids, ketones, and calcium appear to play some role in the development of immunosuppression
- Insulin concentrations and functional activity are decreased at a time coincident with periparturient immunosuppression
- Compounds that alter insulin action also alter the activity of immune cells from periparturient cows
- Future work will determine if exogenous insulin or compounds that affect insulin action can improve immune function around the time of calving
- Metabolites associated with periparturient metabolic disorders also appear to impair immune function. Careful nutritional management to maximize metabolic health is also currently our best recommendation to maximize periparturient immune function

Introduction

Infections of the mammary gland (mastitis) or uterus (metritis) are common sources of inflammation in lactating dairy cows, particularly during the periparturient period. Other health disorders common during this period (e.g., milk fever and ketosis) do not arise from infectious organisms, but instead

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have metabolic origins. Although the etiologies of infectious and metabolic disorders differ, epidemiologists report a significant association between their occurrences. For example, Curtis et al. (1985) reported that cows with milk fever were more than 5 times as likely to contract clinical mastitis as animals without milk fever. These results do not imply cause and effect; however, they suggest an association between the occurrences of one disease with that of a second disorder. Potential causal relationships between periparturient metabolism and immune function have been investigated for about the last 20 years, but this research has intensified recently. More research needs to be performed, but at this time elevated levels of ketones have been the most consistent metabolic variable to negatively impact immunity. Our group is investigating metabolic regulators that may also be important in the regulation of immune function of the transition cow.

■ Aspects of Periparturient Metabolism

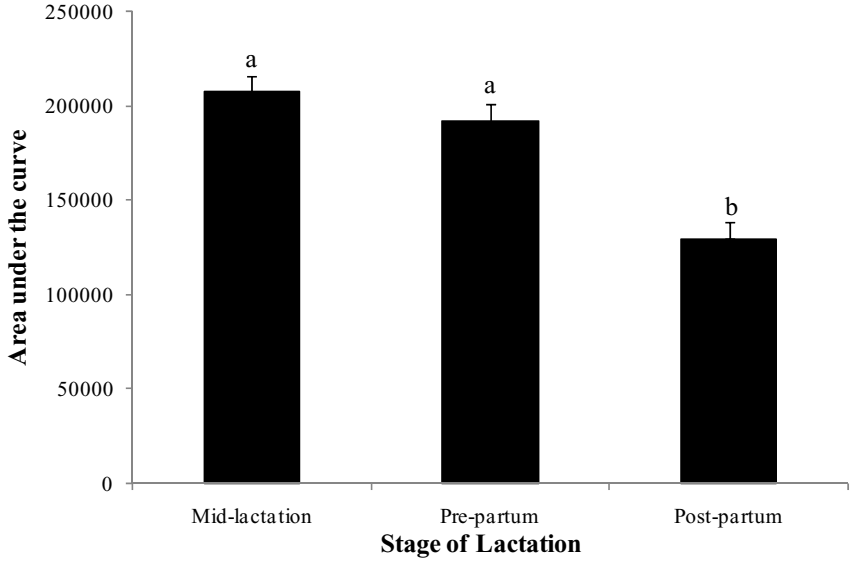
The periparturient dairy cow experiences a dramatic increase in nutrient requirements that cannot be met by feed intake alone as she transitions from pregnancy into lactation (Bell, 1995). Thus, the animal experiences a period of negative energy and nutrient balance requiring the mobilization of body tissue lipid, protein, and calcium in order to sustain productive function (Goff and Horst, 1997). Feeding strategies to optimize these metabolic adaptations have received significant research attention (Overton and Waldron, 2004), but the incidence of periparturient metabolic disorders and infectious diseases in the dairy industry persists (USDA, 2008).

■ Immunosuppression: An Interaction between Metabolism and Immunophysiology?

In addition to the potential metabolic disorders associated with negative energy and calcium balance, periparturient dairy cows also undergo a period of reduced immunological capacity during the weeks around calving. This immune dysfunction is not limited to isolated immune variables; rather it is broad in scope and affects multiple functions of various immune cell types (Sordillo and Streicher, 2002). The combined results of these dysfunctions are that dairy cows may be hyposensitive and hyporesponsive to antigens, and therefore more susceptible to infectious disease such as mastitis during the periparturient period (Mallard et al., 1998). Grommers et al. (1989) reported that fewer mammary quarters responded to low-dose *E. coli* endotoxin, and maximum somatic cell count also was somewhat later and less pronounced during early lactation than during mid-lactation. Furthermore, when live *E. coli* were administered into the mammary gland, periparturient cows experienced more rapid bacterial growth, higher peak

bacterial concentration, higher fever, and equal or greater proinflammatory cytokine concentrations in foremilk than did midlactation cows (Shuster et al., 1996).

Research results from our laboratory are in agreement with this decreased immune function around the time of calving and perhaps give some insights into which mechanisms may be impaired. Neutrophils (PMN) are recognized as being one of the most important cell types in protecting of the mammary gland and uterus from infection (Paape et al., 2002). We isolated PMN from midlactation (220-350 DIM and 100-200 d of gestation, n = 9), prepartum (12 d prior to calving, n = 8), and postpartum (7 DIM, n = 8) cows and studied various functional activities of these cells. The PMN from postpartum cows produced fewer intracellular (data not shown), extracellular (data not shown), and total (Figure 1) reactive oxygen species (ROS). These ROS are compounds such as hydrogen peroxide that kill bacteria upon contact. Production of these ROS is part of how the immune system works to fight infection. This postpartum decrease in ROS expression is in agreement with other reports (Mehrzad et al., 2001) and could contribute to the attenuated pathogen killing capacity that has been reported after calving (Dosogne et al., 2001). A novel finding from our lab relates to the ability of PMN to produce neutrophil extracellular traps (NETs). These bacteriocidal structures were first reported by Brinkmann et al. (2004) and were subsequently reported to be expressed at similar levels in milk and blood (Lippolis et al., 2006), contrary to other antimicrobial mechanisms. Using the same experimental design as above for the ROS production, we report that PMN NETs expression is increased in PMN incubations isolated from cows 12 d prepartum, compared to PMN from postpartum or midlactation cows (Figure 2). This finding, along with the expression of NETs in milk (Lippolis et al., 2006), suggests that NETs expression by PMN is an important protective mechanism for the mammary gland of transition cows.



[1]Neutrophils were collected from midlactation (100-200 days pregnant; n = 9), pre-partum (-12 d; n = 8) and post-partum (7 DIM; n = 8) cows.

*Day of lactation effect, $P < 0.01$. [a,b] Bars with different letters differ ($P < 0.01$).

Figure 1. Effect of stage of lactation[1] on bovine neutrophil total reactive oxygen species production measured by luminol-dependant chemiluminescence.*

The cause of periparturient immunosuppression is not known, but is the subject of much research. Research to date suggests that this immune dysfunction appears to be due to a combination of endocrine and metabolic factors. Glucocorticoids (e.g. cortisol), known endocrine immunosuppressants, are elevated around the time of calving, and have been postulated to be at least partly responsible for periparturient immunosuppression (Burton et al., 1995). Furthermore, changes in estradiol and progesterone just prior to calving may directly or indirectly affect immunocompetence (Weber et al., 2001). However, changes in any of these steroid hormones do not overlap with the entire period of immunosuppression, suggesting that other causes are at least partially responsible for immune dysfunction.

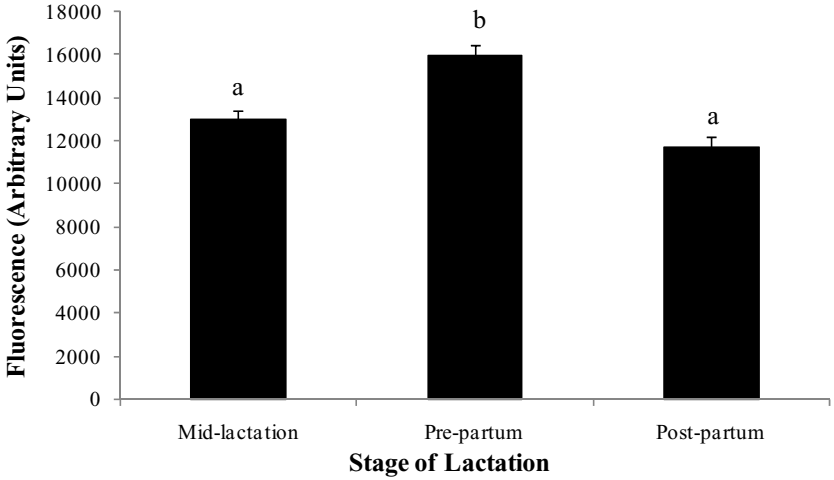


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Figure 2. Effect of stage of lactation[1] on bovine neutrophil extracellular trap formation.*

■ Effects of Metabolism on Immunocompetence

Periparturient negative energy balance has been implicated in contributing to immunosuppression. However, negative energy balance alone had little effect on the expression of adhesion molecules on the surface of bovine leukocytes (Perkins et al., 2001). Furthermore, experimental negative energy balance in midlactation cows did not affect the clinical symptoms associated with an intramammary endotoxin infusion (Perkins et al., 2002). These results are contrary to work in periparturient cows where the presence of a mammary gland (vs. mastectomized cows) and its attendant metabolic demands slowed recovery of neutrophil function, suggesting that the metabolic stress of lactation exacerbated periparturient immunosuppression (Kimura et al., 1999). Other work has investigated individual metabolic components associated with negative energy balance, and has concluded that although hypoglycemia alone is not likely to exacerbate periparturient immunosuppression (Nonnecke et al., 1992), hyperketonemia appears to have multiple negative effects on aspects of immune function (Suriyasathaporn et al., 2000). Ketosis may increase the risk of mastitis in periparturient immunosuppressed cattle because many immune cell types are negatively affected by metabolite levels

typical of a ketotic environment (i.e., low concentrations of glucose and high concentrations of ketone bodies and NEFA). Furthermore, experimental mastitis in ketonemic cows was more severe than mastitis in non-ketonemic cows (Kremer et al., 1993). As reviewed by Suriyasathaporn et al. (2000), impairment of the udder defense mechanism in cows experiencing negative energy balance seems to be related to hyperketonemia.

Another aspect of periparturient metabolism that has the potential to impact immune competence is calcium metabolism. Significant quantities of calcium are required for milk synthesis and an inadequate adaptation to this calcium sink at the onset of lactation results in hypocalcemia (milk fever). Although it is important for milk synthesis, calcium is also important for intracellular metabolism and signaling in most cell types, including the leukocytes of the immune system. Realizing the importance of calcium in leukocyte activation, Kehrli and Goff (1989) hypothesized that low blood calcium around the time of calving could contribute to periparturient immunosuppression. However, they were unable to substantiate this hypothesis when they compared the functional capacity of leukocytes from hypocalcemic cows and cows that were made normocalcemic through the administration of intramuscular parathyroid hormone. This study squelched the theory of a hypocalcemic contribution to immunosuppression for a number of years, until the same group revealed that mastectomized cows were less immunosuppressed than were animals with an intact mammary gland (Kimura et al., 1999). One of the key variables that was different between mastectomized and intact cows was plasma calcium concentration. This revelation rekindled interest in the potential role for calcium metabolism to be causal toward impaired immunity. Recently, Kimura et al. (2006) reported that calcium stores in mononuclear leukocytes are depleted prior to the development of hypocalcemia in the blood, and that this depletion of intracellular calcium does potentially contribute to immunosuppression. Interestingly, it appears that intracellular calcium stores are a more sensitive measure of calcium stress than is blood calcium concentration.

■ New Thoughts Role of Metabolic Regulators on Immunity

The role of insulin in regulating glucose homeostasis and adipose tissue metabolism has received considerable attention relative to periparturient metabolism. In this role, insulin actions result in decreased lipolysis and therefore, fewer NEFA released into the blood. Fewer NEFA in circulation results in less NEFA uptake by the liver with subsequent less hepatic fat accumulation and ketone body release. In this way, insulin is involved in the etiology of energy-related metabolic disorders, and increased insulin action could decrease the incidence and severity of the fatty liver and ketosis

complex (Bobe et al., 2004). However, insulin also promotes the uptake of glucose into non-mammary tissues. Thus, although insulin may attenuate plasma NEFA concentrations, it would also result in partitioning of glucose away from the mammary gland and potentially lower plasma glucose concentrations via increased glucose utilization in non-mammary tissues. Several strategies have been investigated in an attempt to manipulate this "energetic axis" of insulin-regulated fat and carbohydrate metabolism. Slow release insulin administration to dairy cows did result in lower plasma NEFA and hepatic fat accumulation, but also resulted in unacceptable concentrations of circulating glucose except at the lowest levels of insulin release tested (Hayirli et al., 2002). Various strategies to manipulate non-structural carbohydrate and dietary energy intake have also sought to enhance metabolic health either via the modulation of insulin concentrations or via affecting insulin sensitivity or responsiveness (Overton and Waldron, 2004). Finally, nutrients such as chromium (Smith et al., 2008) and several therapeutics have also been studied for their potential effects on insulin action. Among the promising therapeutics, Smith et al. (2007) have reported that the PPAR-γ ligand 2,4-thiazolidinedione (TZD) decreased plasma NEFA and BHBA concentrations without affecting glucose concentrations in periparturient cows.

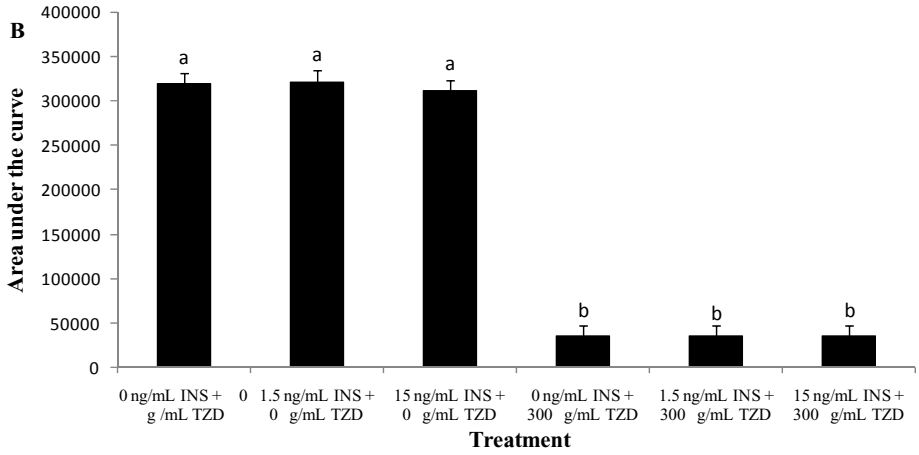
Although regulators of the energetic axis have been studied for their effects on metabolism, the effects of these strategies on the immune function of transition cows has received relatively little attention. One can easily appreciate how manipulation of the energetic axis could indirectly influence immunity. Given the potential negative effects of NEFA and subsequent high ketone body concentrations on leukocytes, a treatment or dietary manipulation that would lower these compounds in blood would likely be beneficial. However, our laboratory is not only interested in these indirect metabolic effects of insulin or its effectiveness, we want to understand how insulin itself (or compounds that result in insulin-like effects) influence leukocyte function and immunity. Insulin concentrations decrease in the weeks around calving and it is generally accepted that insulin resistance also occurs during this period. Is it a coincidence that immune function is decreased during this same timeframe?

Insulin has been shown to be immune supportive in humans and other species. For example, a short term hyperinsulinemic euglycemic clamp resulted in increased concentrations of PMN in blood and increased phagocytic and chemotactic activity of these cells (Walrand et al., 2004; Walrand et al., 2006). Nielsen et al. (2003) reported that bovine PMN possess insulin receptors and preliminary results from our laboratory have demonstrated that PMN from lactating cows not only possess insulin receptors, but that these receptors are active because downstream signaling proteins become phosphorylated within 30 minutes after in vitro incubation of PMN with insulin. Given these findings, we hypothesized that in vitro PMN

incubation with insulin would result in increased functional activity of these leukocytes. Furthermore, we hypothesized that insulin might have differential effects on PMN harvested from cows in different physiological states of pregnancy and lactation due to differences in circulating insulin concentrations and insulin resistance in these different classes of animals.

In addition to the effects of insulin itself, we also investigated the potential role for the PPAR-γ ligand TZD to alter immune function. As discussed previously, TZD has been studied for some of its insulin-like actions influencing the energetic-axis in transition cows. However, in addition to the metabolic effects of TZD which might also have indirect effects on immunity, TZD has been shown to directly affect PMN function. Indeed, PMN are PPAR-γ responsive cells, and in addition to the effects of TZD on insulin action and lipid metabolism, binding of TZD to the transcription factor PPAR-γ results in direct effects on inflammatory and immune-function genes (Houseknecht et al., 2002). Therefore, we hypothesized that in vitro PMN incubation with TZD would result in increased functional activity of these leukocytes. Furthermore, we hypothesized that TZD might have differential effects on PMN harvested from cows in different physiological states of pregnancy and lactation due to differences in circulating insulin concentrations and insulin resistance in these different classes of animals.

To test these hypotheses, PMN harvested from prepartum (12 d before calving), postpartum (7 d after calving), and midlactation (100-200 d of gestation, 220-350 DIM) multiparous dairy cows were incubated for 120 min in vitro with either insulin, TZD, or a combination of the two compounds. The in vitro doses of insulin and TZD used were 0, 1.5, and 15 ng/ml and 0 or 300 μg/ml for insulin and TZD, respectively. Insulin had no effect on PMN functional capacity overall, nor were there any insulin by stage of lactation effects on PMN function. However, TZD decreased PMN total ROS production (Figure 3) without affecting intracellular ROS production , suggesting that extracellular ROS production might be decreased by TZD. Such a scenario might allow for the maintenance of PMN intracellular bacteriocidal activity concomitant with decreased tissue damage from extracellular ROS release - we are currently investigating this possibility. No TZD by stage of lactation effects were evident and incubation of PMN with TZD did not affect NETs formation, phagocytosis, or bacteriocidal activity of these cells.



[1]Neutrophils were collected from cows (n = 25) and incubated for 120 minutes with 0, 1.5 or 15 ng/mL of insulin either alone or in combination with 300 µg/mL of TZD.

*TZD treatment effect, $P < 0.01$. [a,b] Bars with different letters differ ($P < 0.01$)

Figure 3. Effect of insulin and 2,4-thiazolinedione (TZD) on bovine neutrophil total reactive oxygen species production measured by luminol-dependant chemiluminescence.[1,*]

■ Practical Considerations when Feeding for Immunity

Feeding Management

No matter how good the diet is on paper, the nutrients that make it into the blood of the cow are what counts. There is no replacement for watching the cows to truly tell you how good your nutrition program is. Any significant imbalances have the potential to alter immunity. Unfortunately, we don't know all of the imbalances that tip the scale or know how severe the imbalances must be in order to negatively affect immunity.

Stay Ahead of Problems

It's much easier to prevent or catch problems early than to have the proverbial "train wreck".

Avoid Stressors

Stress can be a potent immunosuppressant and the effects of an excellent nutritional program can be negated if the cows are stressed.

Manage for Metabolic Health

At this time, some of the best strategies for us to avoid losses due to infectious disease are to pay strict attention to the details of close-up and fresh cow management such that metabolic disorders are also avoided. Strategies to minimize negative energy balance, and the accompanying fat mobilization and ketone body production, are keys to minimizing immunosuppression. Likewise, management of calcium metabolism to prevent hypocalcemia may have benefits beyond just the avoidance of metabolic disorders. These strategies will minimize nutrient deficiencies and negative metabolic impacts on immune function thereby maximizing the health of the periparturient cow.

■ Conclusions

Research to develop strategies to maximize the metabolic health of transition cows has been emphasized for much of the past 15 years. This line of research has now been extended to determine the role of metabolites, nutrients, and metabolic regulators in supporting immune function. To date, aspects of energy metabolism, especially ketones, have been reported to negatively impact immune function. Although not as well understood, high-levels of circulating NEFA and calcium metabolism may also contribute to periparturient immunosuppression. Our most recent research results suggest that insulin does not have a role in PMN function; however, more research is warranted to ensure that these early results are not artifacts of experimental design or laboratory methods. The PPAR-γ ligand TZD did result in significant changes in PMN function that may result in minimized inflammatory damage and improved health of animals during an infectious insult. The potential beneficial direct effects of TZD on immune function are particularly exciting when coupled with the potential for indirect metabolic effects of this compound on immunity. Given the interplay between metabolism and immunity, strategies to carefully manage metabolic health are also our best recommendation to maximize periparturient immune function of the dairy cow.

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