Dairy Sessions

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Immunity and Inflammation in Transition Cows

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Abstract

Inflammation is an ancient, broadly-conserved set of non-specific responses to infection that contribute to the immune system's ability to clear invading pathogens. However, inflammation is a double-edged sword, and there is growing recognition of the harmful effects of excessive or chronic inflammation for the animal, especially with respect to its metabolic function. Activation of inflammatory pathways is very common among periparturient dairy cows, likely driven by a combination of pathogen challenges, calving-associated tissue damage, endotoxin exposure, and lipid-mediated oxidative stress. Recent evidence suggests that the resulting inflammation may promote metabolic disorders through decreases in feed intake and maladaptive changes in liver metabolism of carbohydrates and lipids. Treatments that successfully prevent the causes of inflammation and/or directly inhibit inflammatory signaling cascades hold promise for improving the health and productivity of dairy cows in early lactation.

Résumé

L'inflammation est une réaction non-spécifique à l'infection, d'origine ancienne et généralement préservée, qui assiste le système immunitaire afin d'éliminer les pathogènes envahissants. Toutefois, l'inflammation est une épée à deux tranchants et on reconnait de plus en plus les effets néfastes d'une inflammation excessive ou chronique pour l'animal surtout au niveau de ses fonctions métaboliques. L'activation de la réaction inflammatoire est très répandue chez les vaches laitières en période périnatale et découle probablement d'une combinaison de plusieurs facteurs incluant la réaction au pathogène, le dommage au tissu causé par le vêlage, l'exposition aux endotoxines et le stress oxydatif induit par les lipides. Des travaux récents suggèrent que l'inflammation qui en résulte peut entraîner des troubles métaboliques par l'entremise d'une réduction de la prise alimentaire et d'un dérèglement du métabolisme des glucides et des lipides dans le foie. Les traitements qui préviennent bien les causes de l'inflammation et/ou qui inhibent directement les cascades de signaux inflammatoires semblent prometteurs afin d'améliorer la santé et la productivité des vaches laitières tôt dans la lactation.

Introduction

The multitude of disorders that dairy cows face during the transition to lactation is a perennial source of concern for dairy producers, nutritionists, and veterinarians. Total disease incidence in the several weeks after parturition accounts for a substantial proportion of all morbidity on many dairies, with particularly high rates of mastitis, metritis, milk fever, displaced abomasum, ketosis, and fatty liver, among other problems. Not surprisingly, these issues have been the focus of much research in recent decades. During that time, substantial progress has been made in some areas (e.g. milk fever); however, incidence of other disorders (e.g. displaced abomasum) may be on the rise.[12]

Recent research highlighted the role of inflammation in infectious diseases and suggested that inflammation is involved in metabolic diseases as well. A key role for inflammation in numerous transition cow disorders may help to explain links between these diverse conditions. On the other hand, inflammatory pathways play important roles in normal immune function, metabolism, and reproduction. An improved understanding of the necessary and pathological aspects of inflammatory pathways in transition cows may improve our ability to predict and prevent transition disorders.

Inflammation and the Acute Phase Response

During infections such as mastitis or metritis, immune cells in the body recognize invading pathogens and become activated. When the infection is caused by gram-negative bacteria, lipopolysaccharide (LPS) released by the bacteria also activates immune cells. The activation of local and systemic host defense mechanisms requires cross-talk between numerous types of immune cells, and one component of this response is inflammation. The host of signaling molecules released by activated immune cells includes inflammatory mediators such as nitric oxide, prostaglandins, and cytokines. While many of these molecules promote local inflam-

mation and increased blood flow to the infected tissue, inflammatory cytokines play a key role in stimulating systemic inflammatory responses, including increased body temperature, increased heart rate, and decreased feed intake.[9] Cytokines can alter many physiological systems because nearly all cell types express cytokine receptors. Key inflammatory cytokines include tumor necrosis factor alpha (TNFα), interleukin (IL) 1b, and IL-6; these inflammatory cytokines act through many of the same signaling cascades and often produce similar responses in cells.

One effect of cytokines is to activate production of acute phase proteins. Primarily produced by the liver, this class of proteins includes haptoglobin, serum amyloid A, and C-reactive protein. Proteins that participate in the acute phase response to infection are generally found in very low abundance in the bloodstream, but are greatly elevated during systemic activation of the immune system. The importance of acute phase proteins in response to infection is somewhat unclear, but they have gained widespread acceptance as markers of inflammation. Furthermore, at least some acute phase proteins further amplify systemic inflammation.[16]

It is clear that mammary and uterine infections result in both local and systemic inflammation. Coliform mastitis results in release of LPS into the bloodstream and increased plasma concentrations of cytokines and acute phase proteins.[14] Likewise, metritis is associated with an acute phase response in transition cows;[15] in fact, plasma haptoglobin is elevated prior to clinical signs of metritis. Furthermore, monocytes are known to become more responsive to inflammatory stimulants during the transition period, resulting in greater secretion of inflammatory cytokines when stimulated.[25] Mastitis and metritis can therefore result in systemic inflammation.

Is There a Role for Inflammation in Metabolic Disorders?

Inflammation has been proposed as a missing link in the pathology of metabolic disorders in transition cows.[10] Recent findings have documented relationships between inflammatory mediators and metabolic disorders. Plasma concentrations of haptoglobin and serum amyloid A were increased in cows that developed fatty liver,[1] and Ohtsuka and colleagues[22] observed increased serum TNFα activity in cows with moderate to severe fatty liver. A retrospective study of cows on three commercial Italian dairies suggested that liver inflammation is associated with a problematic transition to lactation.[3] Cows were classified in quartiles for degree of liver inflammation based on plasma concentrations of acute phase proteins. Those cows with the strongest inflammatory profiles were at eight-fold greater risk for experiencing one or more transition disorders, had lower plasma calcium concentrations, took longer to re-breed, and produced less milk in the first month of lactation.[3] These correlations have driven strong interest in potential mechanisms underlying an inflammation-based pathogenesis of transition cow disorders.

Strong evidence has emerged from two recent studies where inflammatory mediators directly induced metabolic problems. Trevisi and colleagues[27] orally administered interferon-α (a cytokine) daily during the final two weeks of gestation, which caused liver inflammation and release of acute phase proteins. Compared to control cows, treated cows had significantly higher plasma ketone concentrations in the first two weeks after calving. Our own lab recently reported that subcutaneous injection of TNFα for seven days doubled liver triglyceride content in late-lactation dairy cows.[7] We also observed changes in mRNA abundance consistent with transcriptionally-mediated increases in fatty acid uptake and esterification and decreased fatty acid oxidation. These results strongly support the hypothesis that inflammation disrupts normal metabolism, because although both of the above treatments were considered low-dose and short-term, they nevertheless promoted ketosis and fatty liver, respectively.

Non-infectious Sources of Inflammation

Although the importance of inflammation in transition disorders is becoming clear, the pathways that cause this inflammation are less clear. Infections certainly initiate the process in some cows, but this is not likely the cause of metabolic disorders in all cows. In particular, the dramatically higher incidence of transition disorders in cows with excessive body condition is difficult to attribute exclusively to infections.

Lipid peroxides are emerging as likely mediators linking plasma lipids to inflammation. Lipid peroxides are produced when intracellular lipids encounter reactive oxygen species (ROS) such as hydrogen peroxide. Some ROS are always produced in the liver; however, events occurring in early lactation likely contribute to enhanced ROS production. One adaptation to increasing delivery of non-esterified fatty acids (NEFA) to the liver in early lactation is an increase in the capacity of peroxisomal oxidation,[13] an alternative pathway for fatty acid oxidation. Enhanced peroxisomal oxidation increases total oxidative capacity of the cell, but the first step in this pathway produces hydrogen peroxide rather than NADH,[10] and therefore it contributes to ROS production to a greater extent than mitochondrial oxidation.

Increased ROS production in early lactation cows, coupled with increased NEFA concentration, increases lipid peroxide formation; both the transition to lactation and high body condition are associated with increased plasma markers of lipid peroxidation.[2] Lipid peroxides activate inflammatory cascades, which in turn alter

nutrient metabolism. In addition, ROS are especially harmful to immune cells and can decrease the ability of the immune system to respond to infections.[26]

Abrupt dietary shifts during the transition period can also contribute to systemic inflammation. Cows are generally fed diets with greater energy density at the onset of lactation, and if this change is too dramatic, it can result in ruminal production of endotoxin and subsequent transfer of endotoxin into the bloodstream, resulting in activation of an acute phase response.[17]

Requirements for Inflammatory Pathways in the Transition Cow

Although the term "inflammation" conjures thoughts of pain and disease, inflammatory pathways and compounds are critical to many aspects of physiology. In many cases, activation of inflammatory pathways promotes resolution of problems, even those that do not result in apparent disease. During infections, inflammatory signals promote activation and recruitment of immune cells, increasing the delivery of cells that can engulf bacteria, produce extracellular traps, and increase blood flow to the site of infection. Even in conditions that don't involve pathogens, inflammatory pathways can be beneficial. For example, if a liver cell dies, the cellular contents that are released can activate inflammatory pathways in neighboring cells. If the situation is mild, this triggers the cell to turn on protective machinery to aid the cell's ability to survive a cytotoxic challenge. Without this inflammatory response, minor problems that cause cell death could rapidly spiral into broad tissue necrosis.[29]

Another critical role of inflammatory pathways in the transition cow is to promote labor and expulsion of the placenta. Like many reproductive processes, signaling molecules known as prostaglandins are critical in this process. Prostaglandin production requires the presence of long-chain polyunsaturated fatty acids as substrates and a number of enzymes, including cyclooxygenase and prostaglandin synthases. Production of prostaglandins can be limited by availability of fatty acid substrates, but is also highly regulated by enzyme activity. The same inflammatory pathways that alter liver function and activate immune cells also stimulate prostaglandin synthesis. This is a critical process in the term fetus, as prostaglandin E_2 synthesis in the fetal membranes is thought to act directly on cervical tissue and myometrial cells to dilate the cervix and induce contraction, contributing to the initiation of parturition.[8]

Perhaps the clearest indication of the mixed benefits and problems associated with inflammation comes from human genetics. Toll-like receptor 4 (TLR4) is a cellular receptor that recognizes bacterial endotoxin and numerous other ligands, and activates inflammatory cascades in response. A relatively common TLR4 mutation discovered in humans decreases the ability of the receptor to activate inflammation. This mutation has been found to decrease the risk of diabetes and its comorbidities,[18] but also to increase the risk of clinical infections.[30] Balancing inflammation during the transition period may be critical to minimizing overall disease risk during this period.

Potential Interventions

Antioxidants. Dietary antioxidants, notably vitamin E and selenium, are important for their ability to contribute to ROS neutralization, thereby impeding the progression toward inflammation. Interestingly, plasma concentrations of a-tocopherol (vitamin E) decrease through the transition period,[31] and low antioxidant status is associated with transition cow disorders.[19,21] Supplementing vitamin E prepartum improves antioxidant status.[32] Multiple studies have shown that supplementing vitamin E in excess of traditional recommendations decreases the incidence and severity of clinical mastitis.[24,32] Additionally, a meta-analysis showed that supplemental vitamin E is effective at preventing retained placenta.[5]

Although much of the literature on antioxidants in transition cows demonstrates positive effects, these nutrients must be used with caution. In an effort to maximize the odds of observing a response, most studies are designed with rather dramatic treatments; for example, the classic Weiss study cited above[32] compared vitamin E intakes of 574 IU/day (no supplemental vitamin E) to 1474 IU/day (supplementing 88 IU/lb dry matter). In many such scenarios, the control group is fed a diet that is marginally deficient in the nutrient of interest. On most dairies, this is not the case. As a result, adding large amounts vitamin E, for example, can sometimes push the supply of the nutrient high enough to cause mild toxicity. Supplementing 3000 IU/day vitamin E to transition cows with adequate vitamin E status resulted in pro-oxidant responses, increasing markers of lipid peroxidation and the incidence of mastitis.[6] Any treatment that alters oxidative balance should be evaluated carefully.

Non-steroidal anti-inflammatory drugs (NSAIDs). Flunixin meglumine was evaluated in two recent studies in which transition cows were treated prior to any disease diagnosis to assess whether flunixin might prevent disorders. Shwartz and colleagues[23] showed no benefit to administration of flunixin meglumine for the first three days of lactation. In fact, this treatment depressed feed intake and milk yield over the first week of lactation. In a much larger study, Duffield and coworkers[11] demonstrated that flunixin injections in the first two days postpartum significantly increased the risk of retained

placenta and metritis. This negative finding may be due to the ability of flunixin to inhibit cyclooxygenase enzymes, suppressing prostaglandin synthesis, and slowing uterine contractions necessary for expulsion of the placenta.

Salicylates have also been evaluated for use in the treatment of mastitis, and in general they are effective at reducing body temperatures, but do not appear to decrease the severity of the infection.[20] However, this class of NSAIDs shows some promise in regard to metabolic inflammation. Cows treated with acetyl-salicylate (aspirin) for the first five days of lactation had significantly lower plasma concentrations of acute phase proteins and tended to have greater peak milk production than controls.[4] In a similar study, aspirin treatment for five days postpartum improved milk yield in the first two months of lactation and increased first-service conception rates.[28] A relatively small number of cows was included in the study (23/treatment); however, ketosis incidence appeared to decrease with aspirin treatment (4.4% vs. 22.7%) while metritis incidence appeared to increase (30.4% vs. 13.6%). Again, these results point to the tradeoffs between metabolic and immune function associated with decreased inflammation.

Our lab recently completed a study in which 78 transition cows were alternately provided with drinking water containing either 0 or 2.5 g/L sodium salicylate for the first seven days postpartum (Farney and Bradford, unpublished). Consistent with our hypothesis, cows treated with sodium salicylate tended to produce 8% more energy-corrected milk over the first three weeks of lactation, with no overall difference in feed intake or incidence of metabolic or infectious diseases. However, the production response was driven primarily by an increase in milk fat content among the salicylate-treated cows, and metabolic profiling revealed that these cows had sustained elevations of plasma NEFA and ketone concentrations compared to control cows. Nevertheless, salicylate treatment still decreased liver triglyceride content at three weeks postpartum. These findings suggest still more complicated roles of inflammatory pathways; it may be that inflammation provides a "release valve" for the metabolic system, allowing the cow to slow the rate of lipolysis even as negative energy balance continues, albeit at the risk of impairing liver function. We hope that continued investigation of the metabolic and signaling responses to this treatment will help to uncover the role that inflammation plays in regulating metabolism in early lactation.

Conclusions

In summary, research in the fields of metabolism and immunology are uncovering a growing list of physiological functions influenced by inflammatory pathways. The acute phase response is common in transition cows, indicating the presence of an inflammatory state. While this state can help support the cow's ability to calve, expel the placenta, and fight off infection, it may also strain the metabolic system that is critical for handling fatty acids mobilized from body stores and providing nutrients to support lactation. Consistent with this idea, various anti-inflammatory strategies have shown promise for minimizing metabolic disease and improving productivity, while also increasing the risk of retained placenta and perhaps infection. Striking the right balance between immune responsiveness and inflammation may be critical to reducing transition disorders.

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