Novel immunological approaches to improve cattle health

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Abstract

For years veterinarians have understood that stressful experiences increase disease in lightweight, recently transported and commingled cattle. Increased incidence of bovine respiratory disease (BRD) in high-risk cattle has traditionally been attributed to endogenous cortisol, but in fact increased cortisol isoften not measured in cattle presumed to be stressed. While high-risk cattle are often seronegative to common respiratory viruses at receiving,field trials have confirmed they can mount significant humoral response to vaccination at arrival, indicating they are not too immunosuppressed to produce antibodies. However, at-arrival vaccination of high-risk cattle is not reliably associated with improved health over the subsequent 60 - 90 days. Research indicates that stress actually increases the magnitude of inflammatory responses to infection or other stimuli. Taken together, these findings indicate that high BRD incidence in some high-risk cattle may be more attributable to excessive or prolonged inflammation than to immunosuppression. Recent studies demonstrated that high-risk cattle that nontheless stay healthy have increased expression of genes related to production of specific pro-resolving mediators (SPM), which bring inflammatory responses to a close. Research is ongoing to determine whether SPM can be induced by certain management practices, genetic selection, or therapeutic or prophylactic interventions, to improve cattle health.

Key words:inflammation, immunosuppression, respiratory disease, specific pro-resolving mediators

Introduction

Veterinarians often ask whether anything can be done to improve immunity in high-risk cattle, given the the general understanding that the stressful experiences these cattle encounter are immunosuppressive, increasing susceptibility of the animals to disease. However, recent research indicates that stress increases the magnitude of inflammatory responses to infection or other stimuli. Accumulating evidence which will be reviewed here suggests that high incidence of bovine respiratory disease (BRD) in some recently weaned, transported, and commingled cattle may be more due to excessive inflammation than to immunosuppression, per se.

Impact of stress on immunity

It is well known that many factors in the pre- and postweaning period impact the immune response of high-risk cattle, thereby impacting their risk for BRD in the month after they are received. Factors that may have an impact preweaning include the dam's nutrition even before the calf is born, passive immunity obtained from colostrum, presence of persistent bovine viral diarrhea virus (BVDV) infection in the source herd, temperament of the calf and its dam, and whether or not the calf experienced adequate nutrition, parasite control, and vaccination against common diseases.Postweaning factors include the nature of marketing and transportation, receiving program management including metaphylaxis and/or vaccines administered, and whether cattle were castrated or dehorned; and the nature of the receiving diet (reviewed in Duff

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and Galyean, 2007). "Stress" is often invoked as the overarching cause of immunosuppression induced by these and other management practices that can increase BRD risk. However, "stress" is a nonspecific term. For example, the stress induced by commingling and crowding may not have the same physiologic effects, and may not induce the same outcomes, as stress induced by exposure to extreme or abruptly changing weather. The veterinarian should aim to be more precise in defining the forces they assume to be causing stress to cattle, in order to help producers define specific and directed mitigation strategies.

The impacts of stress on immunity are mediated by both endogenous glucocorticoids produced via the hypothalamic-pituitary-adrenal (HPA) axis, and by epinephrine and norepinephrine relased by activation of the sympathetic-adrenal-medullary (SAM) axis (reviewed by Aich et al, 2009).While veterinarians often attribute stress-induced immunosuppression to cortisol, events assumed to be stressful do not always lead to measurable increases in serum or plasma cortisol in cattle. For example, in the week post receiving, single-source calves that had been abruptly weaned and transported in the previous 3 days had significanlty lower serum cortisol concentrations than calves from the same source that were weaned and transported one month earlier.[11]Depending on the degree and duration of the stressful insult, the physiologic responses to stress can suppress immune responses, improve immune responses, or increase inflammatory responses (reviewed by Chen et al., 2015). In one study, calves on their farm of origin were weaned at the time of their first vaccination with a modified-live multivalent viral vaccine containining BVDV2 at weaning, or at the time of their booster, three weeks later.[5] These calves were exposed to no other stressors (such as commingling or transport). While weaning is often considered an immunusppressive stressor, in this study calves weaned at the time of initial

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vaccination had higher titers overall at 3 weeks following their booster than calves weaned at the time of the booster. These results demonstrate that, at least in terms of humoral (antibody) responses to BVDV2 vacination, calves were not too stressed on the day of weaning to mount the better immune response.

The ability of stress to increase respiratory inflammatory responses in cattle was demonstrated elegantly by Mitchell et al.[8]Calves from a single source were assigned to either stressful handling, or control. The calves subjected to stress were abruptly weaned, transported, and fasted overnight; male calves were also castrated. These treatments were intended to replicate typical handling of high-risk cattle. Control calves remained with their dams. After the stressful handling was completed, lung lavage fluid was collected from each stresssed and control calf, then endotoxin was instilled into one lung to induce an inflammatory response; lung lavage fluid was collected again 24 hours later. Neutrophil counts in lung lavage fluid of stressed calves were no different than control calves immediately after the stressful events, but after endotoxin exposure, neutrophil counts in lung lavage fluid were approximately twice as high in stressed calves than controls, which was significantly different ($P < 0.001$).[8] This work demonstrated that the stressful events (weaning, transport, fasting, and castration) did not induce lung inflammation, but once the calves experienced an insult that induced inflammation, their response was significantly greater than the response to the same stimulus by non-stressed calves. Since neutrophils mediate the damage induced in pneumonia following *Mannheimia haemolytica* infection,[24]this stress-induced increasedinflammatory response likely increases the severity of lung pathology in cattle with BRD due to *M. haemolytica* pneumonia, and perhaps other infections, post transport. In summary, while stressful experiences can change immune

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responses, the responses are not always suppressed, and it seems that the impact of stressful events to *increase inflammatory responses*likely contributes as much or more to BRD severity as does immunosuppressionin high-risk cattle.

Response of high-risk beef calves to vaccination

Beef calves are typically defined to be at high risk of developing respiratory disease based on relatively light weight (less than approximately 600 lbs [273 kg]) at arrival, recent weaning, transport and commingling, and uncertain or absent history of vaccination. Research over more than 30 years has repeatedly demonstrated that high-risk calves are largely seronegative to common respiratory viruses at arrival.[11,13,18]While high-risk calves are very often seronegative at arrival, they can, in spite of their recent stressful experiences, generally respond to at-arrival vaccination with substantial antibody responses over the next 14 - 28 days. In one study, mixed source bulls and steers were randomly assigned to be given one dose of modified-live vaccine containing BVDV1, BVDV2, infectious bovine rhinotracheitis virus (IBRV), parainfluenza type 3 virus (PI3V), and bovine respiratory syncytial virus (BRSV) at arrival or not; all cattle were boosted at 56 days.[9]By day 14, serum neutralizing antibodies to IBRV and BVDV in cattle that had been vaccinated at arrival had increased by 3- to 4-fold over baseline, and were significantly higher ($P < 0.05$) than cattle that had not been vaccinated at arrival. At day 85 post arrival, titers to IBRV in cattle vaccinated on arrival were still significantly higher ($P < 0.05$) at day 85 than cattle vaccinated only on day 56, indicating that these high-risk cattle were able to mount a strong memory response to priming vaccine given at arrival. Similarly, cattle vaccinated at arrival with either a multivalent modified-live or inactivated respiratory viral vaccine had serum neutralizing antibody titers aproximately 3- to 6-fold higher than at arrival at day 21 post

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vaccination, with no difference in antibody response for BHV-1, BVDV, or PI3V for stressed cattle than controls.[11] These reports confirm that high-risk cattle can mount brisk and significant antibody responses following at-arrival vaccination. Less information is available regarding cell-mediated immune responses to vaccination in cattle exposed to high-risk handling. It is possible that stressful handling could suppress cell-mediated immune responses more than humoral responses, as stress has been shown in some species to induce a relative increase in T helper type 2 responses,[7] which would be expected to suppress cell-mediated immune responses.[26]More research focused on cell-mediated immune responses to vaccine antigens would improve understanding of the impact of stressful management practices on immune responses of vaccinated cattle.

In spite of multiple studies confirming that seronegative high-risk cattle can readily mount antibody responses to vaccination at arrival, research has generally not shown significant health benefits of at-arrival vaccination over the 60- to 90 days post arrival. In the study demonostrating higher IBRV titers on day 85 in high-risk bulls and steers vaccinated at arrival and 56 days later, vs cattle in the same group vaccinated only on day 56, cattle vaccinated at arrival were *more likely* to be treated for BRD, and more likely to die of BRD, than cattle not vaccinated until day 56.[9]The authors speculated that excessive inflammation induced by vaccination in the cattle, which also had a high rate of post-castration hemorrhage and infection, may have been the cause of increased morbidity and mortality in vaccinated cattle in that trial. In two more replicate trials by these investigators, BRD morbidity and mortality was *not* increased in vaccinated cattle, but there was no difference in morbidity or mortality between vaccinated and controls over the 84 days post arrival (manuscript in progress). Similarly, in a

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large trial of at-arrival vaccination with either parenteral modified-live IBRV/PI3V/BVDV1/BVDV2/BRSVvaccine, or intranasal modified-live IBRV/PI3V/BRSV with parenteral modified-live BVDV1/BVDV2, no effect of morbidity or mortality of vaccination was seen, compared to non-vaccinated control cattle, although all groups seroconverted to BRSV over the first 21 days of the study.[16] Accumulating evidence indicates that delaying vaccination of high-risk cattle for 2 to 4 weeks after arrival will likely be associated with improved outcomes overall.[19]

BRD in high-risk cattle: immunosuppression or excessive inflammation?

It is noteworthy that, in groups of high-risk cattle, while some individuals will be treated one or more times for BRD, other individuals maintain health and a good rate of growth for the 60 to 90 days post arrival. A question worth considering is "Why do some cattle in high-risk populations stay healthy?". Historically, research has typically focused on high-risk cattle that are treated for BRD, but further focus on cattle that resist BRD and grow well in spite of high risk management may provide new insights into mechansisms that could be optimized to keep more cattle healthy. Bassell et al.,[3] drawing on their experience with variability in the responses of uniform groups of calves to a standardized *Mannheimia haemolytica* challenge, proposed that tolerance to bacteria entering the respiratory tract might be one reason that some cattle at risk for BRD stay healthy. These researchers noted that proteins in the respiratory tract, including odorant binding protein and annexin A1, are associated with reduced inflammation.[15,23] Another class of mediators that could limit inflammation in cattle at risk for BRD are the specific pro-resolving mediators (SPM). SPM are lipids molecules similar in biochemical structure to prostaglandins and

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leukotrienes, well-known mediators of inflammation. However, while prostaglandins and leukotrienes *activate* inflammation, SPM*resolve* inflammation. Different families of SPM include lipoxins, resolvins, maresins, and protectins (reviewed in Krishnamoorthy et al., 2018). SPM have a number of actions that limit inflammation in the lung, including decreasing neutrophil degranulation and production of reactive oxygen species (ROS), and increasing the "quiet" uptake of dead neutrophils by alveolar macrophages, termed efferocytosis. Recently discovered evidence supporting a role for SPM in resistance to BRD has been described by Scott et al.,[20]who found that gene expression related to SPM production was increased at arrival in the blood of 5 high-risk cattle that were never treated for BRD over the 85 days after arrival, as compared to 6 cattle in the same group that were treated for BRD 2 or more times in the same period.[20]In two subsequent studies of larger numbers of cattle, expression of the gene for ALOX15, an enzyme which catalyzes production of lipoxins, was consistently increased at arrival in high-risk cattle that did not require treatment for BRD, as measured by whole blood transcriptomes.[21,22]Taken together, these findings suggest that *high-risk cattle that resist BRD may be doing so because of timely activation of responses that resolve inflammation*. In contrast, cattle that develop BRD that requires treatment may do so not so much because they are immunosuppressed, but more because they have excessive inflammatory responses to infection resulting from their stressful experiences.

One may ask: why would some high-risk cattle have excessive inflammatory responses due to the stressful experience of their high risk management, while others do not? If anti-inflammatory proteins and pro-resolving lipid mediators help some cattle to stay healthy, why do only some cattle produce those mediators in the concentrations necessary, or at the time necessary, for this

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beneficial effect? This is the question that will need to be answeredby future research. Possible answers include things cattle experienced prior to marketing, such as nutritional (e.g., omega-3 fatty acid intake supports SPM production[25]) or environmental factors, or perhaps past infections. Genetics seems likely to play a role, as well. While the potential of SPM to modify BRD is an exciting new idea, it will only be useful to know this if SPM production can be leveraged to improve cattle health.

It is important to note that the possibility that SPM may be important to limit inflammation and thereby limit BRD does notimply that treatment with nonsteroidal antiinflammatory drugs (NSAID) to reduce inflammationis necessarily beneficial for BRD. While research has demonstrated that NSAID therapy can decrease fever and improve appearance of cattle in the first day or two immediately following experimentally-induced respiratory infection,[1]or clinical diagnosis of naturally-occurring BRD,[10,27] field trials assessing the effect of NSAID in addition to antimicrobials for BRD have not indicated benefit of NSAID therapy overall.[10,27,28] Because NSAID decrease production of cycloxygenase-2, which catalyzes production of proresolving lipoxin A1.[8,17]NSAID therapy could actually *decrease*resolution of lung inflammation, over days to weeks. A medication or other method to activate SPM in cattle to decrease BRD is not available at this time, but ongoing research may lead to future mitigation strategies that work through SPM activation. Genetic selection for improved respiratory health may also be related to production of SPM or anti-inflammatory proteins such as odorant binding protein or Annexin A1.

Summary and future directions

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Historically veterinarians have recognized that stress can be immunusuppressive, but we may not have adequately understood that stress can also increase inflammatory responses to things that induces inflammation. Accumulating evidence suggests that this hyper-response to inflammatory stimuli may be more relevant to BRD in high-risk cattle than immunusuppression. The inflammation-promoting effects of stress are active at arrival in high-risk cattle, but we are not yet certain how long they persist. Clinical trials comparing at-arrival vaccination with multivalent viral respiratory vaccines to delayed vaccination indicate that delayed administration of multivalent viral vaccines lead to better outcomes overall, and that may be related in part toa hyper-inflammatory state in cattle at arrival. The presence of anti-inflammatory proteins such as annexin A1 in the respiratory tract, or activation of pathways leading to production of specific pro-resolving mediators (SPM), near the time of infectionor at the time of arrival, have been related to improved resistance to BRD in cattle. While evidence indicates that ability to *resolve* inflammation is related to improved health outcomes, NSAID treatment appears to counteract these pro-resolving pathways; this may account for failure of NSAID therapy to be associated with overall benefit in field trials of BRD therapy. Going forward, it will be necessary to confirm exactly how and when SPM and anti-inflammatory proteins need to be produced to improve BRD resistance in high-risk cattle, and to determine whether any management practices, genetic selection strategies, or therapies can modify production of these mediators to meaningfully decrease BRD.

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