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The Immunology of Bovine Respiratory Disease

Recent Advancements

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KEYWORDS

• Bovine • Respiratory disease • Lung • Innate immunity • Adaptive immunity
• Immunomodulation

KEY POINTS

- Bovine respiratory disease (BRD) is a syndrome caused by multiple factors, including environmental and management-related stressors and multiple viral and bacterial pathogens.
- The innate immune system is the first line of defense against BRD. Epithelial cells and immune sentinel cells prevent infection through mucociliary action and secretion of antimicrobial molecules, and secretion of proinflammatory cytokines.
- Neutrophils are essential for eliminating bacterial infections but also play an important role in the pathogenesis of BRD by contributing to lung tissue destruction and inflammation.
- After infection, cattle mount antibody and antigen–specific T-cell responses; however, pathogens frequently evade these immune responses using multiple strategies.
- Immunomodulation and innate training are future alternatives to antibiotics for the prevention and control of BRD.

INTRODUCTION

Susceptibility to bovine respiratory disease (BRD) is multifactorial, influenced by a complex interaction between stress, multiple viral and bacterial pathogens, and the host immune response (**Table 1**). Despite the widespread availability of vaccines and antimicrobial compounds, BRD remains a leading cause of morbidity, mortality, and economic loss to the cattle industry. The continued high prevalence of the disease underlines a fundamental gap in understanding of the host immune response to respiratory infection. In recent years, several advancements have been made in the

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Table 1
The multiple factors with a role in bovine respiratory disease

Stress Factors	Viral Agents	Bacterial Agents
Heat	PIV type 3	*M haemolytica*
Cold	BHV-1, BHV-4	*P multocida*
Dampness	BVDV	*H somni*
Dust	BRSV	*Mycoplasma bovis*
Injury	Adenovirus	*Trueperella pyogenes*
Fatigue	Coronavirus	
Dehydration	Enterovirus	
Nutritional	Reovirus	
Weaning	Influenza D virus	
Shipping		

understanding of the immune system's role in protecting—and potentially harming—the host and how multiple pathogens of the BRD complex interact to evade the host response. There have been comprehensive reviews on the immune response to BRD in previous issues of *Veterinary Clinics of North America: Food Animal Practice*[1,2]; this article focuses on the developments that have occurred over the past decade.

INNATE IMMUNOLOGY OF BOVINE RESPIRATORY DISEASE

Pattern Recognition Receptors

The innate immune system utilizes an array of soluble, surface-bound and intracellular receptors to detect the presence of invading pathogens. These receptors, termed *pattern recognition receptors* (*PRRs*), recognize conserved molecular patterns, known as *pathogen-associated molecular patterns* (*PAMPs*). Common PAMPs include peptidoglycan and lipotechoic acids from gram-positive bacteria, lipopolysaccharide (LPS) from gram-negative bacteria, CpG-rich DNA, and single-stranded and double-stranded RNA. Together, the cells that comprise the bovine respiratory tract express a full arsenal of surface-bound and intracellular PRRs, including Toll-like receptors (TLRs), NOD-like receptors, and RNA helicases, such as RIG-I and MDA-5. All pathogens of the BRD complex produce some type of PAMP that activate the innate immune system.

Infection induces up-regulation of many PRRs, preparing an animal to mount a robust response to the insult. In vitro infection of bovine bronchial epithelial cells by bovine herpesvirus (BHV)-1 or *Mannheimia haemolytica* results in up-regulation of TLR2,[3] a PRR generally involved in the recognition of gram-positive bacteria. In vivo infection with BHV-1 induces up-regulation of TLR3, TLR7, TLR8, and TLR9 in the nasal mucosa, tracheal epithelium, and lung.[4] A transcriptome analysis of the bronchial lymph nodes of calves singly infected with BRD pathogens revealed a global up-regulation of many PRR-associated genes, although there was some specificity in the response to particular pathogens.[5] Although *M haemolytica* infection induced selective up-regulation of TLR1 and TLR6, infection with BHV-1, bovine respiratory syncytial virus (BRSV) or bovine viral diarrhea virus (BVDV) induced more pronounced up-regulation of TLR2 and TLR4.[5] The biological significance of these differences is not immediately clear but warrants further investigation. In an in vitro coinfection model, exposure of alveolar type 2 epithelial cells to *Histophilus somni* induced activation of the type I interferon (IFN) response, which subsequently protected the cells from BRSV infection. Thus, global activation in innate immune sensors may be an

important defense strategy. Other reports, however, have shown that this response may not always be beneficial. Acute stress, such as that caused by abrupt weaning and shipping, results in increased expression of TLR4, CD14, and the IFN-responsive gene 2,5-OAS by circulating peripheral blood mononuclear cells (PBMCs).[6] Although this increase resulted in an enhanced capacity by the cells to respond to LPS (also known as endotoxin) stimulation, a significant positive association was found between PRR expression and risk of mortality from a subsequent BHV and *M haemolytica* challenge.[6]

Airway and Lung Epithelia and Resistance to Bovine Respiratory Disease

Although not often included under the purview of the immune system, airway epithelial cells play a critical role in the first line of defense against infection. The mucociliary escalator is responsible for the removal of inhaled particles, including invading pathogens. One study of healthy animals showed that greater than 90% of aerosolized *M haemolytica* could be eliminated from the lung within 4 hours of administration, primarily due to ciliary action.[7] Viral pathogens, however, such as BRSV, BHV, and parainfluenza virus (PIV), cause ciliary dysfunction and necrosis,[1,8] which can lead to significant delays in the clearance of inhaled particles.[9] Thus, interference with normal ciliary function may be 1 explanation by which primary viral infections predispose cattle to secondary bacterial pneumonia.

Bovine airway epithelial cells express many PRRs, and are responsive to common TLR agonists, such as LPS, which signals through TLR4, and Pam3CSK4, which activates TLR2.[10,11] Epithelial cells of the respiratory tract produce several antimicrobial molecules, including lactoferrin, tracheal antimicrobial peptide (TAP), lingual antimicrobial peptide (LAP), and bovine myeloid antimicrobial peptide (BMAP-28), which accumulate in the mucus and periciliary layers of the air-surface interface. Stimulation with LPS or Pam3CSK induces secretion of LAP, TAP, and lactoferrin, preparing the tissues to ward off invading bacterial pathogens.[11,12] TAP has bactericidal activity against *M haemolytica*, *H somni*, and *Pasteurella multocida*,[13] whereas BMAP-28 can kill *P multocida* in vitro.[14] Viral infections can interfere with the production of antimicrobial peptides by epithelial cells. For example, prior infection with BVDV inhibits pathogen-induced expression of both LAP and lactoferrin by tracheal epithelial cells.[12]

Airway epithelial cells also can play a role in the antiviral immune response. In vivo infection with BHV-1 results in rapid activation of the type I IFN response in the trachea, including secretion of type I and type II IFNs and induction of the interferon-stimulated genes *Mx1, OAS*, and *BST-2*.[15] BHV, and many other viruses of the BRD complex, including BVDV, BRSV, and PIV, however, have mechanisms in place to actively suppress the host IFN response.[16–19] In 1 report, coinfection of bovine epithelial cells with BVDV inhibited type I IFN production, enabling significantly increased replication of BRSV in the same cell cultures,[19] suggesting a synergistic interaction between the 2 viruses.

Perhaps the most critical result of an insult to the airway epithelia is the engagement of the effector arm of the innate immune system. In vitro, invasion of bovine bronchial epithelial cells by *M haemolytica*, or infection with BHV-1, induces rapid secretion of the proinflammatory cytokines interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-8, leading to the recruitment and activation of innate immune effector cells, such as neutrophils.[3,20] Coinfection of bronchial epithelial cells with both BHV-1 and *M haemolytica* has been shown to exacerbate proinflammatory responses by bovine epithelial cells, resulting in greater cytokine expression by dually infected cells compared with either single pathogen alone.[3,20] Similar results also have been shown after coinfection of bovine bronchial epithelial cells with BRSV and *P multocida*.[21] Proinflammatory

cytokines, however, are not the only factors that can contribute to lung damage. In vitro infection of bovine alveolar type 2 cells with BRSV and *H somni* results in significant up-regulation of matrix metalloproteinases 1 and 3, enzymes that break down collagen, thus enhancing the invasion of *H somni* across the alveolar barrier.[22]

Airway epithelial cells also may play a role in regulating the inflammatory response in the respiratory tract. Annexin A1 and annexin A2 are anti-inflammatory proteins produced by airway epithelial cells that regulate neutrophil recruitment and activation, similar to glucocorticoids. Increased concentrations of annexin A1 and annexin A2 in the bronchoalveolar lavage (BAL) fluid prior to challenge have been shown to correlate with improved resistance to the later development of BRD.[23]

Effector Cells of the Innate Immune System

Neutrophils

Neutrophils are among the first cell type to be recruited to the site of infection, migrating from the blood in response to proinflammatory cytokines and chemotactic factors, such as IL-8. Neutrophils are highly phagocytic cells that play an important role in protecting the host against extracellular bacterial infections. It is clear, however, that neutrophils also play a major role in lung tissue destruction during BRD. Depletion of neutrophils,[24] or inhibition of neutrophil infiltration to the respiratory tract,[25] prior to *M haemolytica* infection results in a significant decrease in inflammatory cytokine and lung pathology.

Neutrophil extracellular traps (NETs) have emerged as 1 important factor contributing to BRD pathogenesis. Neutrophils have the capacity to undergo NETosis, a form of cell death in which neutrophils release their nuclear DNA and associated proteins into the extracellular environment. *M haemolytica*[26], *Mycoplasma bovis*,[27] and *H somni*[28] cause neutrophil NET formation in vitro, and evidence of NETS has been observed in the lungs of calves infected with both *M haemolytica*[26] and *H somni.*[28] There is some debate as to whether NETs are an active form of host defense or simply an artifact of neutrophil cell death. In vitro, NETs can kill *M haemolytica* and *H somni*, suggesting some active role in immunity, but the relevance of this antimicrobial activity is difficult to investigate in vivo in the morbid animal. *Mycoplasma bovis* is not susceptible to NET-mediated killing in vitro,[27] potentially due to its ability to release nucleases and degrade the extracellular DNA.[29] Citrullinated histone 3, an indicator of NETs, is increased in the BAL fluid of calves with severe BRSV infection,[30] and NETs have been observed microscopically in the lungs of calves with BRSV infection,[31] demonstrating that NETosis is not specific to bacterial invasion. In calves with BRSV, NETs form dense networks, entrapping mucin and cells, leading to airway occlusion.[31] Consistent with the idea the NETs play a pathogenic role, aerosol administration of dornase alfa, a synthetic form of DNAse I that can degrade NETS, considerably reduced airway obstruction and improved lung pathology in a small group of calves infected with BRSV.[32]

Antigen-presenting cells: monocytes, macrophages and dendritic cells

Antigen-presenting cells (APCs), including monocytes, macrophages, and dendritic cells, are critical in bridging the innate and adaptive immune systems. Dendritic cells in particular are essential to the induction of an effective T-cell and B-cell response. Monocytes and macrophages also fulfill the role of an APC but are similarly active in phagocytosis of dead and dying cells; killing of extracellular pathogens and inflammatory cytokine production.

BVDV infection is a major predisposing factor for BRD due to its known immunosuppressive effects on cells of both the innate and adaptive immune systems. In alveolar

macrophages, noncytopathic BVDV infection suppresses proinflammatory cytokine secretion and reduces phagocytic activity. In vitro infection of monocyte-derived macrophages with both cytopathic and noncytopathic strains of BVDV suppresses responsiveness to ligands for TLR2, TLR3, and TLR4 but does not alter signaling through TLR7.[33] Similarly, in vivo BVDV infection also modulates the capacity of monocytes and macrophages to respond via TLR4.[34]

Like BVDV, several other viruses have an impact on APC activation and function. PIV infection suppresses macrophage phagocytosis and inhibits oxidative burst.[35,36] PIV-infected macrophages, however, are hyperresponsive to LPS stimulation, producing significantly increased quantities of TNF-α.[37] BRSV infection also inhibits alveolar macrophage phagocytosis but does not appear to impair the oxidative burst response.[38] In vitro BRSV infection of ovine alveolar macrophages induces only low-level proinflammatory cytokine expression.[39] In vivo infection of lambs also results in only limited activation of lung-resident dendritic cells, with no significant changes in major histocompatibility complex (MHC) class I or the costimulatory molecules CD80 or CD86. Instead, both lung dendritic cells and alveolar macrophages significantly upregulate gene expression of IL-4 and IL-10.[39] In vivo BHV-1 infection induces recruitment of interstitial and alveolar macrophages to the lungs, and induces production of proinflammatory cytokines, such as TNFα, IL-1α, and induced nitric oxide synthase (iNOS).[40] Calves coinfected with BVDV and BHV-1 show greater numbers of infiltrating macrophages than animals that are singly infected but reduced production of iNOS and the proinflammatory mediators TNF-α and IL-1α.[40]

ADAPTIVE IMMUNOLOGY OF BOVINE RESPIRATORY DISEASE

The development of an adaptive immune response is critical for control and clearance of respiratory pathogens. After infection, cattle mount antibody (Ab) and antigen-specific T-cell responses; however, pathogens frequently evade these immune responses by using multiple strategies. Many of these immune evasion strategies have been covered in reviews of the specific pathogens.[41–44]

B cells and antibody responses

B-cell surface immunoglobulins recognize pathogen epitopes. After antigen recognition and additional downstream signals, B cells terminally differentiate into antibody (Ab)-secreting plasma cells. The Ab secreted play important roles in defending the host from infection with respiratory pathogens. Those roles include neutralizing Ab (nAb), complement activation, Fc Receptor-mediated phagocytosis, and Ab-dependent cellular cytotoxicity. On the other hand, specific Ab against respiratory pathogens and the resultant immune complexes may contribute to BRD pathogenesis.

The protective antigens of Pasteurellaceae family members have not been fully elucidated. There are studies, however, that have shown that Ab that neutralize toxins or Ab against LPS, outer membrane proteins, or secreted antigens can be protective.[45] For example, Ab to *M haemolytica* serotype 1 outer membrane lipoprotein PlpE cross-protects against other serotypes and these Ab promote complement-mediated bacterial killing.[46] Antibodies against the surface exposed outer membrane lipoprotein Gs60 can be protective and have been suggested as especially important in protection against *M haemolytica* when nAb titers to the *M haemolytica* leukotoxin are low.[47] Other Pasteurellaceae, including *P multocida* and *H somni*, have Gs60 homologues,[47] and these also may be targets of protective Ab. In addition, vaccination of calves with sialoglycoprotease enhances protection against experimental disease due

to *M haemolytica*.[48] Fewer studies have been conducted to identify antigens associated with protection from *P multocida* challenge. As was the case for *M haemolytica*, however, Ab generated against outer membrane proteins of *P multocida* have been shown to be an important component of host defense. Intranasal treatment with *P multocida* outer membrane protein H induced both serum IgG and secretory IgA levels that protected calves from experimental challenge with *P multocida.*[49] Although studies have yet to be conducted in cattle, a recombinant outer membrane lipoprotein B from *P multocida* serotype A strain was shown to induce serum Ab in mice with significant bacterial killing activity.[50] Regarding *H somni* immunity, antibodies to a 40-kDa outer membrane protein (OMP) have been found protective, whereas those to a 78-kDa OMP are not.[41] Furthermore, 40-kDa OMP IgG1 antibodies protected less effectively than IgG2. In calves vaccinated with a commercial *H somni* vaccine and then experimentally challenged, IgG2 levels were shown to inversely correlate with disease severity in response to experimental infection.[51]

Seroconversion is detectable 14 days to 28 days after experimental respiratory infection with *Mycoplasma bovis.*[52] The primary serum Ab detected in calves is IgG1,[53] which corresponds with a predominance of IgG1 plasma cells in the lungs of calves experimentally infected with *Mycoplasma bovis.*[43] In cattle, IgG1 is known to be a poorer opsonin for phagocytosis and killing than IgG2, and this may be 1 strategy which *Mycoplasma bovis* uses for immune evasion.[43] Moreover, new antigenically distinct variants of *Mycoplasma bovis* variable surface proteins[54] arise in response to Ab that target these immunodominant surface lipoproteins which further facilitates evasion of host defenses, until adaptive immunity can again respond.

nAb are critical in the response to bovine respiratory viral pathogens. Viral glycoproteins (g) are targets of these Ab against BHV-1, including gB, gC, gD, and gH. Among these, gD has been shown to elicit especially strong nAb titers compared with gC or tegument protein VP8 when delivered via DNA vaccination.[55] Thus, researchers have sought to identify epitopes on gD important for virus neutralization, several of which have been defined, including recently described highly conserved, neutralizing epitopes within the amino and carboxy termini of BHV-1 gD.[56,57] Experimental evidence indicates that BVDV envelope E2 is not only the major immunodominant glycoprotein but also the most variable for BVDV isolates. nAb induced against E2 after natural infection or after vaccination is considered protective against BVDV.[58] To provide information for future vaccines, investigators have mapped neutralizing epitopes and characterized neutralizing monoclonal Ab that bind to E2.[58] Protective Ab responses to BRSV predominately target the F, G, and NP proteins, although calves mount responses to several antigens. Specific Ab can be detected in nasal secretions by day 8 postinfection. Time to detection of BRSV-specific serum IgG1 and IgG2 differs, with IgG1 observed at approximately day 13, whereas IgG2 is not detected until 1 month to 3 months after infection.[59] In either case, the IgG subclass responses wane rapidly. Important in protection from BRSV are nAb against F and G. Neutralizing epitopes have been defined for the prefusion and postfusion F proteins, with the most potent targeting the prefusion protein.[60] In addition, the conserved central core domain of G is an important target of broadly nAb.

Gamma delta T cells

Gamma delta ($\gamma\delta$) T cells play an early role in the host immune response and have functions related to both innate and adaptive immunity. High levels of $\gamma\delta$ T cells are found in the peripheral blood of cattle, especially in young calves, where they can

comprise up to 60% of lymphocyte pool.[61] These cells are found in large proportions at mucosal sites, including the respiratory tract, where they serve as part of a first line of defense against invading pathogens.

Relatively few studies have examined γδ T cell responses to bovine respiratory bacterial pathogens, which is somewhat remarkable given their relative abundance in calves. The authors have shown that *M haemolytica* can exacerbate the expression of the inflammatory cytokine IL-17 induced by BRSV infection and that γδ T cells are a primary producer of IL-17 using an in vitro model system.[62] No enhancement of IL-17 was seen, however, when PBMCs were cocultured with BRSV and *P multocida*. After challenge of previously immunized calves with *P multocida*, an increase in CD5, CD8, and MHC class II expression was found on activated γδ T cells in BAL samples.[63] After *Mycoplasma bovis* lung infection in calves, γδ T cells isolated from peripheral blood and restimulated with heat-inactivated *Mycoplasma bovis* antigen exhibited higher levels of the activation marker CD25.[53] Activated γδ T cells could be 1 source of the intracellular IFN-γ that was measured from in vitro activated PBMCs in that study.

In response to BHV-1 modified live vaccination and subsequent challenge in calves, increased peripheral blood γδ T cells with an activated phenotype were observed.[64] In response to intrabronchial challenge with BVDV1, expansion of γδ T cells in BAL fluid of calves has been reported.[65] The authors' group has found expression of the surface molecule WC1.1 correlates with increased γδ T cell chemokine elaboration during BRSV infection in calves, suggesting that these cells may contribute to recruitment of inflammatory cells.[66] Earlier work of others had shown that depletion of WC1.1-expressing cells did not have an impact on the clinical course of disease in BRSV-infected calves but rather resulted in significantly increased local IgM and IgA responses.[67]

Alpha/beta T cells

As discussed previously for γδ T cells, there has been limited investigation of bovine alpha/beta (αβ) T cells after infection with members of the Pasteurellaceae family. Experimental infection of naïve calves with *P multocida* resulted in a significant increase in the percentage of activated $CD8^+$ T cells in BAL that express MHC II compared with control-naïve calves; however, no significant differences in these cells were seen between immunized control and immunized challenged groups of calves.[63] In addition, increased bronchus-associated lymphoid tissue was noted in lung tissue and an increase in the number of MHC class II–expressing $CD4^+$ T cells was observed in draining lymph nodes after challenge.

Cellular immune responses have been measured using PBMCs isolated from calves after experimental lung infection with *Mycoplasma bovis*.[53] Heat-killed *M bovis* activated $CD4^+$ and $CD8^+$ peripheral blood subpopulations in vitro as measured by flow cytometric analyses. Moreover, as equal percentages of simulated cells produced IFN-γ and IL-4 cytokine responses,[53] indicative of a mixed systemic cytokine response. Local immune responses in lung tissue were evaluated after challenge with *Mycoplasma bovis*; however, no statistical differences in numbers of $CD4^+$ or $CD8^+$ T-cell subsets were noted.[68]

Although nAb are critical in protection against bovine respiratory viruses, there is an important role for cellular immunity involving both $CD4^+$ and $CD8^+$ T cells. $CD4^+$ T cells are considered essential for clearance of BHV-1, with recognition of glycoproteins gB, gC, gD, and VP8 by these immune cells.[44] Defining antigenic regions within these major glycoproteins recognized by $CD4^+$ T cells is important for novel vaccines strategies for BHV-1, and, in this regard, $CD4^+$ T cells epitopes have been mapped on gB[69] and gD.[70] Similarly, gC and gD have been shown to be targets of cytotoxic $CD8^+$

T cells. Importantly, $CD8^+$ T cells may play a role in control of re-establishment of active infection from latency.[44] $CD4^+$ and $CD8^+$ $\alpha\beta$ T cells are critical in the response to BRSV. The F and G proteins are the major class II–restricted targets in cattle, with multiple antigenic regions described for the F protein of BRSV. $CD8^+$ T cells are critical for clearance of BRSV, with M2, F, N, and G targets described.[71] Increased $CD8^+$ T-cell infiltration in several tissues has been seen during BRSV infection, with cytotoxic $CD8^+$ T-cell activity peaking at 7 days to 10 days postinfection.[72] BVDV infections generate peripheral CD4 T cells that can recognize structural and nonstructural epitopes, including those on E2 and NS3 as dominant MHC class II epitopes.[73] Increased numbers of activated CD4 and CD8 T cells have been noted in BAL in response to primary and secondary cytopathic BVDV intrabronchial challenge.[65]

FACTORS THAT HAVE AN IMPACT ON IMMUNITY AND SUSCEPTIBILITY TO BOVINE RESPIRATORY DISEASE

Stress and Immunity

Multiple studies have postulated a link between stress and incidence and severity of respiratory infections. Stress often is a broadly used term to describe adverse circumstances or an alteration induced in an individual as a result of those circumstances. In cases of BRD, the stresses may be categorized generally as psychological, physiologic, and/or nutritional. Thus, stresses in calves can include those associated with weaning, veterinary procedures, transport, comingling, crowding, and dietary changes, among other factors. There is conflicting evidence in the literature on the impact of some of these factors in inducing altered serum stress markers (eg, cortisol levels) and how these may influence bacterial or viral infections or viral-bacterial coinfections associated with BRD.[74] There seems to be strong evidence, however, that weaning and transportation are stressors that contribute to severity of BRD.[75]

Genetics of Disease Resistance

Over the past decades, it has become clear that genetics play a significant role in determining resistance and susceptibility to a wide variety of disease conditions in humans and livestock. In cases of BRD, cattle of the same age and housed under the same conditions vary greatly in their tendency to develop disease, and the severity of the resultant clinical signs. This individual variability strongly suggests some degree of genetic control. Genetic regulation of disease susceptibility was reviewed in a recent edition of *Veterinary Clinics of North America: Food Animal Practice.*[76] This section provides only a brief summary of recent findings related to BRD and the genetic component of disease susceptibility.

Quantitative-trait locus mapping has revealed some regions linked to BRD susceptibility. Two single-nucleotide polymorphisms (SNPs) on BTA20, identified as the *ANKRA2* gene and the *CD180* gene, were shown to associate with susceptibility to BRD.[77] *ANKRA2* plays a role in transcription of the MHC class II genes, whereas *CD180* is a gene in the TLR family and is important for B-cell responsiveness to LPS. Polymorphisms have been identified in other innate bovine PRRs, including TLR, RIG-I, NOD2, and mannose-binding lectin.[78] Although there currently is little evidence to directly link these SNPs to BRD susceptibility, 1 study has suggested that polymorphisms in TLR4 and TLR8 contribute to increased responsiveness to BRSV vaccination.[78] In a recent study, gene set enrichment analysis identified glucose as the most important upstream regulator of BRD susceptibility in dairy cattle. In the same study, TNF was identified as the most significant upstream regulator

in beef cattle, influencing 64 downstream genes that were associated with the immune response.[79] Comparisons between the beef and dairy populations in this studied identified 6 BRD-associated SNPs that were shared between the groups, located in the genes *ADIPOQ*, *HTR2A*, *MIF*, *PDE6G*, *PRDX3*, and *SNCA.*[79] All 6 genes are known to be involved in down-regulating TNF production and in the metabolism of reactive oxygen species.[79] It is expected that the next decade will bring continued advancements in understanding of the genetic components of this pervasive syndrome.

NOVEL INTERVENTION STRATEGIES FOR USE AGAINST BOVINE RESPIRATORY DISEASE

Vaccination and effective management strategies are the foundation of BRD prevention. Metaphylactic use of antibiotics generally is effective against BRD and is an essential tool for controlling outbreaks. There are ongoing concerns, however, regarding the development of antimicrobial resistance, and significant research efforts currently are aimed at designing new approaches for BRD prevention. Vaccines are the focus of another article in this edition. Therefore, this section is focused on a few promising alternative intervention strategies, which are being developed to reduce the impact of BRD in the dairy and feedlot.

Antimicrobial Peptides as Alternatives to Antibiotics for Bovine Respiratory Disease Control

The bovine innate immune system produces several antimicrobial peptides and several have been explored for their use as therapeutic alternatives to antibiotics. NK-lysin is an antimicrobial peptide that has been described in the granules of cytotoxic T cells and NK cells in humans, pigs, and cattle. Although humans and pigs have only a single NK-lysin gene, cattle have 4 functional NK-lysin genes, *NK1*, *NK2A*, *NK2B*, and *NK2C.*[80,81] NK-lysin gene expression is up-regulated in the lungs of animals infected with *Mycoplasma bovis*, *M haemolytica*, *P multocida*, BVDV, BRSV, and BHV-1.[80] In vitro, NK-lysin has antimicrobial activities against *M haemolytica*, *P multocida*, and *H somni,* although susceptibility to the 4 individual NK-lysin peptides differs between species.[80,82] *Mycoplasma bovis* is susceptible only to NK2A and NK2C peptides, and these are effective only at relatively high concentrations,[83] suggesting that NK-lysin may not be an ideal therapeutic candidate for this organism.

TAP is a β-defensin produced by airway epithelial cells. TAP gene expression is induced in bovine epithelial cells in response to TLR stimulation or IL-17A[10,11] and is up-regulated in the lungs of calves with *M haemolytica* pneumonia.[84] In vitro, TAP has potent bactericidal activity against *M haemolytica*, *P multocida*, and *H somni*, although *Mycoplasma bovis* is resistant to TAP treatment.[13] In a recent study, TAP was administered therapeutically, via aerosol or intranasal administration, to neonatal calves that had been challenged with *M haemolytica.*[85] Unfortunately, TAP treatment had little effect on *M haemolytica* disease. Further investigation revealed that physiologic concentrations of sodium chloride, such as the concentrations present in nasal secretions or serum, inhibited TAP-mediated bactericidal activity in vitro.[85]

Innate Immunomodulation as a Novel Strategy for Controlling Bovine Respiratory Disease

Although vaccine development continues to be an active area of research, the past decade has seen increasing interest in strategies to influence the innate immune system. Immunology dogma has long taught that the innate immune system is

nonspecific and does not improve with repeated exposure. It has become apparent, however, that, in fact, the innate immune system can be primed, or trained, by exposure to certain organisms or molecules, that results in an enhanced state of responsiveness to secondary stimuli. This enhanced state of responsiveness, termed trained immunity, is induced primarily in myeloid cells (monocytes and macrophages) and NK cells[86] and results in superior cytokine expression and ultimately, enhanced capacity to prevent infection. Mechanistic studies have demonstrated that trained immunity is independent of adaptive immunity and is caused by epigenetic reprogramming and alterations in basal intracellular metabolic pathways, which result in changes in gene expression and cell physiology leading to increased innate immune cells' capacity to respond to stimulation.[86] The idea of enhancing an animal's innate state of disease resistance is appealing, particularly during well-defined periods of stress, such as during weaning and shipping. Several recent therapies have emerged with potential to train or enhance the innate immune system during times of stress. One such DNA-based immunostimulant, marketed as the commercial product Zelnate (Bayer Animal Health, Shawnee Mission, KS, USA), has been shown to reduce lung pathology scores in cattle experimentally challenged with *M haemolytica*[87] and significantly reduce mortality in high-risk cattle after feedlot placement.[88,89] Although the product's exact mechanisms of action is not well defined, it may be stimulating the immune system via the innate cytosolic DNA sensing cGAS-STING pathway.[90] Another immunomodulatory product, marketed as Amplimune (Novavive, Inc, located in Napanee, Ontario, Canada), is a mycobacterium cell wall fraction derived from the nonpathogenic *Mycobacterium phlei*. Amplimune nonspecifically activates the innate immune system and has been successfully applied for prevention of K99 *Escherichia coli* in preweaned calves. A promising study revealed, however, that Amiplimune had beneficial effects in reducing incidence and mortality associated with BRD in newly received, light-weight beef calves.[91]

SUMMARY

The innate and adaptive immune systems are well equipped to protect the lung from pathogen invasion. BRD is a complex syndrome, however, caused by multiple factors, including environmental and management-related stressors and viral and bacterial pathogens. In combination, these factors overwhelm and dysregulate host immunity and lead to disease. Although vaccination and antimicrobial therapy remain the primary methods for controlling BRD, several novel strategies currently are being investigated as alternatives, including innate immunomodulation and selection of genetically resistant stock.

DISCLOSURE

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