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THE IMMUNOLOGY OF THE BOVINE RESPIRATORY DISEASE COMPLEX

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The bovine respiratory disease complex (BRDC) continues to be a major cause of morbidity, mortality, and economic loss in cattle production systems, despite the widespread use of antibiotics and vaccines. Notwithstanding the likelihood that many management practices that are currently used in cattle production (such as vaccination on arrival to feedlots) do not provide the necessary or optimal conditions for the development of protective immune responses,[61] a better understanding of the role of the immune response in the pathogenesis and prophylaxis of BRDC will likely aid in better control of the syndrome. Moreover, the increasing concerns over the use of antibiotics in livestock and the perceptions and possible negative implications of animal agriculture–related antimicrobial resistance for human medicine indicate that manipulation of the bovine immune system may become increasingly important in disease management in the future. Therefore, these concerns dictate that a better understanding of the ruminant immune system in health and disease is likely to become more of a priority and the focus of continuing research. This article concerning the role of the immune system in pathogenic and disease-sparing roles in the BRDC focuses on recent advances in our knowledge since the previous issue of the *Veterinary Clinics of North America: Food Animal Practice* that dealt with respiratory disease in cattle.

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THE IMPORTANCE OF NONSPECIFIC DEFENSE MECHANISMS IN THE RESPIRATORY TRACT

The respiratory tract is a mucosal surface that is different from other body surfaces where infectious agents interact with the host and diseases may occur. Because of the requirement for unimpeded access of air to the alveoli and the resultant close contact between the external and internal environments that is required for gaseous exchange to occur, this mucosal surface has evolved a filtering system that is capable of removing particles as small as 5 μm before they reach the alveoli. The gross structural basis for this filtering system is the confirmation of the turbinates, trachea, and bronchi, and the turbulence created by movement of air within these structures. Microscopic features of this filtering system comprise cilia that move particulate debris toward the external nares. The mucosal lining of the respiratory tract is coated by mucous that contains antimicrobial soluble factors, such as lysozyme and immunoglobulin A, and it performs trapping and antiseptic functions.[60] The role of viral agents in altering or destroying the structural integrity of the filtering system, such as cilia and lining epithelial cells, is well established.[8, 35] The importance of the nonspecific phagocytic and digestive functions of resident and infiltrating bronchoalveolar macrophages (BAM) and neutrophils and the effect of various viruses and bacterial virulence factors on these functions are well recognized.[35, 48, 60] Recent studies have better addressed how various host and pathogen factors interact at the molecular level to alter the efficacy of the scavenging features of the phagocytes.

THE ROLE OF NONSPECIFIC IMMUNE RESPONSES IN THE PATHOGENESIS OF BOVINE RESPIRATORY DISEASE COMPLEX

The role of bacterial virulence factors, such as the ruminant-specific leukotoxin (Lkt) produced by *Mannheimia (Pasteurella) haemolytica* has been recognized since the 1980s.[48] Recent studies have begun to better elucidate the molecular basis of the interaction of Lkt with the bovine immune system, by demonstrating that this toxin binds specifically to a group of adhesion molecules known as the beta-2 integrins, notably CD18.[4, 41] Specific binding of Lkt to this cellular receptor results in the upregulation of various metabolic pathways associated with an increase in intracellular calcium and tyrosine kinase activity.[34]

In addition to destruction of the target cells, the specific binding of Lkt has recently been shown to upregulate the transcription and translation of several cytokines, including interleukin (IL)-1, tumor necrosis factor-α, and IL-8, all of which mediate proinflammatory functions in tissue.[15, 38, 47] Pretreatment of isolated BAM with interferon-α has been shown to enhance the production and secretion of these inflammatory medicators.[47] The practical implications of the latter in-vitro results may

further explain the pathogenic synergism between bovine herpesvirus-1 and bacteria in BRDC; viral induction of interferon may actually enhance the pathologic inflammatory process in the lung.

Interleukin-8 is a member of a family of low molecular weight cytokines, known collectively as *chemokines*.[16] Recent studies in cattle document that bovine IL-8 functions as a primary chemoattractant for neutrophils in inflammatory sites, including the lung, where *M. haemolytica* apparently upregulates the production of this cytokine, thereby increasing the migration of neutrophils into the infected tissue.[15, 16] In contrast, in calves with experimental bovine respiratory syncytial virus (BRSV) infections in which neutrophil infiltration of the airways and parenchyma is not as prominent as in *M. haemolytica*–associated pneumonias, IL-8 expression was reduced. Studies in calves with bovine leukocyte adhesion deficiency that congenitally lack CD18 expression have demonstrated that this adhesion molecule is essential for the initial passage of neutrophils through the extensive extracellular matrix of the pulmonary airways.[1] In addition, the expression of the adhesion molecule intracellular adhesion molecule-1 on bronchiolar and alveolar epithelium, arterial and venous endothelium, and on BAM and neutrophils is upregulated in calves experimentally infected with *M. haemolytica*, compared with saline-inoculated controls.[52] These results are compatible with the hypothesis that this molecule also plays an important role in leukocyte infiltration, and thereby is important in the pathogenesis of inflammation and tissue damage in the lung. Relatedly, recent investigations document that in addition to controlling the growth of *M. haemolytica*, tilmicosin, a commonly used antibiotic, may reduce pulmonary inflammation by inducing apoptosis (programmed cell death) of neutrophils and reducing leukotriene B4 secretion.[17] Apoptotic neutrophils do not lyse and release tissue-damaging proteolytic enzymes, so further amplification of inflammatory injury in *M. haemolytica*–infected lungs is prevented. These changes occurred without effects on neutrophil infiltration or function.

The identification of specific host molecules involved in pathogenic inflammation raises the possibility that specific blockade of their expression or blocking of their activity by ligand or antibody binding may be used as an approach to reduce disease in infected animals[1, 15]; however, it remains to been seen whether such a reductionist approach that targets one component in an obviously complex interaction comprising soluble mediators with overlapping activities produces practical preventive or therapeutic results. Despite some recent results demonstrating the positive effect of orally administered human recombinant interferon alpha on weight gain in feedlot cattle with BRDC,[20] the almost complete failure of the application of recombinant bovine cytokines (such as IL-2) as therapeutic or preventive agents in cattle attests to the difficulty of this reductionist approach. Another related complicating factor in the application of molecularly targeted therapies in cattle is the difficulty in accurately assessing the stage of disease in individual animals. Continuing work aimed at the possibility of more practically measuring acute-

phase proteins may provide a better means of identifying candidates for therapy. Acute-phase proteins such as serum amyloid A, haptoglobin, and alpha 1-acid glycoprotein are produced in the liver in response to proinflammatory cytokines such as IL-1 and tumor necrosis factor-α.[55] Recently, serum concentrations of serum amyloid A and haptoglobin were used to discriminate between acute and chronic inflammatory conditions in cattle.[33] The practical application of such testing methods almost certainly depends on the development of cow-side tests so that intervention decisions can be made at the time cattle are processed.

The role of nonspecific immunosuppression mediated by bovine herpesvirus-1, bovine viral diarrhea virus, and to a lesser extent, parainfluenza-3 virus in respiratory disease in cattle is part of the current dogma in veterinary medicine, it is supported by in-vivo and in-vitro data, and it was reviewed in a previous issue of the *Veterinary Clinics of North America: Food Animal Practice*.[35, 50] Recent studies have added little to the knowledge in this area, except to suggest that BRSV may also be immunosuppressive,[37] and that bovine viral diarrhea virus and BRSV may synergistically negatively affect local and systemic immune function.[11, 42] Currently the mechanism of BRSV-mediated immunosuppression is poorly understood, but available data indicate no apparent role for prostaglandin secretion or inhibition of IL-1 or IL-2 secretion.[36]

THE ROLE OF SPECIFIC IMMUNE RESPONSES IN THE PATHOGENESIS OF BOVINE RESPIRATORY DISEASE COMPLEX

Specific immune responses to pathogens are generally thought to confer protective immunity; however, some recent data highlight the disease-causing potential of pathogen-specific immune responses.

The role of specific immune responses in the enhancement of bovine respiratory syncytial virus (BRSV)–associated respiratory disease remains controversial, despite voluminous epidemiologic data indicating that RSV-associated disease becomes less severe as age and herd immunity increase, in both cattle and humans.[8] Conflicting results were reported in recent studies using formalin-inactivated, alum-adjuvanted BRSV vaccines[25, 62] that were formulated similarly to the inactivated human respiratory syncytial virus (HRSV) vaccine that was reportedly associated with the enhancement of respiratory disease in human pediatric patients with HRSV infections.[8] The experimental inactivated BRSV vaccines tested stimulated the production of high concentrations of non-neutralizing antibodies in vaccinated calves; however, one study reported significantly more severe pulmonary lesions,[25] and another reported sparing of BRSV-associated lung lesions subsequent to challenge with virulent virus.[62] The dose of BRSV in the respective vaccines and in other inactivated vaccines may, in part, account for the disparate results.[23, 62] Relatively low-dose (BRSV antigen content) vaccine may be associated with disease enhancement, whereas high-dose vaccine

apparently stimulated disease-sparing immune responses; however, this possibility remains to be confirmed.[23, 62] The mechanism of enhancement of pulmonary disease following the administration of the inactivated BRSV vaccine remains to be determined. In one study this phenomenon was significantly correlated with high concentrations of BRSV-specific non-neutralizing antibodies and decreased levels of production of interferon-γ by circulating lymphocytes.[25, 65] Additional experiments examining the constituents of thoracic lymph correlated disease severity with concentrations of BRSV-specific immunoglobulin E and IL-4.[24] Based on these results, it was hypothesized that respiratory disease associated with BRSV infections is an immunopathologic event resulting from the stimulation of BRVS-specific T-helper 2 lymphocytes.[24] The relative importance of this putative mechanism versus the cytolytic effects of the virus on airway and parenchymal epithelium remains to be determined.

The role of BRSV infection and BRSV-specific immune responses has been proposed in pathogenesis of atypical interstitial pneumonia (AIP) of feedlot cattle, based largely on the similarity of gross and histologic lesions in BRSV-infected cattle and in cattle with AIP.[6, 8] In contrast to previous findings,[18] recent investigations have failed to detect active infection by BRSV in most of the cases of AIP in feedlot cattle that were examined retrospectively; however, the BRSV-immune status of these cattle was not examined.[7, 56] Further supportive of a primary role for pneumotoxicants such as 3-methyl indole in the pathogenesis of AIP[6] are data that documented increased plasma concentrations of the latter rumen metabolite in feedlot cattle with AIP compared with controls.[7] This occurred in the absence of apparent pulmonary involvement of BRSV or other significant respiratory pathogens. In contrast, prospective experiments demonstrated that mild or subclinical BRSV infection can synergize with sublethal intoxication with 3-methyl indole, the classic prototype of a toxic rumen metabolite to enhance pulmonary disease.[9] Whether or not this synergism involves an immunopathogenic component was not determined, so the role of BRSV in AIP remains unresolved.

It has been proposed recently that specific antibodies to *M. haemolytica* and immune complexes that result from the interaction between the bacteria and antibodies in the lung may be a component of the pathogenesis of BRDC.[44] In a retrospective immunohistologic examination of lung tissue from 44 cases of naturally acquired bovine pneumonic mannheimeosis, immune complexes were observed in alveolar spaces and walls in 88% of the animals. To further study this potential immunopathologic effect prospectively, mice were immunized with purified outer membrane proteins (OMPs) from *M. haemolytica* and subsequently challenged intratracheally with the live bacteria or OMPs. Immunized mice developed high serum immunoglobulin G responses to the OMPs and necrotizing bronchointerstitial pneumonic lesions. Similar to the findings in the cases of BRDC, immune complexes and complement were identified in alveolar walls in damaged pulmonary tissue. Based on these findings, it was proposed that immune complex disease might be a component of the pathogenesis of BRDC associated with *Mannheimia*

infections. This is logical from an immunologic standpoint, based on the nature of the inflammatory lesions in which complement fixation could mediate neutrophil infiltration and the likely possibility that specific antibodies to *M. haemolytica* could result from previous natural exposure or vaccination and could be present in many cattle that develop pneumonic mannheimiosis. These results may explain previous observations that the administration of early, crude *Mannheimia* vaccines was associated with disease enhancement in cattle that subsequently developed *Mannheimia*-associated BRDC. These findings support the concept that better defined or subunit *Mannheimia* vaccines that apparently do not contain the offending bacterial components are associated with improved efficacy and fewer adverse reactions.[48]

Several species of mycoplasma have been recognized as etiologic agents in calf pneumonia.[5, 48] There is increasing evidence that *Mycoplasma bovis* is an emergent, or at least better recognized, respiratory pathogen in older (feedlot) cattle in North America and elsewhere.[26, 38] Several factors could contribute to this apparent emergence: better control of other bacterial pathogens through the use of vaccines and antibiotics, or interaction with other pathogens such as bovine viral diarrhea virus,[26] both of which could allow *M. bovis* to better assume a role as an opportunistic pathogen; increased virulence of *M. bovis*; or as-yet-unidentified host or management factors. Studies conducted in the 1980s suggest that the immune response to *M. bovis* may be contributory to lesion development.[29, 31] Based largely on the prominence of immunoglobulin G1 and, to a lesser extent, immunoglobulin G2–containing cells at the periphery of characteristic, necrotizing *M. bovis*–associated lesions, it was proposed that a specific immunoglobulin, rather than a cell-mediated immune response, is at least partly responsible for the lesions seen in calves infected with *M. bovis*, although its specific role was not further identified.[29, 31] In contrast to murine mycoplasmosis caused by *M. pulmonis* and *M. pneumoniae* that colonize the epithelium of small airways and cause hyperplasia of bronchus-associated lymphoid tissue (BALT), *M. bovis* is invasive, and hyperplasia of BALT is not a prominent feature of the pulmonary lesion in cattle.[29, 31] Consistent with this is the lack of mitogenic activity of at least some strains of *M. bovis* for cultured bovine lymphocytes, indicating that any lymphoid involvement in the lesions is not caused by a nonspecific mitogenic effect.[29]

THE ROLE OF SPECIFIC IMMUNE RESPONSE IN THE PROPHYLAXIS OF BOVINE RESPIRATORY DISEASE COMPLEX

As the term BRDC implies, this syndrome is the result of a complex interaction of host, pathogen, and environmental factors, rendering the understanding of the probably multifactorial nature of disease-sparing immunologic responses difficult. Traditionally, identification of protective immune responses has been approached in the context of vaccine

trials, in which protection or disease sparing is correlated with various immune responses, usually in the context of experimental infections. Correlation of reduction of naturally acquired disease with immune responses, primarily serologic, is a more epidemiologic, if also less than perfect, approach to this problem. Recent studies have continued to use both approaches to better understand the immune response in BRDC and are reviewed in a pathogen-specific manner.

Bovine Coronavirus

Based on seroconversion and virus isolation, bovine coronavirus (BCV) has become recognized as a potential etiologic agent in BRDC.[35, 39, 40, 57] Whether this pathogen has emerged in the wake of improved control of the other respiratory virus infections or is simply better recognized or diagnosed remains to be determined. To date, there are no published prospective studies that address protective immunologic mechanisms in BCV infections in the respiratory tract. There are no data indicating that any of the commercially available vaccines containing either modified live or inactivated BCV have been applied in the prophylaxis of BRDC. Although discussion of protective immunologic mechanisms would be largely speculative at this point, there is some evidence, similar to the case with BCV-associated enteric disease,[35] that maternal antibodies have a disease-sparing effect on BCV-associated respiratory disease in young calves (John A. Ellis, DVM, PhD, unpublished data, 2000). Seroconversion has been associated recently with a reduced risk of BCV shedding in feedlot calves.[39] Given the genetic and antigenic relatedness of the BCV isolates causing enteric and respiratory disease[27, 35] and, consistent with the quasispecific nature of BCV, it is likely that neonatal enteric infections with BCV can induce mucosal and systemic antibodies that reduce respiratory disease resulting from subsequent respiratory BCV infections.[27, 35]

Bovine Herpesvirus-1

Previous studies have provided evidence that both cell-mediated and antibody responses are associated with disease sparing in bovine herpesvirus-1 (BHV-1) infections.[27] Cellular responses comprise cytotoxicity mediated by CD8+ lymphocytes, natural killer–like cells, and the secretion of interferon and other cytokines. Cellular and cytokine responses to the virus may also contribute to pathology in infected cattle. Protective antibodies recognize major surface glycoproteins of the virus and function in virus-neutralization, cell-mediated cytotoxicity, and, possibly, in blocking virus-mediated immunosuppressive effects.[27] There have been no substantial recent advances related to better understanding specific immunologic mechanisms involved in protection from BHV-1–associated respiratory disease or in the duration of protective re-

sponses conferred by infection or vaccination. Most recent vaccine trials have examined new vaccine technologies including DNA[66] and vector vaccines[67] and continue to support the concept that cell-mediated immunity and local and systemic antibody responses are associated with protection.[27] The use of a nonspecific immune stimulator at the time of experimental BHV-1 infection or 2 days after infection was associated with disease sparing and reduced viral shed; however, the immunologic mechanisms involved were not examined.[14] Recent seroepidemiologic studies provide supportive evidence that BHV-1 continues to be an important pathogen in feedlot cattle and that systemic antibody responses are associated with disease sparing.[10] Cattle that seroconverted to BHV-1 G-IV glycoprotein in the feedlot had a decreased risk of undifferentiated fever, whereas high concentration of BHV-1 specific antibody on arrival, possibly suggesting previous exposure and latent infection, was associated with increased mortality.[10]

Bovine Respiratory Syncytial Virus

The recent development of challenge models[62, 64] that result in clinical disease and lesions similar to those observed in naturally acquired BRSV infection has allowed considerable progress to be made in the understanding of protective immunity in BRSV-associated BRDC. In the case of primary BRSV infection in susceptible, naive cattle, several lines of evidence indicate the local cell-mediated immunity is the critical protective immunologic mechanism in animals that recover from infection, as has been previously hypothesized.[8] Demonstration of genetically restricted cell-mediated cytotoxicity by cells in pulmonary lavage fluid[62] and phenotypic analyses using monoclonal antibodies[46] indicates that CD8+ T lymphocytes are the primary cells mediating a disease-sparing response. This response by CD8+ T cells has been shown to occur in the absence of significant local or systemic antibody responses.[62] In the case of cattle that have been primed immunologically to respond to BRSV by vaccination with modified live vaccines and probably by naturally acquired infections or exposure, both antibody and cell-mediated responses were associated with disease sparing.[62] Viral clearance and clinical protection was coincident with the simultaneous appearance of mucosal antibody (immunoglobulin A and immunoglobulin G), cytotoxic cells in the lung, and primary or anamnestic serum antibody responses to the virus. Interestingly, parenteral vaccination primed for anamnestic mucosal antibody responses.[62] Vaccine-associated secretion of interferon-γ by peripheral blood leukocytes, assumedly by CD4+ T cells, was a further correlate of protection in vaccinated calves[62] and can be detected in the lung along with other cytokines in naive cattle.[45] The antigen specificity of these antibody responses associated with protection was not determined, but results of other studies[55] continue to support the concept[8] that neutralizing antibody responses to epitopes on the fusion (F) protein of the virus are protective and that responses to

epitopes on the F protein may differ in vaccinated and infected cattle. It has been shown recently that responses to specific epitopes on the F but not the G protein in infected cattle may be related to bovine leukocyte antigen (BoLA) haplotypes,[55] which may relate to differences in disease severity among BRSV-infected cattle.

In contrast to previous expectations,[8] recent studies have also documented that significant disease sparing and reduction in the duration and magnitude of shedding of BRSV can be engendered by a commercially available adjuvanted, inactivated vaccine.[23] Protection was highly correlated with high concentrations of non-neutralizing antibodies and a primed interferon-γ response at the time of challenge. Whether the correlation of non-neutralizing antibodies with protection is indicative of a causal relationship remains to be determined, because it is not readily apparent how non-neutralizing antibodies prevent viral replication or mediate viral clearance. Moreover, supportive of previous concepts[8] concerning non-neutralizing antibodies, it has been demonstrated recently that passive protection of BRSV-infected calves was mediated by F protein-specific neutralizing and fusion-inhibiting monoclonal antibodies, but not by non-neutralizing monoclonal antibodies.[58]

Parainfluenza-3 Virus

Whether parainfluenza-3 virus (PI_3V) is a significant respiratory pathogen continues to be a matter of debate, despite its ubiquitous presence in cattle populations, its identification in the lungs of cattle dying with pneumonia, and the fact that under experimental conditions at least some isolates of the virus can produce clinical disease and pulmonary lesions.[12, 35] Recent investigations have shed little additional light on the nature of protective immunity from PI_3V infections in cattle. One recent study demonstrated the significant disease-sparing effect of intranasally administered temperature-sensitive PI_3V vaccine in small numbers of calves using a challenge model that produced severe clinical disease and lung lesions.[12] There was no significant correlation between reduced pulmonary pathology and PI_3V-specific serum antibody at the time of challenge, and the specific protective immunologic mechanism was not identified.

Being related paramyxoviruses, the biology of BRSV and PI_3V is similar.[8, 35] There is, however, more evidence, including the results of recent studies,[2] that PI_3V infects bronchoalveolar macrophages and is more likely to have immunosuppressive effects in the bovine immune system than those recently documented in the case of BRSV.[36, 37] Nevertheless, given the biologic similarities, it is likely that immune responses similar to those recently identified in BRSV challenge studies are likely to be important in conferring protection from PI_3V infection; however, to date, detailed immunologic studies have yet to be conducted in the context of experimental PI_3V challenge models that result in significant

clinical disease and pulmonary lesions, as has recently been accomplished with BRSV.

Bovine Viral Diarrhea Virus

Whether bovine viral diarrhea virus (BVDV) is a pneumotropic virus and hence a true respiratory pathogen is somewhat debatable.[50] Although direct infection of cells of the respiratory system (i.e., epithelial cells in the airways and pulmonary parenchyma) has been documented uncommonly in BVDV infections in cattle, BVDV is generally thought to be a respiratory pathogen in cattle.[50] Data from recent studies with a virulent type II BVDV isolate in which gross and histologic lesions of pneumonia were commonly found in experimentally infected cattle are compatible with the idea that at least some isolates of the BVDV quasispecies are respiratory pathogens.[22] In those cattle, there was often immunohistochemical evidence of BVDV antigen found in bronchoalveolar macrophages and in the pulmonary microvasculature and other lymphoid tissues.[22] These findings, together with the common isolation of bacteria in those cases and recent evidence associating BVDV and respiratory (and arthritic) *Mycoplasma bovis* infections in cattle,[26] support the concept that BVDV acts as a respiratory pathogen primarily in the context of local and systemic immunosuppression by virtue of infection of mononuclear phagocytes, including bronchoalveolar macrophages.

Several studies, both published[19, 21] and unpublished in the form of in-house studies conducted at biologics companies, have now substantiated that vaccines containing modified live BVDV type I can confer significant protection from the development of clinical disease and lesions subsequent to infection with emergent virulent BVDV type II. These data from experimental infections are supported by retrospective epidemiologic studies that document the disease-sparing effect of vaccination in protecting cattle from respiratory and other clinical manifestations of infection with emergent BVDV type II strains.[13] Available data suggest that currently available vaccines can protect cattle against pneumotropic BVDV type I strains as well. The possibility that newer vaccines containing both types of BVDV engender improved protective immunity from acute BVDV (compared with monotypic vaccines) has not been tested in direct comparative experiments.

The protective immunologic mechanism conferred by vaccination has not been specifically identified, but it is likely to involve anamnestic systemic antibody responses and perhaps local immune responses that are stimulated by systemic replication of vaccine virus.[19] Such antibody responses are likely to involve CD4+ T lymphocytes in a helper function[32, 53]; however, to date, there is little direct evidence of a role for cell-mediated immunity in conferring protection from BVDV infection or disease.[32] The correlation of higher arrival BVDV-specific antibody with decreased risk of undifferentiated fever in feedlot cattle is recent epide-

miologic evidence that supports the role of systemic antibody in protection from BVDV-associated disease.[10]

Mannheimia haemolytica

The role of virulence factors of *M. haemolytica*, including Lkt and lipopolysaccharide in the pathogenesis of BRDC, is well established and has been reviewed in the *Veterinary Clinics of North America: Food Animal Practice*.[48] Evidence from experimental and epidemiologic studies documents the relationship between protection and high concentrations of passively (maternal) or actively derived antibodies specific for these virulence factors, primarily Lkt and various OMPs of the bacteria.[48] Recent studies have continued to identify specific antigens for inclusion in improved vaccines, including transferrin-binding proteins.[49, 51] Available data continue to support the concept that the optimal immunogen to protect cattle from *M. haemolytica*–associated respiratory disease will contain Lkt and various OMPs.[48]

Pasteurella multocida

Pasteurella multocida is generally considered to be less pathogenic for the respiratory tract than *M. haemolytica* and necessitates more organisms to initiate primary infection.[48] This may be attributable to the number and efficacy of its virulence factors and may explain its association with subacute and chronic bronchopneumonias rather than acute fibrinous pneumonias.[48] The immunogens that stimulate protective immune antibody responses against *P. multocida* are less well studied than those of *M. haemolytica*, but they are thought to comprise a similar array of OMPs, including iron-regulated OMPs.[48] Recent studies[3] with vaccines designed to prevent hemorrhagic septicemia, the systemic form of *P. multocida* infection in cattle caused by Asian strains of the bacteria, have identified transferrin-binding proteins as new vaccine candidates similar to *M. haemolytica*.[49]

Hemophilus somnus

Hemophilus somnus is recognized as a primary cause of bronchopneumonia in cattle that is generally thought to be more subacute to chronic than that associated with *M. haemolytica*.[48] There is an apparent geographic distribution to the prevalence of reported pulmonary (and cardiac) disease in feedlot cattle associated with this pathogen in North America, with an increased recognized prevalence in northern latitudes (Canada). The reason for this is not known but could relate to differences in management practices, including antibiotic usage, as-yet-unidentified differences in geographically distributed strains of *H. somnus*, or simply

recognition of the infection and disease caused by a bacterium that can be difficult to isolate in the laboratory. Nevertheless, this organism possesses a complement of virulence factors, including lipooligosaccharide, with similar properties to *M. haemolytica* lipopolysaccharide; and OMPs, including iron-binding proteins and an Fc receptor–like protein that contribute to virulence and survival of the organism.[48] Previous studies have documented at least partial efficacy of *H. somnus* vaccines and identify OMPs, including iron-binding proteins, as immunogens that stimulate protective antibody responses.[48] As with *M. haemolytica* and *P. multocida,* antibody responses against the liposaccharide are thought to have no disease-sparing effect.[48] There have been no recent published studies that further knowledge in the area of specific immune responses that engender protection against *H. somnus*–associated BRDC.

Mycoplasma bovis

Although there has been continued work on the biology and immunology of *M. mycoides* infection, the cause of contagious pleuropneumonia in cattle, this agent is currently exotic to North America. In contrast, there have been few recent studies concerning the immunology of *M. bovis* infections, which may be an emerging pathogen in feedlot cattle.[26] Currently there are no vaccines containing *M. bovis* that are commercially available in North America. Studies completed and published in the United Kingdom in the 1980s documented the efficacy of combination vaccines containing *M. bovis* in reducing respiratory disease that was associated with *M. bovis* infection alone or together with BRSV.[30] Detailed immunologic studies indicated that protection from *M. bovis* infection is dependent on killing of the pathogen through antibody-mediated cellular cytotoxicity.[28, 29, 31] Purified *M. bovis*–specific immunoglobulin (Ig) G1 and IgG2 promoted killing by macrophages, whereas IgG2 promoted killing by neutrophils. *M. bovis*–specific IgM was ineffective in promoting cellular cytotoxicity by either phagocyte, and incubation of *M. bovis* with specific IgG1, IgG2, or IgM alone had no effect on the viability of the organism. In experimentally infected calves, IgA-containing cells, lymphocytes, and plasma cells were present in the submucosa of tissues from the nasal cavity and trachea, and *M. bovis*–specific IgA was present in tracheobronchial washings and sera. IgA-containing cells, however, were not a prominent infiltrating cell in lesions, and the role of this immunoglobulin isotype in killing of *M. bovis* was not documented. There have been no recent published studies examining the mechanisms of protection or pathogenesis in *M. bovis* infection in cattle; however, some recent studies have focused on a better definition of potential molecular vaccine candidates for *M. bovis*,[54] but the antigen specificity of the bovine immune responses to *M. bovis* and their role in protection and pathogenesis are currently poorly understood.

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