

The host gene expression response to specific BRD pathogens

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Abstract

Bovine respiratory disease complex (BRDC) is an important cause of economic loss to the cattle industry. Despite the development of numerous vaccines and much scientific investigation, the impact of BRDC on cattle producers remains high. Increased pressure to decrease the use of antibiotics in livestock medicine contributes to the urgency to find a better way to decrease the incidence of BRDC. A consortium of investigators funded by the USDA National Institute of Food and Agriculture was assembled to address this issue using the most recent genetic technology. The work presented herein summarizes a series of experiments in which steers were infected with single pathogens of BRDC (bovine respiratory syncytial virus, infectious bovine rhinotracheitis, bovine viral diarrhea virus, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Mycoplasma bovis*) and their bronchial lymph node RNA transcriptomes were then analyzed to determine gene usage in infected vs control animals. Results showed activation of multiple pathways related to innate and acquired immune responses.

Key words: cattle, genetics, genomics, BRD

Résumé

Une maladie respiratoire chez les bovins complexe (BRDC) est une cause importante de pertes économiques pour l'industrie du bétail. Malgré le développement de nombreux vaccins et beaucoup d'investigation scientifique, l'incidence des producteurs sur les bovins BRDC demeure élevé. Une pression accrue pour diminuer l'utilisation d'antibiotiques dans l'élevage de la médecine l'urgence d'contribue à trouver une meilleure manière de diminuer l'incidence de BRDC. Un consortium de chercheurs financés par l'Institut national de l'USDA de l'alimentation et l'Agriculture a été formé pour traiter ce problème à l'aide de la plus récente technologie génétique. Le travail présenté dans ce document résume une série d'expériences dans lesquelles les bouillons étaient

infectés par des agents pathogènes d unique BRDC (virus syncytial respiratoire bovin, la rhinotrachéite infectieuse bovine, le virus de la diarrhée virale bovine, *Mannheimia haemolytica*, *Pasteurella multocida*, et *Mycoplasma bovis*) et leurs ganglions lymphatiques bronchiques RNA transcriptomes ont ensuite été analysées afin de déterminer l'utilisation de gènes chez les infectés vs animaux témoins. Les résultats ont montré l'activation de multiples voies liées aux réponses immunitaires innés et acquis.

Introduction

The bovine respiratory disease complex (BRDC) is a major cause of morbidity and mortality for dairy calves and feedlot cattle. Often referred to as "shipping fever" the BRDC is caused by infection with one of several viruses, followed by the movement of one or more bacterial pathogens from the upper respiratory tract to the lung. Stress associated with weaning, co-mingling, over-crowding, shipping, castration, dehorning, and other environmental factors facilitate the development of the resulting fulminant bronchopneumonia. The viruses involved in BRDC include bovine respiratory syncytial virus (BRSV), bovine herpes type 1 virus, also known as Infectious bovine rhinotracheitis virus, bovine diarrhea virus, and sometimes bovine parainfluenza type 3 virus. The bacterial agents include: *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, and *Histophilus somni*.¹² Each of these pathogens has a unique method of disease causation and interaction with the immune system of the host. Our group has endeavored to investigate these interactions by creating single pathogen infections and studying the RNA sequences that are expressed in lung associated lymph nodes of the infected cattle. The purpose of this work was to determine which host genes are activated in defense of these various pathogens.

Bovine respiratory syncytial virus (BRSV) is a paramyxovirus, a single stranded RNA virus. It is capable of causing severe disease in the absence of stress and secondary bacterial infection, but it is also an important contribu-

tor to BRDC. The immune response to BRSV is often part of the disease process, as the host response and resultant inflammatory infiltrates in the lung causes decreased lung function. The appropriate immune response to a viral infection involves stimulation of T lymphocytes that are able to kill virus infected cells and thus control viral progression. In contrast, a different type of T lymphocyte (a T helper type 2 cell) is often stimulated by BRSV – these cells create an immune environment that facilitates inflammation without the cytotoxic T cell response. Individuals that produce the latter type of response will do more poorly than those that produce the cytotoxic T cell response. The cytokines interferon gamma dominates the T cytotoxic type response whereas the cytokine Interleukin 4 facilitates the inflammatory type (Th2) response.⁶

Infectious bovine rhinotracheitis (IBR) virus is an α -herpes virus, bovine herpes virus type 1, subtype 1 (BHV-1), which causes upper respiratory tract disease. Clinical signs of IBR include cough, fever, conjunctivitis, increased salivary and lacrimal secretions, and often purulent laryngeal lesions. The interaction of this virus with the host induces an immunosuppressive state by interfering with the transport protein for viral peptides that must be coupled to major histocompatibility proteins (MHC class I) for antigen presentation to T lymphocytes that are needed to generate a cellular immune response. Similar to BRSV, BHV-1 causes disease as a single pathogen, but can also facilitate commensal bacterial pathogens to move from the upper respiratory tract into the lung and cause BRDC.⁹

The third virus of importance in BRDC is BVDV. This virus causes abortion, persistent infection, diarrhea and immunosuppression. Infection associated with abortion or persistent infection occurs through the intrauterine route, but infection after birth through the nasal cavity can cause destruction of lymphoid tissue in Peyer's patches in the intestine and severe immunosuppression. The immunosuppression facilitates bacterial infection in the lung.¹

The nasal cavity and pharyngeal mucosa harbors bacteria that are considered commensal, living in harmony with the bovine host until incited to travel to the lower respiratory tract. It is a member of the family Pasteurellaceae, and as such is a gram negative rod. *Mannheimia haemolytica* is an important component of nasopharyngeal bacterial population and can cause enzootic pneumonia in neonatal calves as well as BRDC in feedlot cattle. *M. haemolytica* has several virulence factors that allow it to effectively ward off the bovine immune defenses. One of these is a leukotoxin (LT), which is produced during logarithmic growth. This 104 kD exotoxin is able to insert into the membrane of host target cells causing lysis and cell death.¹⁴ Neutrophils are important in host defense against bacterial pathogens and the *M. haemolytica* LT is able to attack and induce apoptosis in these cells. This virulence factor of *M. haemolytica* contributes to the fibrinous pleuropneumonia that comprises much of the pathology in BRDC.

Pasteurella multocida is also a member of the Pasteu-

rellaceae and is part of the respiratory microbiota. This gram negative coccobacillus causes disease in a variety of species; it is an important component of BRDC. The presence of a polysaccharide capsule is a major virulence factor of *P. multocida*; it allows the bacterium to evade phagocytosis by neutrophils. The lipopolysaccharide and extracellular enzymes are additional virulence factors. Cattle that have antibodies to the outer membrane proteins of *P. multocida* have increased resistance to infection.³

Another member of the Pasteurellaceae that is a pathogen of BRDC, *Histophilus somni*, formerly named *Hemophilus somnus*, is a gram negative pleomorphic rod. In addition to respiratory disease it causes septicemia, thrombotic meningoencephalitis, myocarditis, abortion, and arthritis. Immunoglobulin binding proteins (Ibbs) are important virulence factors. Antibodies to the Ibbs have been shown to be protective.¹⁰ Another virulence factor is antigenic variation of lipopolysaccharide (LOS). LOS facilitates adhesion to the epithelial cells of the upper respiratory tract and colonization. Binding of antibodies to the LOS can depress colonization and facilitate host defense; thus alteration of the LOS and inhibition of antibody binding can enhance the colonization of the respiratory tract with *H. somni*.²

Mycoplasma bovis is a common pathogen of cattle, causing a variety of syndromes including arthritis, mastitis and respiratory disease in calves and feedlot cattle. Infection with *M. bovis* results in caseonecrotic bronchopneumonia. Pathogenic mechanisms of *M. bovis* include variation of membrane surface proteins; variable membrane surface lipoproteins (vsps) are critical for adhesion of *M. bovis* to the host cells. This is the first and critical step for infection of the host with *M. bovis*. The ability to survive in phagocytes is another pathogenic mechanism of *M. bovis*. There are some reports of immunosuppression by *M. bovis*, caused by induction of production of immunosuppressive cytokines including IL-10. Down regulation of lymphocyte proliferation has also been suggested as a cause of systemic dissemination of *M. bovis*.¹¹

Despite the development of multiple vaccines and a considerable body of information on each pathogen compiled by researchers, BRDC remains a huge problem to the cattle industry. In an effort to take a different approach to control of this disease a consortium of several University researchers came together to evaluate genetic components of the response to BRDC. One part of this study involved production of single pathogen infection and analysis of the genes that were differentially expressed in each disease. The molecular basis of the immune response was initially examined using bronchial lymph node tissue obtained from the challenged animals.

Materials and Methods

Single pathogen infection with agents of the BRDC: six to eight month old Angus-Hereford cross steers were obtained from the Sierra Field Station, Brown's Valley CA,

and maintained at the University of California-Davis. Single pathogen infections were performed with BRSV, IBR, BVDV, *Mannheimia hemolytica*, *Pasteurella multocida*, and *Mycoplasma bovis*. All steers were determined to be antibody negative for the viral pathogens that they were infected with. Steers infected with viral pathogens received a nebulized dose of the virus through a face mask; those infected with bacteria received a dose of the bacteria by the intra tracheal route through a foal nasogastric tube inserted into the ventral nasal meatus. Doses and strains of the virus and bacteria are presented elsewhere.⁷ There were 3 control steers for the bacterial infection and 3 for the viral infection – these animals received either media without virus or diluent for the bacteria using a similar route. Six steers were infected with each pathogen and tissues were recovered at necropsy for RNA sequencing. Details of the clinical and pathological results of the infections are presented elsewhere.^{7,15} RNA isolation and sequencing: tissue samples harvested at necropsy were frozen in liquid nitrogen for RNA isolation at the University of Missouri. Details of RNA processing, sequencing, and transcript analysis are presented elsewhere.¹⁵

Results

The data obtained from challenged animals was analyzed with pairwise comparisons between bacterial challenged steers and controls, virus challenged steers and controls, and between bacterial and viral challenged steers. Clear differences were present in each of these comparisons. When comparing the BRSV infected steers with controls, there were over 1,500 genes that were up-regulated and over 1,900 genes that were down-regulated in infection. There were several immune response pathways identified by gene expression in the pathogen challenged animals. There included many genes related to the immune response; the detailed results are presented in detail elsewhere.¹⁵ For IBR there were over 2,000 genes up regulated and down-regulated; some of these were related to B cell signaling and acute phase proteins. Steers infected with BVDV showed over 1500 genes up-regulated and over 1700 genes down-regulated. Pathways involved with cell adhesion molecules, toll-like receptor signaling, T cell receptor signaling, mitogen activated protein kinases (MAPKs) and TGF β signaling were several of the most prominent pathways activated. Pathways identified in the bacterial infections were similar to those for viral infections in that they involved elements of the immune response, particularly innate immunity. *M. haemolytica* infected steers showed enhances expression of genes involved in cell adhesion, chemokine signaling, antigen processing and presentation, Toll-like receptor (TLR) signaling, diapedesis, and TGF β signaling, to name just a few. For *Mycoplasma bovis* and *P. multocida* many of the gene usages were similar to those identified for *M. haemolytica*. Interleukin 1 β , a cytokine important in the innate and acquired immune response, was up-regulated in *M. bovis* infected steers. Pattern recogni-

tion receptor expression is important in the innate immune response; several of these pathways were shown to be activated. There were common pathways of innate immunity that were expressed in more than 1 pathogen infection. These include the pathway TLR and the TGF β signaling pathways.

Discussion

The current state of knowledge of the bovine genome and improved techniques for the identification of gene usage have made it possible to determine differential gene expression in animals experimentally infected with individual pathogens of the BRDC. The work summarized here is presented in detail in 2 publications.^{7,15} There were differences in genes expressed in the bronchial lymph nodes between pathogen infected steers and controls that were common to all pathogen infected steers and differences that were specific to certain pathogens. Most of the commonly up-regulated differentially expressed genes were related to the innate immune response. One of the pathways identified as important in all of the pathogen infections is the response to lipopolysaccharide (LPS). LPS stimulation activates the innate immune response and also stimulates several anti-viral genes.

The innate immune response is important for the initial response to pathogens and for priming of the adaptive immune response. For example, expression of pattern recognition receptors (PRR) on sentinel cells (dendritic cells and macrophages) of the immune response allows the host to recognize the presence of pathogens in the body. The TLR molecules are 1 family of PRR's; they recognize structurally conserved molecules on microbes.¹⁶ Each TLR recognizes a specific ligand. There are 9 TLR molecules with different binding ligands (Table 1) and several more with less well-identified function. TLR4 is important in recognition of gram negative bacteria (such as *M. haemolytica* and *P. multocida*). The bacterial lipopolysaccharide (LPS) from the bacteria binds to a LPS binding protein and then complexes with CD14 on the macrophage surface. This complex then activates TLR4 and the associated kinases, which ultimately activate MAPKs and cause activation of the genes that stimulate development of inflammatory and immune responses.⁵ Our data suggested that TLR 1 and TLR 6 were involved in mediating the immune response to *M. haemolytica*.

TLR molecules have been shown to be very important for the initial host response to viral infection. Viral components recognized by TLR's include nucleic acid and envelope glycoproteins. There are 4 TLR's that recognize viral nucleic acids; these include TLR 3 (double stranded RNA), TLR 7 and 8 (single stranded RNA), and TLR9 (double stranded viral DNA containing unmethylated CpG segments). These 4 TLR's recognize their ligands within the endosomal compartment of the cell, whereas the other TLR's recognize ligands that bind to the cell surface. TLR 2 and 4 can bind to glycoprotein components of the viral envelope.¹⁶ We found that TLR 2 and TLR4 were up-regulated in response to the 3 viral pathogens.

Table 1.

Toll-like receptor	Ligand
TLR1	Bacterial lipoproteins
TLR 2	Lipoteichoic acid, bacterial peptidoglycan, bacterial lipopolysaccharide, zymosan
TLR 3	Double stranded RNA
TLR 4	Lipopolysaccharides, viral glycoproteins, RSV F protein
TLR 5	Flagellin
TLR 6	Bacterial diacyl lipoproteins
TLR 7	ssRNA
TLR 8	Imidizaquinolines, GU containing ssRNA
TLR 9	Non-methylated CpG oligodeoxynucleotides in dsDNA

Transforming growth factor β (TGF β) has been shown to be involved in the regulation of immune responses to many pathogens, both bacterial and viral. It is a negative regulator of the immune response, and often the activation of this molecule is more favorable to pathogen survival than to host defense. For example, TGF β has been shown to dampen CD8 T cell mediated cytotoxicity by inhibiting the expression of perforins (molecules that enable killing of target cells) and interferon γ , which is important for the differentiation of T cells into cytotoxic T cells.¹³ This is important for all 3 of the viral pathogens in our study.

The gene coding for Interleukin 1 β was identified as being up-regulated in at least 2 of the bacterial infections. It is produced by activated macrophages and is an important mediator in the inflammatory response. In 1 study of human pneumonia patients, the bacterial burden in the lung correlated with the amount of IL-1 in the lung lavage fluid.¹⁷ Studies with *M. haemolytica* and IL-1 β have shown that IL-1 β enhances adhesion of bovine neutrophils to the leukotoxin and thus facilitates cell death.⁸

Genes related to both T and B cell signaling were identified as up-regulated for the viral pathogens. These are important processes for development of a cellular and humoral acquired immune response.

The pathways discussed above are just a few of those that were altered by either bacterial or viral infection, or both. The implications of these findings is that upon further analysis of this data target molecules can be identified that might become excellent targets for development of therapeutic drugs or biologics. Inhibitors of certain cytokines have been found to have therapeutic benefit when administered to human patients with diseases that have been shown to be exacerbated by expression of these cytokines. One example is the use of an IL-1 β antagonist to treat rheumatoid arthri-

tis.⁴ With a current national emphasis on the reduction of antibiotic usage implementation of genetic information to identify alternative means of disease control would be highly advantageous.

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