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Research paper

Gene expression profiling of bovine bronchial epithelial cells exposed in vitro to bovine herpesvirus 1 and *Mannheimia haemolytica*

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ABSTRACT

Bovine respiratory disease (BRD) often occurs when active respiratory virus infections (BHV-1, etc.) impair resistance to *Mannheimia haemolytica* infection in the lower respiratory tract. The interactions that occur when the respiratory epithelium encounters these viral and bacterial pathogens are poorly understood. We used Agilent bovine gene microarray chips containing 44,000 transcripts to elucidate bovine bronchial epithelial cell (BBEC) responses following in vitro exposure to BHV-1 alone, *M. haemolytica* alone, or both BHV-1 and *M. haemolytica*. Microarray analysis revealed differential regulation (>2-fold) of 978 transcripts by BHV-1 alone, 2040 transcripts by *M. haemolytica* alone, and 2189 genes by BHV-1 and *M. haemolytica* in combination. *M. haemolytica* treatment produced significantly greater inductions (>10-fold) of several inflammation associated genes, such as CXCL2, IL-6, IL-1α, e-selectin, and IL-8, than to BHV-1 alone. Functional analysis of the microarray data revealed a significant upregulation of genes involved in important biological processes such as inflammation (TNF-α, IL-8, Tlr-2, IL-1, CXCL2, CSF2), vascular functions (VEGF, EDN2) and leukocyte migration (ICAM1, IL-16) during a co-infection with BHV-1 and *M. haemolytica* compared to either pathogen alone. This study provides evidence to support that lung epithelial cells are a source of mediators that may promote inflammatory changes observed during bovine respiratory disease.

1. Introduction

Bovine respiratory disease (BRD) is a multi-factorial disease complex that involves interactions among stressors, management factors, and viral and bacterial pathogens (Car et al., 1991; Hodgson et al., 2005; Ohmann and Babiuk, 1985). The main bacterial pathogen of BRD is *Mannheimia haemolytica*, *which* produces a potent leukotoxin that is its principal virulence factor (Fedorova and Highlander, 1997; Highlander et al., 2000). In its most severe manifestation, infection with *M. haemolytica* can cause fibrinous pleuropneumonia (Car et al., 1991; Hodgson et al., 2005; Loneragan et al., 2001; Ohmann and Babiuk, 1985; Yates, 1982). It is clear that in cattle, as in humans and other mammalian species, active viral infection dramatically increases susceptibility to bacterial pneumonia. This has been demonstrated experimentally in cattle infected with any one of several bovine respiratory viruses such as bovine herpesvirus 1 (BHV-1) and bovine respiratory syncitial virus (BRSV), which renders them highly susceptible to challenge with *M. haemolytica* (Hodgson et al., 2005; Yates, 1982). In marked contrast, far greater numbers of *M. haemolytica* cells are required to cause pneumonia in

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the absence of viral infection, even when the bacterial cells are inoculated into a bronchus (Ohmann and Babiuk, 1985; Yates, 1982). These observations indicate that viral infection impairs host defense mechanisms against *M. haemolytica*, or amplifies undesirable aspects of the host response to this bacterial pathogen.

We have a limited understanding of how respiratory virus infection increases the susceptibility of cattle to bacterial pneumonia with *M. haemolytica*. Earlier in vivo and in vitro studies identified functional defects in bovine leukocytes exposed to BHV-1 or other viruses (Forman et al., 1982; Hinkley et al., 1998; Leite et al., 2002; McGuire and Babiuk, 1984; Noel et al., 1988). However, these relatively modest alterations do not sufficiently explain the increased susceptibility to bacterial pneumonia that is observed in the field.

BHV-1 does not result in a productive infection in bovine leukocytes. However, it infects other cell types including bovine epithelial cells (Babiuk et al., 1996; Thaker et al., 1994). Thus, one might infer that the effects of BHV-1 on resistance to bacterial pneumonia are indirect and may result in part from epithelial cells releasing chemical mediators during viral infection (Babiuk et al., 1996; Brown and Shin, 1990; Leite et al., 2004a,b; Raz et al., 1993). These mediators in turn can alter the activity of bovine neutrophils and mononuclear phagocytes. For example, we demonstrated previously that exposure of leukocytes to BHV-1 virus -induced cytokines, alters leukocyte expression (or activation) of β-integrins in ways that increase their susceptibility to *M. haemolytica* leukotoxin (LKT) and their adhesion to epithelial cells in vitro (Rivera et al., 2009; Leite et al., 2004a,b).

Previous studies have focused on the direct interactions of bovine respiratory pathogens (both viral and bacterial) with bovine leukocytes (Forman et al., 1982; Leite et al., 2004a,b). Less attention has been paid to the interplay among viral and bacterial pathogens and respiratory epithelial cells. Recent reports from our laboratory and others (Gershwin et al., 2005; Hodgson et al., 2005; Rivera et al., 2009; Leite et al., 2004a,b; Raz et al., 1993; Wilson et al., 2005) provide evidence for viral and bacterial pathogen interactions with bovine respiratory epithelial cells. These findings suggest that BHV-1 infection of respiratory epithelial cells results in the release of mediators that attract leukocytes, and with subsequent exposure to *M. haemolytica* LKT, intensify the inflammatory process that characterizes BRD.

In this study we investigated the gene expression response of bovine bronchial epithelial cells to the bovine respiratory pathogens BHV-1 and *M. haemolytica*. We use a bovine gene microarray platform to assess expression of more than 44,000 gene targets by primary bovine bronchial epithelial (BBE) cells exposed in vitro to BHV-1, *M. haemolytica*, or the two agents in combination (Fig. 1). The results of this analysis demonstrate significant changes in gene expression as a result of epithelial cell encounter with BHV-1 and *M. haemolytica*. These observations will inform subsequent efforts to assess how products of these genes alter the response of bovine leukocytes to respiratory pathogens.

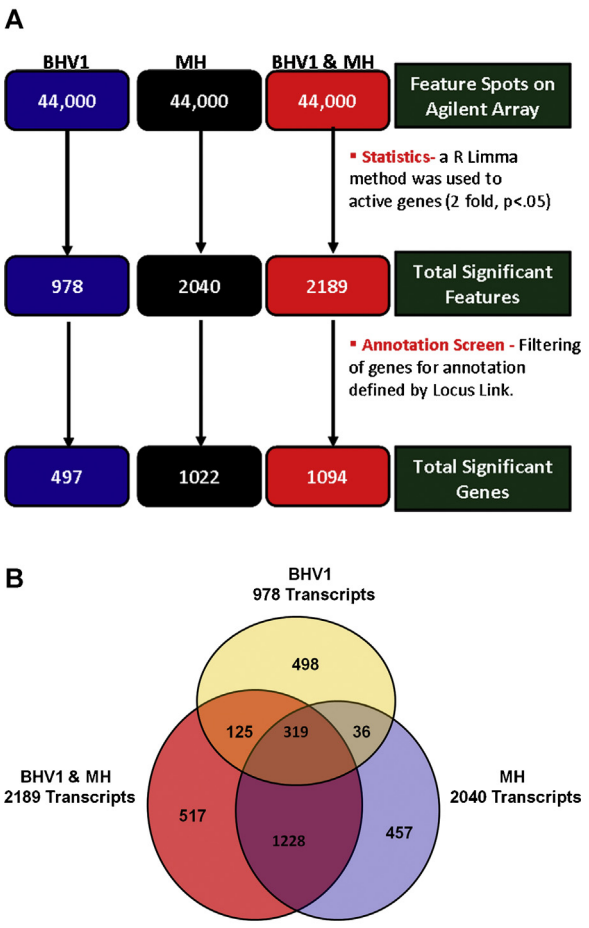

Fig. 1. Overview of differential gene expression by bronchial epithelial cells exposed to BHV-1, *M. haemolytica* (MH) or BHV-1 and *M. haemolytica* in combination (BHV1 + MH). (A) Identification of differentially expressed features at each treatment by annotation screening ($p < 0.05$ and fold change ≥ 2.0). (B) There were 978, 2040, and 2189 differentially expressed transcripts identified for BHV-1, MH, and BHV-1 + MH, respectively. Three hundred and nineteen transcripts were differentially regulated by all treatments ($p < 0.05$, and fold change ≥ 2.0).

2. Methods

2.1. Bovine bronchial epithelial cell culture

Primary bovine bronchial epithelial cells (BBEs), generously provided by Dr. Allen-Gipson (University of Nebraska Medical Center), were maintained in Dulbecco's Modified Eagle's Medium/F12 (DMEM) (Cellgro; Mediatech, Inc., Herndon, VA) containing 10% Fetal Bovine Serum (FBS) (Atlanta Biologicals, Lawrenceville, GA), 2 mM L-glutamine (L-glu) (Mediatech), 200 ng/l epithelial growth factor (EGF) (Sigma, St. Louis, MO), 1% penicillin and streptomycin (Cellgro) at 37 °C and 5% CO_2.

2.2. M. haemolytica

M. haemolytica A1 isolate D153 (a plasmid-negative strain isolated from the lung of a steer that died of pneumonia) was a gift from R. Briggs (Ames, IA). *M. haemolytica*