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Host response to bovine respiratory pathogens

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Abstract

Bovine respiratory disease (BRD) involves complex interactions amongst viral and bacterial pathogens that can lead to intense pulmonary inflammation (fibrinous pleuropneumonia). Viral infection greatly increases the susceptibility of cattle to secondary infection of the lung with bacterial pathogens like *Mannheimia haemolytica* and *Histophilus somni*. The underlying reason for this viral/bacterial synergism, and the manner in which cattle respond to the virulence strategies of the bacterial pathogens, is incompletely understood. Bovine herpesvirus type 1 (BHV-1) infection of bronchial epithelial cells *in vitro* enhances the binding of *M. haemolytica* and triggers release of inflammatory mediators that attract and enhance binding of neutrophils. An exotoxin (leukotoxin) released from *M. haemolytica* further stimulates release of inflammatory mediators and causes leukocyte death. Cattle infected with *H. somni* frequently display vasculitis. Exposure of bovine endothelial cells to *H. somnii* or its lipooligosaccharide (LOS) increases endothelium permeability, and makes the surface of the endothelial cells pro-coagulant. These processes are amplified in the presence of platelets. The above findings demonstrate that bovine respiratory pathogens (BHV-1, *M. haemolytica* and *H. somni*) interact with leukocytes and other cells (epithelial and endothelial cells) leading to the inflammation that characterizes BRD.

Keywords: *Mannheimia haemolytica*, *Histophilus somni*, leukotoxin, viral–bacterial synergism, leukotoxin

Introduction

Resistance to respiratory disease in cattle requires orchestration of host defense mechanisms that protect against viral and bacterial pathogens. There is substantial evidence that active viral infection, with any of a number of bovine respiratory viruses, predisposes cattle to secondary bacterial pneumonia with *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*) and *Histophilus somni* (formerly *Haemophilus somnus*) (Hodgson *et al.*, 2005). The challenge for the animal is to initiate an innate immune response that overcomes the virulence mechanisms utilized by the viral and bacterial pathogens without eliciting extensive inflammation that can compromise lung function and lead to decreased weight gain.

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Results and discussion

Our laboratory has been studying events related to involvement of leukocytes and other cells in the pathogenesis of bovine respiratory disease (BRD). We have established an *in vitro* model for viral–bacterial synergism in which bovine bronchial epithelial cells are infected with bovine herpesvirus type 1 (BHV-1). Viral infection results in increased release of chemokines and cytokines, which in turn attract, activate and increase the adhesion of neutrophils (Rivera *et al.*, 2009). These events lead to inflammation, as seen in BRD. BHV-1 infection also results in greater adherence of *M. haemolytica* to bronchial epithelial cells. *M. haemolytica* adhesion appears to be dependent largely on two outer membrane proteins, OmpA and Lpp1 (Kisiela and Czuprynski, 2009). This finding is interesting in light of previous evidence that a serological response to *M. haemolytica* outer membrane protein correlated with resistance following immunization or natural infection (Ayalew *et al.*, 2004). The net result

of BHV-1 infection of epithelial cells is increased adhesion of *M. haemolytica* under circumstances in which a vigorous inflammatory response is also initiated. Although this scenario could be beneficial in host defense against *M. haemolytica*, it also establishes conditions that predispose to poorly regulated inflammation in the lung. The sum of these events could lead to the intense inflammation that characterizes fibrinous pleuropneumonia, the most acute form of BRD (Hodgson *et al.*, 2005).

Once *M. haemolytica* is established and begins to multiply in the lung, the bacterial cells release a protein exotoxin (leukotoxin or LKT) that has potent effects on bovine leukocytes. Epithelial cells are resistant to the LKT, although they can be affected by the lipopolysaccharide in the outer envelope of *M. haemolytica* (McClenahan and Czuprynski, 2008). Small amounts of LKT can activate Neutrophils (PMNs) and mononuclear phagocytes to release cytokines and reactive oxygen intermediates that might further exacerbate the inflammatory response in the respiratory tract (Czuprynski *et al.*, 1991; Stevens and Czuprynski, 1996). Greater amounts, or sustained exposure to LKT, impairs leukocyte function and leads to leukocyte death. These events, together with those initiated by viral infection, set the stage for a vigorous inflammatory response that might not be effective in eliminating the bacterial cells from the respiratory tract.

Formerly it was thought that the LKT exerted its effects solely at the surface of leukocytes, causing membrane lesions (pores) that led to calcium influx, efflux of macromolecules and cell death (Jeyaseelan *et al.*, 2002). Other evidence demonstrates that LKT binds to the CD18 chain on adhesion molecules (β2 integrins) on the surface of bovine leukocytes (Ambagala *et al.*, 1999; Dassanayake *et al.*, 2007; Shanthalingam and Srikumaran, 2009). This in turn initiates a series of intracellular events that compromise mitochondrial function and lead to release of cytochrome *c*. The latter in turn causes leukocytes to die by a programmed cell death pathway (apoptosis or pyroptosis) (Atapattu and Czuprynski, 2005). Our laboratory has shown that after binding to CD18, the LKT is internalized into bovine leukocytes and is then transported via the cytoskeleton to the mitochondria. The LKT damages the outer mitochondrial membrane, which impairs mitochondrial membrane potential and leads to cell death (Atapattu *et al.*, 2008).

Evidence from other laboratories shows that viral infection can also enhance the severity of *H. somni* infection in cattle (Gershwin *et al.*, 2005). One common characteristic of *H. somni* infection is formation of thrombi in blood vessels (Gogolewski *et al.*, 1987). Our laboratory has shown that *H. somni* adheres to, but does not invade, bovine endothelial cells *in vitro* (Behling-Kelly *et al.*, 2006). This attachment makes the endothelial cell surface procoagulant, which promotes thrombus formation (Behling-Kelly *et al.*, 2007a). *H. somni* also triggers cytoskeletal alterations that increase the permeability of the endothelium (Behling-Kelly *et al.*, 2007b). The effects of *H. somni* on endothelial cells are amplified by its interactions with blood platelets (Kuckleburg *et al.*, 2005). Phosphorylcholine on *H. somni* cells is required for platelet aggregation, a process that can be blocked by inhibitors of the platelet activating factor receptor (Kuckleburg *et al.*, 2007). Activated platelets stimulate endothelial cell expression of adhesion molecules, inflammatory cytokines and tissue factor, and cause some endothelial cells to undergo apoptosis (Kuckleburg *et al.*, 2008).

The ability of bovine leukocytes to ingest and kill *M. haemolytica* and *H. somni* is limited due to properties of these bacterial pathogens (Chiang *et al.*, 1986; Czuprynski *et al.*, 1987; Gomis *et al.*, 1997). We have been investigating further the interaction of bovine neutrophils with *M. haemolytica* and *H. somni*. Confocal microscopy reveals that exposure to *H. somni* leads to redistribution of PECAM−1 on the surface of bronchial endothelial cells, and that neutrophil transmigration across *H. somni*-treated monolayers is reduced by anti-platelet endothelial cell adhesion molecule-1 (PECAM-1) antibodies (Tiwari *et al.*, 2009). Recently, we investigated the role of neutrophil 'nets', extracellular structures composed of DNA and protein extruded from neutrophils (Wartha *et al.*, 2007), in BRD. We find that both the *M. haemolytica* LKT and intact *H. somni* cells stimulate net formation by bovine neutrophils. We are in the process of investigating the antimicrobial activity of these nets for *M. haemolytica* and *H. somni*.

Conclusion

Collectively, the above events result in an inflammatory response to bovine BRD pathogens that is pro-inflammatory. The ability of *M. haemolytica* to circumvent leukocyte antibacterial activity via its LKT, and of *H. somni* to resist leukocyte killing while creating a pro-inflammatory and pro-coagulative environment on the endothelial cell surface, likely contribute to the intense inflammation that characterizes BRD. Efforts to reduce the severity of BRD through vaccination must take these events into account and strive to eliminate the bacterial cells without further exacerbating pulmonary inflammation.

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