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Antimicrobial peptides and surfactant proteins in ruminant respiratory tract disease

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Abstract

In ruminants, respiratory disease is multifactorial and a leading cause of morbidity and mortality. Pulmonary innate immunity is the first line of defense for the respiratory tract. Alteration of regulation, expression, and function of these factors may be important to disease development and resolution. Many antimicrobial peptides and surfactant proteins are constitutively expressed in the respiratory tract and expression levels are regulated. Beta-defensins are cationic peptides with broad antimicrobial activity against bacterial, viral and fungal pathogens. Beta-defensins are primarily expressed in mucosal epithelia (and in some species leukocytes); where they may also participate in chemotaxis, wound repair and adaptive immune responses. Surfactant proteins A and D are secreted pulmonary surfactant proteins that have antimicrobial and immune regulatory activity. Anionic peptide is a constitutively expressed, aspartate-rich peptide that has antimicrobial activity and is most prominent during reparative epithelial hyperplasia. Regulation of these immune defense components by stress, pathogens, and inflammatory cytokines may play a role in the susceptibility to, severity and resolution of respiratory infection. The expression patterns of these molecules can be specific for host-species, class of pathogen and stage of infection. Understanding the regulation of antimicrobial peptide/protein expression will further enhance the potential for novel prophylactic and therapeutic modalities to minimize the impact of respiratory disease.

Keywords

Antimicrobial peptides; Surfactant proteins; Ruminant; Pneumonia

1. Introduction

Respiratory disease is an important cause of ruminant morbidity and mortality leading to significant economic losses (Ames et al., 2002). Bovine respiratory disease accounts for nearly 60% of cattle death in the first few weeks entering the feedlot (Loneragan et al., 2001). It is often multifactorial in origin and can have many participating etiologic agents (Brogden et al., 1998; Cusack et al., 2003; Leite et al., 2004). A common pathway suggested in the development of lower respiratory tract disease is reduced innate immunological function (Ames et al., 2002).

Innate pulmonary defense can roughly be divided into three basic levels: anatomic, cellular and molecular. The upper respiratory tract is anatomically designed to promote particle (bacteria, dust, etc.) deposition along with humidification and warming of inspired air. The

ciliated epithelial cells then move the deposited material on the mucociliary blanket for clearance or macrophages in the terminal airways phagocytize the particles for clearance (Lopez, 2002). At the molecular level, secretions in the lung serve many purposes including antimicrobial activity. This was first recognized in the early 20th century as Dr. Alexander Fleming characterized a component of respiratory secretions he termed “lysozyme” (Flemming, 1922; Flemming and Allison, 1922). Over the past decade, identification and characterization of antimicrobial peptides/proteins (AMP) in the respiratory tract have been increasingly appreciated for their role in homeostasis of pulmonary health. In humans, cystic fibrosis patients are suggested to be at higher risk for pulmonary infection due to inactivation of antimicrobial activity of beta-defensin peptides (Goldman et al., 1997). Furthermore, pulmonary supplementation of surfactant proteins A and D (SP-A and SP-D, respectively) promotes clearance of respiratory syncytial virus and alleviates clinical disease (Hickling et al., 1999; LeVine et al., 1999). An important question is to what extent does AMP expression by respiratory epithelia prevent, reduce severity of, or enhance resolution of respiratory infection? Three general subclasses of respiratory AMP to be discussed and that are studied in ruminants include beta-defensins, surfactant proteins and anionic peptides.

2. Beta-defensins

Defensins are cationic peptides that are widespread in nature ranging from mammals to insects to plants (Raj and Dentino, 2002). Defensins are characterized by six conserved cysteine residues that form intramolecular disulfide bonds. Depending on the pairing of these bonds these peptides are further classified into alpha, beta and theta defensins (Yang et al., 2001). Beta-defensins are typically expressed in the epithelia of mucosal surfaces such as the respiratory, urinary, and gastrointestinal tracts where they are thought to be the first line of defense for pathogen interaction (Schutte and McCray, 2002). In some species such as cattle, certain beta-defensins are also expressed in leukocytes (Selsted et al., 1993).

Beta-defensins are most recognized for their broad antimicrobial functions against bacteria, fungi, protozoa and viruses (Joly et al., 2004; Schroder, 1999; Zaalouk et al., 2004). As cationic peptides, beta-defensins are able to interact with pathogens having a negative surface membrane charge (Peschel et al., 2001). The mechanism for microbicidal activity is believed to be through membrane disruption; however, other mechanisms such as antiviral cell signaling have been suggested for defensins (Chang et al., 2003; Koczulla and Bals, 2003; Lehrer and Ganz, 2002). In addition, beta-defensins may act as a ligand to CC-chemokine receptor-6 (CCR6) to recruit memory T-cells, mast cells, natural killer cells, and TNF-alpha primed neutrophils (Niyonsaba et al., 2002, 2004; Yang et al., 1999). Beta-defensins may also direct the immune response, for instance beta-defensin binding to antigen can enhance antibody response, or a beta-defensin binding with Toll-like receptor-4 on dendritic cells may induce a polarized Th1 response (Biragyn et al., 2001, 2002). Interestingly, beta-defensins may participate in epithelial proliferation and differentiation as seen during wound healing or development (Frye et al., 2001; McDermott et al., 2001; Meyerholz et al., 2004a).

The first well-characterized beta-defensin was tracheal antimicrobial peptide (TAP), a bovine beta-defensin of the respiratory tract (Diamond et al., 1991, 1993). The peptide is expressed in ciliated respiratory epithelium of the trachea and connecting airways. Lingual antimicrobial peptide (LAP) is another bovine beta-defensin found on the tongue and airway epithelium (Russell et al., 1996). The TAP peptide has a broad spectrum of antimicrobial activity against Gram-negative and Gram-positive bacteria along with fungal agents (Diamond et al., 1991). Lung tissue from *M. haemolytica* infected calves show an upregulation of TAP and LAP mRNA expression and the inducibility of TAP was coordinated with inflammatory mediators such as nuclear factor-kappaB (NF-kappaB) and IL-8 (Caverly et al., 2003; Stolzenberg et al., 1997). This is consistent with previous work in which LPS induction of TAP mRNA expression was

suggested to be mediated by transcriptional regulation through NF-kappaB elements (Diamond et al., 2000).

In sheep there are two recognized beta-defensins, sheep beta-defensin-1 and -2 (SBD1 and SBD2, respectively). Both appear to be developmentally regulated with SBD1 having constitutive expression in pulmonary tissues while SBD2 pulmonary expression is sporadic and weak, but is consistently expressed in the gastrointestinal tract (Huttner et al., 1998; Meyerholz et al., 2004a). During parainfluenza virus-3 (PI-3) infection of neonatal lambs, pulmonary SBD1 mRNA expression increased through the course of infection (3, 6 and 17 days post-inoculation) (Grubor et al., 2004a). However, acute *M. haemolytica* infection in 8-month old lambs caused a dose-dependent decrease in SBD1 mRNA and increase in IL-8 mRNA expression (Ackermann et al., 2004). These results suggest differential regulation of beta-defensin expression dependent on host species and class of pathogen.

3. Surfactant proteins A and D

In the lung, surfactant is composed primarily of phospholipids, cholesterol and surfactant proteins (Phelps, 2001). Two of these proteins, surfactant proteins A and D (SP-A and SP-D, respectively) are hydrophilic calcium-dependent lectins called collectins (LeVine and Whitsett, 2001). These proteins are secreted by type II pneumonocytes and to a lesser degree by other respiratory epithelia such as Clara cells and tracheobronchial glands. SP-A and SP-D mRNA can also be found in tissues with mucosal surfaces such as the urinary and gastrointestinal tracts (Hansen and Holmskov, 2002; Lin et al., 2001). SP-A and SP-D have a broad range of antimicrobial activity against bacteria, fungi and viruses (LeVine and Whitsett, 2001; McCormack and Whitsett, 2002). The mechanism of antimicrobial activity is suggested to be through the formation of aggregates with the pathogen to inhibit cellular invasion, enhance phagocytosis and alter inflammation such as production of reactive oxygen species or cytokines expression (McCormack and Whitsett, 2002).

SP-A and SP-D are increasingly recognized for their antimicrobial and immunomodulating roles in humans and other species. The studies done thus far in ruminants are beginning to highlight their regulation and role(s) during disease. In neonatal lambs with PI-3 infection, SP-A and SP-D mRNA expression was increased (along with SBD1) during the course of infection and expression correlated with development of inflammation and clearance of the virus (Grubor et al., 2004a). In a similar study of neonatal lambs infected with PI-3 with concurrent adenoviral-mediated gene therapy, SP-A and D expression proportionally increased with the degree of PI-3/adenoviral inflammation (Meyerholz et al., 2004b). Interestingly, the increased SP-D mRNA expression paralleled pulmonary neutrophil infiltration which is consistent with previous reports that LPS-recruited pulmonary neutrophils cleared SP-D protein and increased SP-D mRNA expression (Meyerholz et al., 2004b; Herbein and Wright, 2001). Conversely, *M. haemolytica* infection in 3-month old lambs caused minimal to reduced SP-D mRNA expression and SP-D protein antigen detected by immunohistochemistry was inversely correlated with inflammation and reparative epithelial hyperplasia (Grubor et al., 2004a, 2004b). This suggests pathogen-elicited differential regulation of SP-D expression with viral infection increasing expression, but Gram-negative infection decreasing expression.

4. Anionic peptide

Anionic peptide (AP) belongs to a class of antimicrobial peptides that are unusual in that they have aspartate rich regions, require zinc as a cofactor and are present in pulmonary secretions of humans and ruminants (Brogden et al., 1996, 1997, 1999). These peptides are expressed constitutively and mediate antimicrobial activity against Gram-negative and Gram-positive bacteria (Fales-Williams et al., 2002b; Brogden et al., 1996, 1997).

Ruminant anionic peptides have been studied in relation to respiratory disease and its role during infection is apparently different from other AMPs. During acute *M. haemolytica* infection of calves, anionic peptide expression was not increased suggesting constitutive expression (Fales-Williams et al., 2002a). A similar study in sheep suggested anionic peptide was increased only during chronic *M. haemolytica* pneumonia the source of the anionic peptide was reparative hyperplastic epithelium (Fales-Williams et al., 2002b). Efforts to assess activity against *M. haemolytica* bacteria showed within minutes of inoculation anionic peptide bound to the bacteria causing degenerative ultrastructural changes suggesting bacteriocidal effects (Heidari et al., 2002). This evidence suggests that anionic peptide is a constitutively expressed molecule that is effective against *M. haemolytica* and may be important in resolution of infection near epithelial surfaces undergoing reparative hyperplasia.

5. Antimicrobial peptide/protein regulation

Innate antimicrobial molecules have the potential to be the next generation of antimicrobials used in prophylactic and therapeutic settings (Koczulla and Bals, 2003). Understanding AMP regulation is only the first step as additional hurdles to overcome include making these molecules effective for delivery and treatment. Exogenous AMP administration for respiratory tract disease is challenged by the need to identify proper recombinant AMP synthesis methods, adequate delivery systems and to avoid potential toxicity to the host. Another potential mechanism is that of gene therapy for enhanced AMP expression at sites of infection. Unfortunately, viral vectors, which are more efficient than direct methods at gene transfer, retain the potential to elicit additional inflammation or cause disease. For example, recent work has shown enhanced clinical disease and lesions with adenoviral delivery of a beta-defensin gene during PI-3 infection of neonatal lambs (Meyerholz et al., 2004b). A more practical option may be that of enhancing host AMP expression by dietary supplementation of a putative AMP regulator during times of susceptibility or clinical disease to elevate the status of host resistance. By enhancing AMP expression, the susceptibility to disease would diminish and synergistic antimicrobial activity amongst multiple AMPs may minimize the development of pathogen resistance to therapy. Evaluation of exogenous regulators of AMP expression will help future efforts to manipulate these regulatory pathways for optimum health status. Vascular endothelial growth factor, retinoic acid derivatives, glucocorticoids, nicotine and alcohol are some of the early regulators being assessed in human and animal models (Gallup et al., 2002; Guidot and Brown, 2000; Raoul et al., 2004; Tan et al., 1999; Wuenschell et al., 1998). Even cytokines participate in regulation as TNF-alpha suppresses SP-A expression by p38 mitogen-activated protein kinase (Miakotina and Snyder, 2002). Additional research is needed to elucidate pathways by which to enhance respiratory defense.

6. Summary

The significance of beta-defensins, anionic peptide, SP-A and SP-D in ruminant respiratory host defense is increasingly appreciated. The expression of these AMPs appears to be complex with altered regulation by host species and pathogen. As these protective molecules are further characterized, defining the positive and negative regulation of AMP can help identify causes of reduced pulmonary immunity and augment future pharmaceutical development. Novel compounds that enhance AMP expression may in the future reduce agricultural antibiotic use and provide a more esthetic and economical consumer product.

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Abbreviations

AMP, antimicrobial peptides/proteins; AP, anionic peptide; PI-3, parainfluenza virus-3; SBD1, sheep beta-defensin-1; SBD2, sheep beta-defensin-2; SP-A, surfactant protein A; SP-D, surfactant protein D; TAP, tracheal antimicrobial peptide; LAP, lingual antimicrobial peptide.

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