Cell Host & Microbe
Review

The Interaction between Respiratory Pathogens and Mucus

Mark Zanin,[1,*] Pradyumna Baviskar,[1] Robert Webster,[1] and Richard Webby[1]
[1]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: mark.zanin@stjude.org
http://dx.doi.org/10.1016/j.chom.2016.01.001

The interaction between respiratory pathogens and their hosts is complex and incompletely understood. This is particularly true when pathogens encounter the mucus layer covering the respiratory tract. The mucus layer provides an essential first host barrier to inhaled pathogens that can prevent pathogen invasion and subsequent infection. Respiratory mucus has numerous functions and interactions, both with the host and with pathogens. This review summarizes the current understanding of respiratory mucus and its interactions with the respiratory pathogens *Pseudomonas aeruginosa*, respiratory syncytial virus and influenza viruses, with particular focus on influenza virus transmissibility and host-range specificity. Based on current findings we propose that respiratory mucus represents an understudied host-restriction factor for influenza virus.

Introduction

The classical roles of respiratory mucus are to maintain the hydration of the respiratory tract and to act as a protective barrier against the external environment by trapping particulate matter, including pathogens. Trapped matter can then be expelled from the airways by mucociliary clearance, the rhythmic beating of cilia bundles on the airway epithelium. It is now clear that this classical model is not complete and that mucus is a complicated, multicomponent secretion with numerous functions. The functions of respiratory mucus now include immune response regulation, the presentation of molecules that are inhibitory to pathogens, the regulation of cell differentiation and proliferation, and the maintenance of the barrier function of the epithelium.

Respiratory tract mucus is the first interaction that inhaled agents have with a potential new host. Accordingly, this mucus layer can determine the infectivity, and potentially the transmissibility, of respiratory pathogens including influenza viruses, respiratory syncytial virus (RSV) and rhinoviruses as well as pathogenic lung colonization by the opportunistic bacterial pathogen *Pseudomonas aeruginosa* (PA), particularly in patients with cystic fibrosis (CF). Here, we review the current understanding of respiratory tract mucus and its interactions with respiratory pathogens. We pay particular attention to studies investigating the interactions between influenza viruses and mucus and their importance in understanding influenza virus pathogenesis and host-range restriction.

Respiratory Tract Mucus

The epithelium of the respiratory tract is coated with mucus, a multicomponent secretion that has the properties of a viscous fluid or a soft, elastic solid depending on shear stress (reviewed in Lai et al., 2009). This secretion exists in two layers: a more viscous mucus gel layer on top of a periciliary liquid layer (PCL), where the cilia lie. The PCL is generally as deep as the cilia bundles are high, and the reduced viscosity compared to the top layer facilitates the beating of cilia bundles. While the PCL layer is less viscous than the gel layer above, diffusion of molecules into the PCL layer is impeded by membrane-spanning mucins and mucopolysaccharides associated with the cilia and the epithelium. These molecules form a type of molecular "brush" that helps maintain the boundary between the mucus and PCL layers. This is the "gel-on-brush" model of the mucus barrier (Button et al., 2012) (Figure 1A).

Mucins comprise a significant portion of airway mucus and contribute to the barrier function of mucus. Mucins are among the largest macromolecules encoded in the mammalian genome, being 200 kDa to 200 MDa in size. The majority of this weight, approximately 80%, is comprised of carbohydrate chains. Due to their size, the initial polymerization of mucins to form homodimers, which occurs after their translation, can expose mucin-producing cells to endoplasmic reticulum and Golgi stress (Martino et al., 2013). The size of mucins after their secretion exceeds the average size of the secretory vesicles in which they are packaged. This necessitates that mucins be packaged in a condensed and dehydrated state, which is thought to be mediated by calcium-dependent crosslinking within the molecule and by charge shielding the sialic acids and sulfate groups with calcium ions (Ridley et al., 2014; Verdugo et al., 1987a, 1987b). The release of mucins from secretory granules follows a rise in pH and a fall in calcium ion concentration, which mediates mucin unfolding and rapid hydration and leads to expansion and the formation of higher-order oligomers (Ambort et al., 2012b; Ridley et al., 2014). Mucins must adsorb in excess of 1,000 times their mass in water to achieve a viscoelasticity that facilitates mucociliary clearance (Button et al., 2012).

Mucins share a general multidomain structure (Figures 1B and 1C). The centers of these molecules are rich in serine and threonine residues interspersed with proline residues, termed PTS sequences. Mucins contain greater than 100 PTS sequences, which are important, as glycan chains are anchored onto the serine and threonine residues (Ambort et al., 2012a). This region is flanked by domains with sequence homology to von Willebrand domains, similar to those found on von Willebrand factors in the blood, which facilitate clotting in the event of vascular injury. These domains are involved in polymerization via disulfide linkages between mucin molecules (reviewed in Thornton et al., 2008).

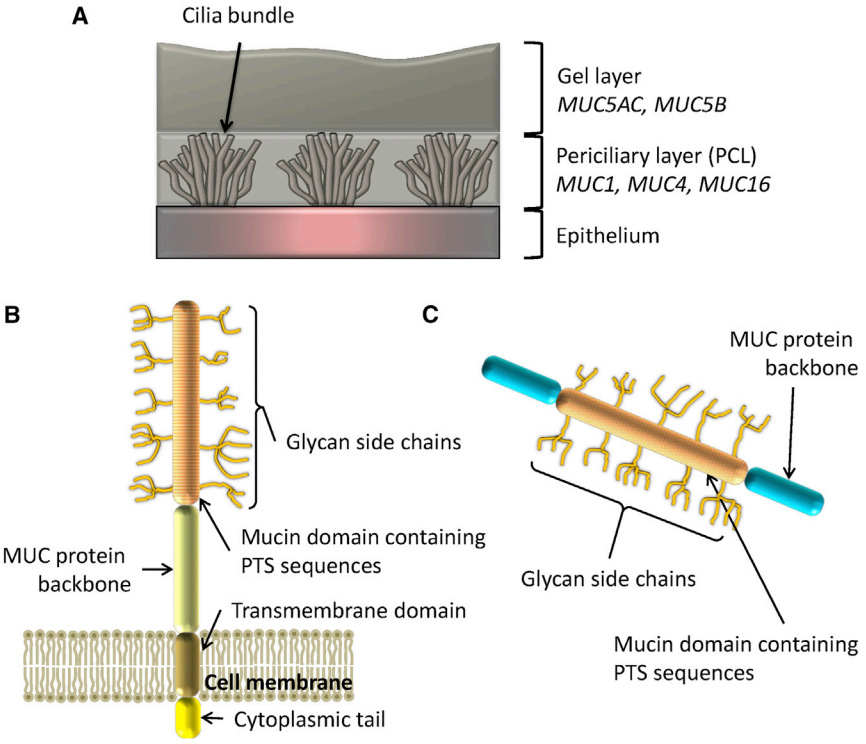

Figure 1. The "Gel-on-Brush" Model of Respiratory Mucus and the Structure of Mucins

(A) The gel-on-brush model of respiratory mucus describes mucus existing in two discreet layers, a more viscous gel layer on top and a periciliary layer (PCL) below. The gel layer contains the secreted mucins MUC5AC and MUC5B, while the PCL contains the membrane-tethered mucins MUC1, MUC4, and MUC16. The reduced viscosity of the PCL in comparison to the gel layer facilitates the beating of cilia and mucociliary clearance.

(B) Generic structure of a membrane-bound mucin.

(C) Generic structure of a secreted mucin.

Mucins are heavily glycosylated, such that there are 25–30 carbohydrate chains per 100 amino acids comprising 80% of the dry weight of the molecule, and this is important for their function (Brockhausen et al., 2009; Stanley et al., 2009). Mucins contain complex glycan chains consisting mainly of *O*-glycans to which *N*-acetylgalactosamine (GalNAc) is added, followed by the additional glycan moieties galactose, *N*-acetylglucosamine (GlcNAc), fucose, and sialic acids (Brockhausen and Schachter, 1997). The complexity and diversity of mucins offers a high degree of resistance to microbial proteases, as a number of diverse enzymes including proteases, glycosidases, and sialidases are needed to totally degrade the various bonds present in mucins (Corfield et al., 1992; Hoskins and Boulding, 1981). Most mucins have a high sialic acid content which, along with the high sulfate content, results in a strongly negative surface charge, increasing the rigidity of the polymer via charge repulsion (Shogren et al., 1989). As such, the sialic acid content of mucins is important, as it is thought to be an important determinant of the viscosity and elasticity of mucus (Puchelle et al., 1973).

There are at least 15 mucins in the human lung; MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC7, MUC13, MUC15, MUC16, MUC18, MUC19, MUC20, MUC21, and MUC22 (reviewed in Lillehoj et al., 2013). The major airway mucins are MUC1, MUC4, MUC5AC, MUC5B, and MUC16. MUC1, MUC4, and MUC16 are membrane-tethered mucins, while MUC5AC and MUC5B are the major secreted mucins, secreted by goblet cells and the submucosal glands, respectively (Hovenberg et al., 1996; Wickström et al., 1998). Secreted mucins are evolutionarily old, as they can be traced back to early metazoan evolution, while tethered mucins are much younger, appearing in vertebrates (Lang et al., 2007). This history could reflect the functionality of tethered mucins being specific for vertebrates.

Experiments with knockout mice have demonstrated that Muc5b is particularly important for normal airway function, while Muc5ac is beneficial but not essential. Overexpression of *Muc5ac* in mice was protective against influenza virus infection, as discussed later, and *Muc5ac*$^{-/-}$ mice were viable and were capable of mucociliary clearance (Ehre et al., 2012; Roy et al., 2014) (Table 1). In contrast, *Muc5b*$^{-/-}$ mice showed a number of severe deficiencies in airway function and succumbed to bacterial infections (Roy et al., 2014). Mucociliary clearance was severely reduced in *Muc5b*$^{-/-}$ mice, despite the presence of functional ciliated cells in the airway, such that breathing was impaired due to obstruction of the upper airways. Inflammatory infiltrates and bacteria were also present in the lower airways of *Muc5b*$^{-/-}$ mice, particularly *Staphylococcus aureus*, which was an important cause of mortality in *Muc5b*$^{-/-}$ mice (Roy et al., 2014). There were also aberrations in the immune response in the lungs of *Muc5b*$^{-/-}$ mice, which were particularly evident by the accumulation of macrophages with impaired phagocytic functions. IL-23, a mediator of antimicrobial inflammatory responses, was also dramatically decreased in *Muc5b*$^{-/-}$ mice (Roy et al., 2014) (Table 1). Therefore, there appears to be a link between mucins and the innate immune response, two important aspects of the "first line of defense" against pathogens.

In addition to secreted mucins there are also membrane-tethered mucins that exist in the PCL, these being MUC1, MUC4, MUC16, and MUC20. Tethered mucins play a number of roles, including the activation of intracellular signal transduction pathways, regulation of the immune response, and cell differentiation and proliferation (reviewed in Lillehoj et al., 2013). MUC1 appears to be more strongly associated with microvilli, while MUC4 is more strongly associated with cilia (Sheehan et al., 2006). A PA challenge model using *Muc1*$^{-/-}$ mice revealed a role for MUC1 in regulating the inflammatory response. Postchallenge, these mice showed greater numbers of macrophages and greater amounts of the cytokines tumor necrosis factor alpha (TNFα) and keratinocyte chemoattractant (KC) in bronchoalveolar