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Possible role of amino acids, peptides, and sugar transporter in protein removal and innate lung defense

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Abstract

This review presents the hypothesis that removal of polypeptides and glycoproteins from the alveolar space and airways is mediated in part by enzymatic degradation, followed by transporter mediated transepithelial transport of amino acids, peptides and sugar residues. Furthermore, the activity of these transporters ensures low availability of nutrients, and decrease bacterial growth. Thus, airway epithelial transporters for sugar, amino acids, peptides and other nutrients can contribute to the innate lung defense.

Keywords: Amino acid; Peptide; Innate lung defense

1. Introduction

Throughout the lung, proteins are secreted into the airway lumen and alveolar space and are involved in multiple lung functions. In the alveoli, the dominant proteins are surfactants, secreted by type II pneumocytes, which serve to regulate alveolar surface tension. Additionally, albumin and other plasma proteins are filtered into the alveolar space from the alveolar capillaries (Trapnell and Whitsett, 2002; Folkesson et al., 1996). In the airway, multiple proteins involved in the innate lung defense such as mucins, lysozyme, and defensins are secreted by specialized cells and glands (Whitsett, 2002).

While the luminal proteins are essential for lung function, excess protein will obstruct the airways (Rose et al., 2001) and hinder gas exchange in the alveoli (Carraway et al., 2001; Shah et al., 2000). Thus, to eliminate protein accumulation, the rate of protein secretion should be equal to the rate of protein removal. We will consider two classes of protein clearance; the axial transport mediated by the mucociliary escalator that transports proteins from the lower to the higher airway, and transverse transport, in which proteins or their degradation products will cross the epithelium. The relative contribution of each of these processes to luminal protein clearance is not known, but as will be discussed below, the architecture of the lung required that most of the luminal protein transverse the epithelium, or at least the apical membrane (Kilburn, 1968).

The branching anatomy of the airway leads to a large decrease in the aggregate surface area of the airway (or perimeter, when normalized to a unit of length) when moving from the distal airways to the bronchi and trachea. The surface area decreases from ~ 2 m^2 at 20th to 25th generation, down to only ~ 50 cm^2 at the third generation (Kilburn, 1968; Knowles and Boucher, 2002), a ~ 400-fold decrease in airway surface area. Since proteins are constantly moving up along the airways while the surface area and the airway surface liquid (ASL) volume decreases (Kilburn, 1968; Knowles and Boucher, 2002), we would expect that moving from the 25th generation to 3rd generation, the protein concentration will increase ~ 400 fold and will obstruct the upper airways. Moreover, protein secretion along the airway would further increase the total protein concentration at the upper airways. Yet, protein concentration does not change markedly along the airways. It is therefore required that most of the luminal proteins be absorbed by, or traverse the epithelia (Sloan et al., 2003).

To better estimate the extent of protein absorption by the airway epithelia, an accurate measurement of the depth,

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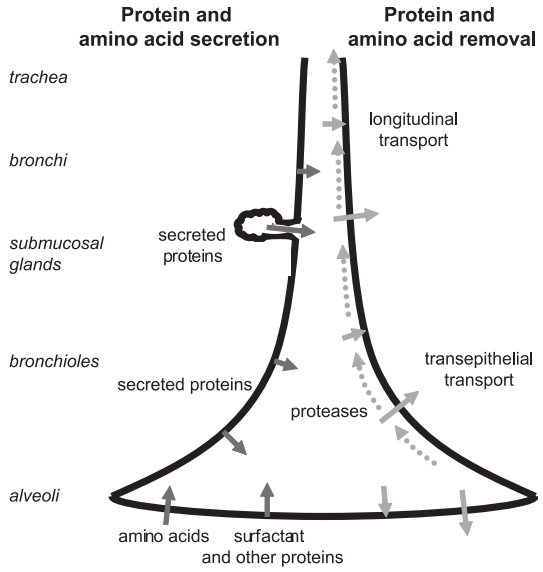

Fig. 1. Model of proteins and amino acids removal in the lung. Throughout the lung, proteins are secreted from epithelial cells and submucosal glands. In the airway, proteins are transported cephalad by the mucociliary escalator. However, moving from alveoli to the trachea, the airway surface area decreases exponentially. To prevent accumulation of proteins and obstruction of the higher airways, these proteins must be removed. We propose that most of the luminal proteins must transverse the epithelium or be absorbed by epithelial cells and recycled for novel protein synthesis.

protein content and longitudinal transport rate of the airway protein is required. Nevertheless, from the discussion above, it is quite clear that most of the airway luminal proteins are absorbed by the epithelia, and are not transported all the way up. In the alveoli, there is no significant longitudinal protein transport, and therefore, in steady state, most proteins secreted or filtered into the alveolar space are probably reabsorbed (Folkesson et al., 1996) (see Fig. 1).

2. Mechanisms of protein transport across the epithelium

While the discussion above strongly suggests that most luminal proteins must be absorbed or transverse the epithelia, little is known about the extent of luminal protein removal, the mechanism by which it occurs, and the physiological significance of this process. Several possible mechanisms can be considered: (1) Paracellular diffusion or transport (2) Transcytosis (3) Endocytosis of the proteins at the apical membrane, followed by enzymatic degradation (4) Enzymatic degradation of the proteins in the lumen followed by absorption of small peptides, amino acids, and other residues such as sugars and by specialized transporters (Fig. 2).

The paracellular junction of epithelial cells is a complex structure, and possess selectivity for small molecules and ions based on size, charge and molecular structure (Anderson, 2001). Moreover, larger molecules were shown to cross the junctions in rat alveolar monolayer (Matsukawa et al., 1997). Several reports show that in alveolar epithelium in culture, small proteins and polypeptides such as granulocyte-macrophage colony stimulating factor and cytochrome *C* are removed from the alveolar apical side via the paracellular pathway (Crandall and Matthay, 2001; Dodoo et al., 2000; Folkesson et al., 1996; Kim et al., 2001a,b).

Large proteins are excluded from the paracellular pathway but can traverse the cell by transcytosis (Matsukawa et al., 2000). In rat alveolar epithelial cell monolayers, apically applied horseradish peroxidase was transported across the epithelium by a nonspecific endocytosis (Matsukawa et al., 1996). In airway epithelial cells in culture, apically applied albumin was transported to the basolateral side by nonspecific protein transcytosis (Deffebach et al., 1996; Johnson et al., 1989). Therefore, the epithelium might be able to recycle specific proteins that enter the alveolar space back into the bloodstream.

In alveolar preparations, several studies that utilized exogenously applied labeled proteins showed that albumin, transferrin and immunoglobulin G are cleared by specific processes, possibly receptor mediated endocytosis (Crandall and Matthay, 2001; Hastings et al., 1994; John et al., 2001; Kim et al., 2001a,b). Some of the endocytosed proteins might be degraded inside cells. In alveoli, the surfactant proteins are endocytosed in part by alveolar type II cells (Trapnell and Whitsett, 2002).

3. The role of transporters in protein removal from the airways

An additional possible mechanism of protein removal is enzymatic degradation followed by transport of the degradation products (Sloan et al., 2003). To better understand the contribution of this pathway to protein removal, it is important to (1) determine the identity, location and amount of the degraded proteins, (2) to identify the specific enzymes involved in degrading proteins and (3) to identify the transporters involved in the removal of the degradation products.

The extent of protein degradation in airway and alveolar lumen is not known, but several indications suggest the importance of this process. First, several studies reported the expression of multiple proteases and peptidases in the lung (Caughey, 1997; Kido et al., 1992; Kim et al., 2001a,b; Takahashi et al., 2001; van der Velden and Hulsmann, 1999; Yamaoka et al., 1998). The functional role of these proteases is not known, but some of them might be involved in protein degradation. Second, a high concentration of free amino acids has been detected in the airway lumen (Thomas et al., 2000; Barth and Pitt, 1996) and it is likely that the source for these amino acids is protein degradation. Third, transporters for amino acids, peptides, and sugars have been identified at the luminal membrane of airway and alveolar epithelial cells. These transporters might be involved in removing proteins degradation products from airway lumen (Sloan et al., 2003;

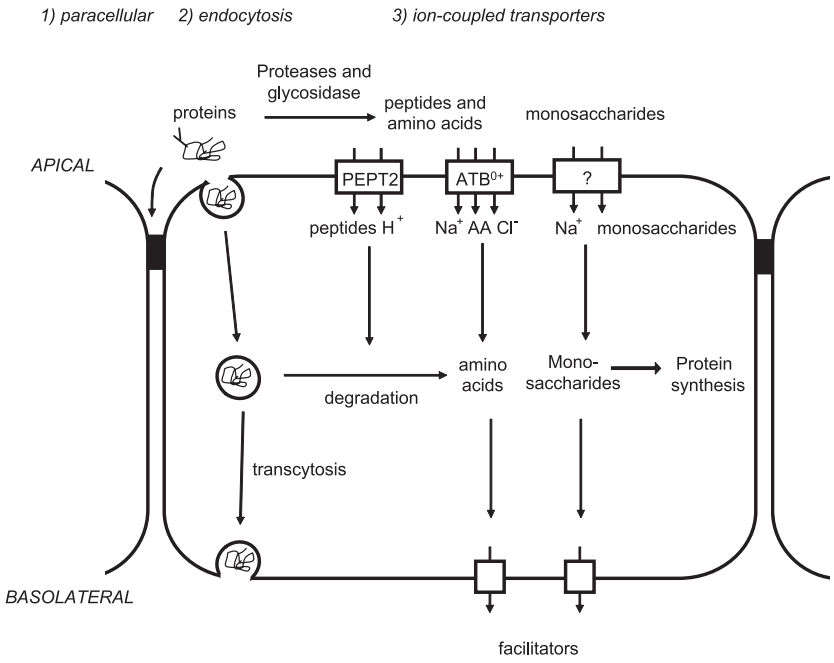

Fig. 2. Mechanisms for protein removal from the airway lumen. Proteins can cross the epithelium via the paracellular pathway or by transcytosis. Alternatively, proteins might be degraded by proteases, the amino acids peptides are then removed by specific transporters into the epithelial cells. Similarly, endocytosed proteins might be degraded inside the cell. The cytoplasmic amino acids can then be used for synthesis of novel proteins or transported through the basolateral membrane and into the blood. Likewise, sugar transporters might remove the degradation products of the saccharide moiety of glycoproteins.

Groneberg et al., 2002). By mRNA and immunocytochemical analysis, the broad specificity neutral and cationic amino acid transporter ATB^{0+} is highly expressed in human distal lung and trachea (Sloan and Mager, 1999; Sloan et al., 2003). Immunohistochemical studies in mouse showed that ATB^{0+} protein is predominantly localized to the apical membrane of ciliated epithelial cells lining the airways and in alveolar type I cells (Sloan et al., 2003). Measurements of amino acid induced short circuit current in mouse trachea (Sloan et al., 2003), cultured human airway epithelia (Galietta et al., 1998), and cultured rat alveolar cells (Jiang et al., 2000) are in agreement with the immunolocalization. Additionally, the H^+ coupled peptide transporter PEPT2 is expressed in alveolar type II pneumocytes and the bronchial epithelium where it is localized to the apical membrane of the epithelial cells (Groneberg et al., 2002, 2001). Functional measurements with a fluorophore-conjugated dipeptide showed uptake from the lumen into the epithelium. The pharmacological properties of this transport suggest that it is mediated by PEPT2. Therefore, ATB^{0+} and PEPT2 are likely to play a predominant role in protein removal following degradation into amino acids and peptides.

In addition to the clearance of amino acids and peptides, transporters for mono- and oligo-saccharides can mediate the removal of sugar moieties of degraded glycoproteins. Glucose-induced, Na^+-dependent short circuit current was reported in mouse and horse trachea preparations (Sloan et al., 2003; Joris and Quinton, 1989). These studies suggest expression of a member of the Na^+-dependent sugar transporter family, SGLT (Wright et al., 1994), but the molecular identity of the lung transporter is not known. Similarly, lipid transporters might play a role in luminal clearance. Surfactant removal is mediated in part by endocytosis into alveolar macrophages and type II pneumocytes (Trapnell and Whitsett, 2002). Future studies will show whether lipid transporters are also involved in surfactant removal. Interestingly, mutations in the amino acid transporter y^+LAT1 result in lysinuric protein intolerance; a human disease characterized multiple symptoms that include alveolar proteinosis (Palacin et al., 2001). The distribution of y^+LAT1 in the lung has not been studied but this phenotype suggests that amino acid transporters play important roles in protein removal from the alveolar space.

4. Amino acids transport mediated by ATB^{0+}

Analysis of the transporters kinetic can provide further insights into protein and amino acids clearance. Kinetic measurements of human ATB^{0+} expressed in *Xenopus* oocytes suggest a stoichiometry of 2–3 Na^+, one Cl^- and one amino acid. This stoichiometry predicts 1–2 unitary charges for neutral amino acids, and 2–3 charges for cationic amino acids. For the calculation below, we will assume two charges per amino acid. Functional measurements from tracheal preparations allow us to estimate the extent of amino acid removal. In mouse tracheas mounted in Ussing chamber, super maximal concentration of amino acids induced a short circuit current of ~ 2.5 μA/cm^2

(Sloan et al., 2003). Thus the transporter can remove 7.5 nmol amino acids per cm^2 per minute. Taking an ASL depth of 20 μm, and amino acid concentration of 20 mM (Thomas et al., 2000; Barth and Pitt, 1996), the amino acid clearance time would be 5 min. In the absence of continues supply, this clearance rate should be sufficient to maintain low luminal amino acid concentration. Thus, the presence of a substantial amino acid concentration, despite an effective removal system suggests a renewable amino acids source. A likely source of these amino acids is protein degradation. Moreover, using the equilibrium equation for ion coupled transporters (Lester et al., 1994), and acceptable values for Na^+, Cl^-, apical membrane potential, and assuming epithelial cytoplasmic amino acid concentration of 10 mM, the luminal equilibrium concentration of amino acids would be below the μM range. These values are much lower then the measured amino acid concentration, and again suggest that the system is not in equilibrium, and there is a continuous supply of amino acids in the airway lumen.

5. Transporters and the innate lung defense

Removal of amino acids might not only be important for protein recycling, but may also contribute to the innate lung defense. The airway is exposed to continuous assault by airborne pathogens. The first line of defense is mucociliary clearance, but clearance of a single particle may take several hours (Knowles and Boucher, 2002). Under optimal growth conditions, the bacterial number can increase substantially during this period. Nevertheless, antimicrobial factors such us lactoferrin, lysozyme, secretory leukoproteinase inhibitors, and defensins in the airway may suppress bacterial growth (Ganz, 2002; Cole et al., 1999). The bacterial growth rate and clearance time are quite important since residual bacteria might acquire resistance to these defense mechanisms (Cole et al., 1999) and thus colonize the airway. Nutrient availability might be an important parameter in determining bacterial growth. While the airway contains a large amount of nutrients in the form of polypeptides and glycoproteins, these macromolecules are not readily available for the bacteria. As discussed previously, multiple proteases reside in the airway lumen and others might be secreted by the bacteria. Enzymes involved in degradation of the post-translation modification moieties of proteins such as polysaccharides might also be present. We hypothesize that ion coupled transporters for amino acids, peptides and sugar residues keep the availability of nutrients low, and by that mechanism, inhibit bacterial growth (Sloan et al., 2003).

Thus, the removal of nutrients from the ASL and alveolar space may be critical for maintenance of a low nutrient environment and consequently prevention of bacterial growth. We speculate that there may be an interaction between pathogenic bacteria and luminal epithelial transporters. First, in response to bacterial invasion the host will increase the activity of the luminal transporters in order to maintain a lower nutrient environment and decrease bacterial growth. Second, pathogenic bacteria might utilize specific mechanisms to decrease the host transporter activity in order to increase nutrient availability and facilitate their growth. In support of this hypothesis, preliminary studies showed significant increase in ATB^{0+} mRNA in cultured human airway epithelium treated with supernatant of *Pseudomonas aeruginosa* culture (Personal communication, Wanda O'Neal, Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill). On the other hand, the amino acid concentration in the ASL of cystic fibrosis patient is higher than in the normal population (Barth and Pitt, 1996). Moreover, increased ASL amino acid concentration correlated with severity of the respiratory disease (Barth and Pitt, 1996; Thomas et al., 2000). This increase in amino acid concentration might be related to decreased activity of amino acid transporters, and might contribute to the sustained bacterial infection in cystic fibrosis.

6. Future directions

To fully understand the role of transporter for amino acids, peptides and sugars in lung function, it is now possible to use genetic tools to develop animal models. Then, to determine if genetic elimination of the transporters will increase the susceptibility of the lung to bacterial infection. At a later stage, it might be possible to utilize pharmacological agents that stimulate transport activity in order to decrease bacterial growth in the airways.

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