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Topical Review

Mechanisms of Acid and Base Secretion by the Airway Epithelium

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Abstract. One of the main functions of the airway epithelium is to inactivate and remove infectious particles from inhaled air and thereby prevent infection of the distal lung. This function is achieved by mucociliary and cough clearance and by antimicrobial factors present in the airway surface liquid (ASL). There are indications that airway defenses are affected by the pH of the ASL and historically, acidification of the airway surfaces has been suggested as a measure of airway disease. However, even in health, the ASL is slightly acidic, and this acidity might be part of normal airway defense. Only recently research has focused on the mechanisms responsible for acid and base secretion into the ASL. Advances resulted from research into the airway disease associated with cystic fibrosis (CF) after it was found that the CFTR Cl^- channel conducts HCO_3^- and, therefore, may contribute to ASL pH. However, the acidity of the ASL indicated parallel mechanisms for H^+ secretion. Recent investigations identified several H^+ transporters in the apical membrane of the airway epithelium. These include H^+ channels and ATP-driven H^+ pumps, including a non-gastric isoform of the H^+-K^+ ATPase and a vacuolar-type H^+ ATPase. Current knowledge of acid and base transporters and their potential roles in airway mucosal pH regulation is reviewed here.

Airway Epithelium and Airway Surface Liquid

In the human lung, there are 23 generations of dichotomously branching tubes. From generations 0 (trachea) to between 8 and 11 (depending on location within the lung), the airways contain both cartilage and mucous glands, and are termed bronchi. From about generation 10 to 16 the airways lack cartilage, glands and alveoli, and are known as conducting bronchioles. From generation 17 on, the airways become respiratory bronchioles with increasing numbers of alveoli budding off them. By generation 20, the entire wall of the airway is occupied by alveolar openings and the airway has become an "alveolar duct", which will end in an alveolar sac at generation 23 [100].

By definition airways conduct gases, but their epithelium is too thick (10 – 50 μm) to allow exchange of gases between the airway lumen and blood. By contrast, alveoli are where gas exchange occurs, and are lined with an epithelium that is only a fraction of a micrometer thick. This review is concerned solely with airway epithelium. In the human trachea, the epithelium is pseudostratified and about 50 μm in height. Numerically its three main cell types are ciliated, basal and goblet (i.e., mucus-secreting). With increasing airway generations, the height of the epithelium decreases progressively until it reaches about ~10 μm in the respiratory bronchioles, by which point it has assumed a columnar morphology. Not only does the form of the epithelium change along the airways, but so also does its cellular composition. Though ciliated cells account for ~50% of the cells in the epithelia of all airway generations [43], basal cells are lost completely before the respiratory bronchioles are reached. Similarly, the number of goblet cells declines progressively to reach zero by the terminal bronchioles (i.e., the smallest bronchioles lacking alveoli). After this, in the respiratory bronchioles, they are replaced by Clara cells [9]. This switch from goblet to Clara cells may reflect the fact that mucus is primarily a defense against particles, and these are deposited predominantly in the larger airways. Clara cells, by contrast, defend mainly against toxic vapors [78]. Occasionally within the surface epithelium of human bronchioles are serous cells [84], specialized for water secretion [95].

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On the apical surface of the airway epithelium is a film of liquid, varying from 5 to 40 μm in depth [104], corresponding to volumes of 0.5 to 4 μl per cm^2 of surface. Visual inspection of the surface of monkey nasal mucosa led Lucas and Douglas [67] to propose that this airway surface liquid (ASL) consisted of two layers: a watery sol between the cilia and a mucous blanket over their tips. They proposed that the low viscosity of the periciliary sol allowed the cilia to beat, and that during their effective strokes the tips of the cilia contacted the underside of the mucous blanket, propelling it, together with entrapped particles, to the mouth, where it was swallowed or expectorated. This process of "mucociliary clearance" thus kept the surface of the airways clean [88, 99]. Subsequently, many ultrastructural studies have confirmed the presence of two layers of ASL [104]. In most cases, the depth of the putative sol is approximately the same as the length of the cilia.

ASL is about 96% water, 1% salts, 1% lipids, 1% proteins and 1% mucus [7] and leukocytes are also present. Both ASL composition and volume are considered critical for its function [17, 56, 91, 96, 102, 103]. How does pH affect this mixture, and thus, its function? Firstly, pH-dependent changes of ion conductances [4, 14, 82] could alter salt and water movement and the concentrations of all components. Secondly, the function of several components is pH-sensitive. Mucus, produced mainly by glands in the larger airways, and by goblet cells in the smaller, contains acidic groups that are involved in the interactions between individual molecules. Its properties are well known to be pH-sensitive. A number of proteins involved in antimicrobial defense are secreted by airway surface epithelium and glands [34]. Protein functions are potentially sensitive to pH. For example, the function of the antibacterial lactoperoxidase secreted by airway glands has a pH optimum at a slightly acidic pH [15, 18, 26]. In a recent review, Ricciardolo et al. [83] examined the role of excessive acidity on airway function, including 1) effects of H^+ on sensory neurons and associated bronchoconstriction, 2) induction of cough by acid, 3) microvascular leakage and mucus production caused by excessive acidity, 4) effects on leukocyte function, and 5) interaction with nitric oxide and superoxide metabolism of the airways (reviewed in [83]). Thus, the regulation of the pH of the ASL is critical for its normal function.

pH of the Airway Surface Liquid

Normal Mucosal Airway pH

Normal ASL pH is considered acidic compared to a blood and interstitial pH of 7.4. There are indications that in airway diseases ASL pH is comparably alkaline (e.g., chronic bronchitis) or more acidic (e.g., CF or pneumonia), suggesting breakdowns in the pH regulatory mechanisms. Table 1 summarizes studies performed over the past 70 years in which ASL pH was determined using different techniques. The most common means of measuring ASL pH has been by direct placement of a pH electrode on the nasal mucosa. pH electrodes of various sizes and materials have been used, but measurements are confounded by electrode design, immersion depth, placement of the reference electrode, and possibly mechanical disturbance of the epithelial layer. Measurements have also been made on expectorated sputum or in situ with the use of pH-sensing fluorescent probes. Astoundingly, despite improvements in the measurement techniques over the years, reported pH values measured on the airway surface are quite consistent and repeatable, averaging ~6.6 in healthy airways (Table 1).

In 1941, Fabricant [30] placed a pH electrode in the nostril of healthy subjects to contact the mucosa directly and found a pH range of 5.5 to 6.5. Because in this study the external reference electrode was placed on the skin of the subject, the acidity of these measurements was possibly overestimated (owing to the higher negative skin potential compared to the nasal potential [3]). Nevertheless, this study established early on the acidity of the normal nasal mucosa. Using a similar electrode placement, England et al. [29] recently measured the same pH range for the nasal mucosa using an antimony pH probe. To avoid the confounding effect of an externally placed reference electrode, McShane et al. [68] used a glass pH electrode with an internal reference electrode which was placed together with the pH sensor on the nasal mucosa. Normal nasal pH was 6.6, and the pH was similar along the respiratory mucosa down to the 3rd generation bronchus. Using an endoscopically guided pH electrode Steinmann [93] measured endobronchial pH values of 5.7 to 6.6. In a more extensive study, where many segments of the bronchial tree were compared, Bodem et al. [8] found an average endobronchial pH of 6.6 and, again, no difference was found between various distal bronchial segments. A number of additional studies found similar tracheal and bronchial mucosal pH values. Although there is some variability, which may in part be due to technical differences between studies, these measurements show consistently that the normal mucosal pH of the airways is quite acidic, averaging pH 6.6 for all investigations listed in Table 1. Interestingly, one study grouped measurements of nasal pH by ethnicity [54] and found that the nasal pH in people of African descent was significantly more acidic (pH 6.4) than in Caucasians (pH 6.9). The potentially confounding factor of ethnicity had not been considered in earlier studies.

The relative contribution of different resident or mobile pH buffers has not been well investigated but both mucus and HCO_3^- are likely significant

Table 1. pH of the airway mucosa

	pH	Method	Citation
Human			
Nasal			
	5.5–6.5	pH electrode	[30]
	6.6	pH electrode	[45]
	6.44 /6.91	pH electrode, measurements grouped by ethnicity (African descent *vs*. Caucasians)	[54]
	5.5–6.5	pH electrode	[29]
	6.6	pH electrode	[68]
	6.4–7.9	pH electrode	[12]
	6.4–6.5	pH electrode	[107]
Tracheal			
	6.1–7.9	pH electrode	[41]
	6.71	micropipette sampling	[42]
Bronchial			
	5.7–6.6	pH electrode	[93]
	7–7.5	pH electrode	[41]
	6.7	pH electrode	[73]
	7.1	pH electrode	[68]
	6.25	micropipette sampling, primary cell cultures	[17]
	6.78	BCECF dextran	[58]
	6.58	pH electrode	[8]
Human, disease			
Smokers			
	7.25	Sputum	[48]
	6.82	Sputum	[49]
Chronic bronchitis			
	7.59	Sputum, mucoid	[2]
	7.83	Sputum, purulent	[2]
Rhinitis			
	7.2–8.3	pH electrode, nasal	[29]
Common cold			
	7.2–8.3	pH indicators	[46]
Cystic fibrosis			
	6	micropipette, primary cell cultures	[17]
	6.8	pH electrode, nasal	[68]
Bacterially infected			
	6.48	pH electrode, bronchial	[8]
	5.6–6.2	pH electrode, bronchial	[41]
	7.2–7.4	pH electrode, bronchial	[93]
Non-human			
Rabbit			
	6.73	pH electrode	[41]
	7.7	pH electrode	[36]
Rat			
	7.52	pH electrode	[35]
Ferret			
	6.85	pH electrode	[63]
Mouse			
	7.28	BCECF dextran, isolated lungs	[92]
	7.14	BCECF dextran	[57]
	6.95	BCECF dextran, in situ	[56]
Pig			
	6.93	pH of bronchial perfusate	[53]
Cow			
	6.81	BCECF dextran, primary cell cultures	[57]
	6.94	BCECF dextran, primary cell cultures	[56]
Dog			
	7.09	pH electrode, bronchial	[8]

"pH electrode" refers to measurements where an electrode was placed on the airway mucosa.

contributors to ASL pH and its buffer capacity. Healthy airways have very little mucus and therefore its role in ASL pH has not been analyzed. In contrast, inflamed airways consistently secrete large amounts of mucus. Airway mucins are acidic and show low isoelectric point values [85] and thus may contribute to low pH buffering. Holma et al. [48, 49] used induced sputum from smokers to determine the contribution of airway mucus to ASL pH. Sputum reflects in large parts mucous secretions from the upper airways with variable contamination of saliva. In an initial study average sputum pH was 7.25 and the buffer capacity, usually expressed as the amount of acid needed to achieve a change of one pH unit, was ~7 mM/pH in the pH range from pH 5 to 6.5 [48] and increased to ~15 mM/pH at increasing pH values up to 7.5. (calculated from Table 1 in ref. [48]). In a second study by these investigators it was found that the average sputum pH (6.82) and buffer capacity (6 mM/pH) were somewhat lower and declined with increasing pH to 2.5 mM/pH at pH 7.5 (calculated from Table 1 in ref. [49]). These two studies established the buffer capacity of human airway mucus using measurements of sputum pH. The differences between these two studies are consistent with differences in HCO_3^- levels. Specifically, the increased baseline pH and increased buffer capacity at high pH values in the initial study [48] is consistent with elevated levels of HCO_3^- or additional buffers. More recent estimates of buffer capacity are similar to Holma's. The buffer capacity of ASL of bovine tracheal cultures was 14 mM/pH at pH 7 [57]. In human gland secretions induced by pilocarpine the buffer capacity was found to be 12 mM/pH at pH 7. This value was determined in the presence of HCO_3^-. When the buffer capacity of pig gland secretions was determined in the absence of HCO_3^- it was found to be substantially lower, 3.7 mM/pH, [91], indicating HCO_3^- as a substantial contributor to the buffer capacity of airway gland secretions.

Mucosal Airway pH in Disease

As early as the 1930s, nasal secretions were sampled and their pH determined using indicator dyes. In one early study, nasal secretions were found to be acidic during acute inflammation [71], while pH alkalinized over the course of the common cold to pH 7.2 to 8.3 [46]. This resulted in the proposition that the nasal pH could be used as an indicator for airway disease [30]. More recent measurements also reveal changes in ASL pH in disease (Table 1). Induced mucoid or purulent sputum from chronic bronchitis patients was found to be alkaline (7.6 and 7.8, respectively [2]), tracheobronchial mucus samples suctioned from lungs of acutely ill patients showed an average pH of 7.7 [70], endobronchial pH in pneumonia averaged 7.2–7.4 [93], and nasal pH from rhinitis patients measured by placing an electrode onto the nasal mucosa resulted in pH values of 7.2–8.3 [29]. These studies indicated quite consistently that pH of inflamed or infected airways is alkaline, supporting the notion that airway infection activates a mechanism that alkalinizes the mucosal pH. In contrast, one study of endobronchial pH during active bacterial infections resulted in quite acidic values (5.6–6.2, ref. [41]). Also measurements on CF primary cell cultures using a capillary sampling technique resulted in ASL pH values of 6.0 [17], while in nasal pH recordings in CF patients, McShane et al. [68] found no increased acidity compared to normal. The wide variation of ASL pH in disease supports the notion that the activity of epithelial acid and base transporters is differentially affected in disease.

Recently, a number of cellular mechanisms of acid and base transport have been identified in the airway epithelium and apical localization suggests a direct role in ASL pH regulation. Although their relative importance in health and disease are as yet uncertain, the measured changes in ASL pH likely reflects a selective regulation of specific transporters. These pH-regulating transport mechanisms are discussed individually in the next section.

Mechanisms of Base Secretion

CFTR

The cAMP-activated Cl^- channel CFTR (cystic fibrosis transmembrane conductance regulator) is expressed in the apical membrane of ciliated surface cells and gland ducts [61]. The permeability of CFTR to HCO_3^-, the relative acidity of the pH of CF gland secretions, and its apical localization support the notion that CFTR activity alkalinizes ASL pH. In a seminal study using normal and CF primary human cultures, Smith and Welsh [89] described cAMP-stimulated HCO_3^- transport that was inhibited by the CFTR blocker DPC (diphenylaminecarboxylate) and was absent in CF airways, implicating CFTR in HCO_3^- secretion. In addition, block of carbonic anhydrase by acetazolamide effectively blocked HCO_3^- secretion. These data suggested a model where intracellular carbonic anhydrase generated HCO_3^- that was released apically through CFTR.

Conduction of HCO_3^- by CFTR has been debated intensely [81]. Initial observations by Gray et al. [39] in pancreatic duct cells showed a small-conductance Cl^- channel expressing a relative permeability to HCO_3^- with respect to Cl^- of 0.13. Firm evidence for conduction of HCO_3^- across CFTR was presented by Poulsen et al. [79] in a patch clamp study of recombinantly expressed CFTR, and a number of further studies investigating the HCO_3^--to-Cl^-

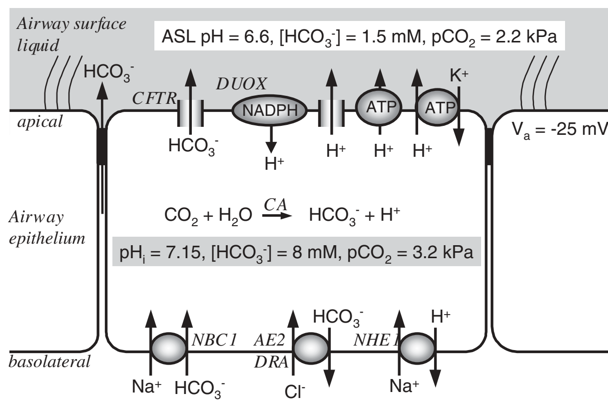

Fig. 1. Major acid and base transporters of airway epithelium. HCO_3^- is generated both intracellularly by carbonic anhydrase (CA) activity and enters via the basolateral membrane by way of NBC1, whose Na^+-to-HCO_3^- stoichiometry may be 1 : 2 or 1 : 3 [62, 94]. HCO_3^- is released apically via CFTR. Additional gradient-driven HCO_3^- secretion across the paracellular pathway into an acidic ASL is likely. Direction of HCO_3^- movement by the basolateral anion exchanger is determined by the respective gradients for Cl^- and HCO_3^-. Intracellular H^+ is produced by CA and an apical DUOX-based NADPH oxidase. Three apical H^+ secreting mechanisms have been identified: H^+ channels, V-ATPase, and H^+-K^+ ATPase. The role of the basolateral NHE1 is likely to maintain intracellular pH. Concentrations given are explained in text and may vary considerably. For abbreviations *see* text.

permeability of CFTR resulting in ratios of 0.13 to 0.25 [39, 50, 51, 65, 79].

However, the actual rates of Cl^- and HCO_3^- secretion across CFTR are determined not only by their relative permeabilities but also by the electrochemical driving forces for both anions. Interestingly, under unstimulated conditions, driving forces for Cl^- movement are small. With an apical membrane potential of airway cells of −25 mV [16, 19, 105, 106], and intracellular [Cl^-] of 43 mM [106] and ASL [Cl^-] of 140 mM [58], a net electrochemical driving force for inward Cl^- movement of 5 mV can be estimated. By comparison, HCO_3^- concentrations in the cytosol (8 mM, [77]) and the ASL (1.5 mM; at pH 6.6 and pCO_2 = 2.2 kPa, *see* also Fig 1) result in a large outwardly directed electrochemical driving force for HCO_3^- across the apical membrane of −68 mV. Therefore, in the presence of an acidic ASL pH, HCO_3^- is predicted as the major secreted anion through CFTR despite its relatively low permeability compared to Cl^-. In support of this notion, it has been shown that primary airway epithelia or the Calu-3 cell line (a human airway cell line with characteristics of submucosal gland serous cells [27, 87]) secrete significant amounts of HCO_3^- and the relative contribution of Cl^- and HCO_3^- to CFTR mediated current is governed by the respective driving forces [24, 25, 51, 62, 64, 94].

Cl^--HCO_3^- Exchange

The Cl^--HCO_3^- exchanger (anion exchanger, AE) isoform 2 (AE2) is broadly expressed in epithelia and has been consistently found in human trachea, bronchi and Calu-3 cells using PCR techniques [28, 52]. When human primary airway epithelial cultures were investigated using separate perfusion of the mucosal and serosal compartments [77], electroneutral Cl^--HCO_3^- exchange activity was found to be present in the basolateral membrane, whereas HCO_3^- release across the apical membrane was well explained by CFTR activity, based on ion replacement studies, block by DIDS, and use of CF epithelia [24, 77]. Basolateral localization of AE2 was found in the Calu-3 cell line and rat tracheal submucosal glands, and ciliated surface cells (showing less expression) using immunocytochemistry and sensitivity to serosal DIDS and DNDS in Ussing chambers [66]. The direction of transport by AE2 is determined by the respective gradients for Cl^- and HCO_3^-. In connection with an apical exit step, basolateral AE2 may contribute to HCO_3^- secretion when the HCO_3^- gradient over the basolateral membrane is larger than the Cl^- gradient. This would be the case, for example, during intracellular acidification and concomitantly reduced intracellular HCO_3^- concentrations.

DRA (downregulated in adenoma [69]) was initially cloned from the colon and was found to be expressed in the normal colon but not in most adenocarcinomas. DRA is widely expressed in intestinal epithelia [98] and apical localization of DRA has been shown by immunocytochemistry in intestinal epithelia [13] and in mouse pancreas [40]. Null mutations in the DRA gene result in congenital Cl^- diarrhea [47], resulting from defective Cl^--HCO_3^- exchange, and recombinant expression of DRA in HEK293 cells resulted in the appearance of Cl^--HCO_3^- exchange activity [69]. In airway epithelia, DRA has been found in mouse trachea and CFTR-corrected CFT1 airway cells using Northern hybridization [101]. In analogy to its function in HCO_3^- secretion by the pancreatic duct epithelium [90], a model has been proposed by Wheat et al. [101] for the airway epithelium in which Cl^--HCO_3^- exchange activity governed by DRA located in the apical membrane secretes Cl^- into the ASL with Cl^- recycling through CFTR [101]. However, that study was done under conditions that did not allow distinction of apical from basolaterally located transporters, and studies in polarized airway epithelia have not found Cl^--HCO_3^- exchange activity in the apical membrane [77]. Therefore, at present it appears that expression of Cl^--HCO_3^- exchange in airway epithelia is limited to the basolateral membrane where it only indirectly contributes to base secretion in conjunction with a separate apical exit step.

Na^+-HCO_3^- Cotransport

In the Calu-3 cell line, the Na^+-HCO_3^- cotransporter (NBC) isoform 1 (NBC1) has been detected by RT-PCR [52]. These cells secrete HCO_3^- and contain high levels of apical membrane CFTR, and a basolaterally localized NBC has been identified using the sided effects of anion replacements and small-molecule blockers in Ussing chambers [25]. Thus, movement of HCO_3^- into Calu-3 cells on NBC is followed by efflux through apical CFTR [25]. In human primary airway cultures, NBC activity was identified by blocking HCO_3^--dependent transepithelial currents with high concentrations of DNDS [24]. The same study introduced the notion that basolateral NBC activity coupled with an acetazolamide-sensitive carbonic anhydrase each provide fractions of the apically secreted HCO_3^-. This extended the initial model of HCO_3^- secretion by Smith and Welsh [89] by adding basolateral NBC as an additional source of intracellular HCO_3^-. A role for carbonic anhydrase activity as a source of HCO_3^- was later also suggested by Krouse et al [62]. Similarly, in isolated pig bronchi [6] both NBC and carbonic anhydrase were determined to be HCO_3^- sources supporting HCO_3^- secretion into the lumen via CFTR. Obviously, when carbonic anhydrase generates intracellular HCO_3^- it generates equal amounts of intracellular H^+, and the resulting effects on ASL pH will therefore be determined by the localizations (apical vs. basolateral) and rates of turn over of both HCO_3^- and H^+ transporters.

Paracellular Pathway

The airway epithelium is moderately tight, the paracellular pathway expresses a relatively high permeability, and ion conduction across the paracellular pathway supports secretion or absorption of approximately isotonic salt solution [10]. With a transepithelial potential of $V_t = -32$ mV (lumen negative) in normal human trachea in vivo [59] and a serosal pH of 7.4, HCO_3^- can be calculated to be in equilibrium across the epithelium at an ASL pH of 6.85, i.e., at more acidic ASL pH, there is a net driving force for HCO_3^- flux from the serosa into the ASL across the paracellular pathway. A paracellular HCO_3^- permeability has been suggested in CF airway epithelial cultures [17] by measuring the effects of transepithelial HCO_3^- gradients on transepithelial potentials. Using Calu-3 epithelia the characteristics of anion permeation across the paracellular pathway was investigated and the paracellular HCO_3^- permeability was found to be quite high (0.94×10^{-6} cm/s, compared to the paracellular Cl^- permeability of 6.28×10^{-6} cm/s, calculated from data in [50]). For this permeability, an average ASL pH of 6.6 in normal airways (i.e., the average of measurements in humans listed in Table 1) can be calculated to drive a HCO_3^- current of 3.2 $\mu A/cm^2$ across the paracellular pathway (using the Goldman-Hodgkin-Katz equation and $V_t = -32$ mV, $[HCO_3^-]_{serosal} = 21$ mM and $[HCO_3^-]_{mucosal} = 1.5$ mM at a time-averaged pCO_2 of 2.2 kPa in the airway lumen). In comparison, in forskolin-stimulated primary airway cultures, a transcellular HCO_3^- current of 3.7 $\mu A/cm^2$ was measured, identified by its sensitivity to acetazolamide and serosal DNDS (4,4′-dinitrostilben-2,2′-disulfonate, a blocker of NBC) [24]. Although the driving forces for HCO_3^- transport are quite different in this comparison, it suggests that in presence of an acidic ASL pH a substantial part of transepithelial HCO_3^- secretion may be via the paracellular pathway.

A paracellular HCO_3^- permeability has considerable implications for airway acidification, which is predicted to be limited by the gradient-driven paracellular HCO_3^- flux into an acidic ASL. An efficient passive HCO_3^- flux into an acidic ASL (which may include a paracellular component) is consistent with the finding that CO_2-induced acidification of ASL in ferret trachea resulted in minute changes of ASL pH [63]. On the other hand, in similar measurements using bovine tracheal cultures, when the ASL was acidified with HCl, pH recovery was largely blocked by DIDS [57], indicating that in these cells the transcellular pathway is the major route for HCO_3^-. Bovine tracheal cultures express very high transepithelial resistances [60], which is expected to limit the paracellular HCO_3^- permeation.

Mechanisms of Acid Secretion

Initially, Smith and Welsh [89] measured HCO_3^- secretion across primary cultures of human tracheal epithelium and predicted that it would contribute to alkalinization of ASL in vivo. Then Poulsen et al. [79] showed that CFTR was permeable to HCO_3^- and concluded that CFTR activity might contribute to an alkalinization of ASL pH. However, normal steady-state ASL pH is consistently found to be acidic (Table 1) and mucosal acidification of airway epithelial cultures or native tissues is apparent. When the mucosa of human airway epithelial cultures was mildly alkalinized to pH 7.5, it re-acidified at initial rates of ~0.2 pH/h [17] which predicts a rate of net acid secretion of 3.5 $nmole \cdot h^{-1} \cdot cm^{-2}$ (assuming an ASL volume of 2.5 $\mu l/cm^2$ and a buffer capacity of 7 mM/pH). In contrast, basal rates of epithelial acid secretion by human primary airway cultures in HCO_3^--free conditions were 170 $nmole \cdot h^{-1} \cdot cm^{-2}$ [31] under conditions where both secretion of HCO_3^- and the buffering of secreted acid in the ASL by HCO_3^- was minimal. The difference between net acid secretion in the presence and absence of HCO_3^- suggests that the airways constitutively secrete both acid and HCO_3^- in parallel at substantial rates. The investiga-

tion of the cellular mechanisms involved in acid secretion into the ASL has only recently focused on acid transporters known from other epithelial tissues. Several acid transporters have been identified and localized in airways using both molecular and functional approaches. However, at this point we still lack a precise understanding of the regulation of the acidification of the ASL, which necessarily includes the activity of both acid and base transporters. Below, we discuss the roles in acid secretion of the major acid transporters that have been identified in airway epithelial cells.

Na^+-H^+ Exchange

The ubiquitously expressed Na^+-H^+ exchanger (NHE) releases H^+ from cells across the plasma membrane driven by the large electrochemical gradient for Na^+ entry. Its major function is the maintenance of intracellular pH by protecting the cytosol from acidification. NHE might participate in epithelial acid secretion if expressed in the apical membrane. For example, in the intestinal epithelium NHE activity has been found in the apical brush border membrane where it functions in Na^+ absorption from and H^+ secretion into the intestinal lumen [5]. The NHE1 isoform was detected by RT-PCR in all bronchial regions of the human lung [28]. Amiloride-sensitive NHE activity has been identified in isolated primary human airway epithelial cells using intracellular pH measurements [74]. In a study using bilateral perfusion of intact epithelia amiloride-sensitive NHE activity was localized to the basolateral membrane [75], without any measurable apical activity. Basolateral localization of NHE indicates its role in intracellular pH regulation, rather than in H^+ secretion.

Interestingly, when using mucosal amiloride or its analogue as tools to identify possible apical NHE activity, a number of studies found small transient effects. Acid secretion across intact tracheal tubes isolated from sheep recorded using the pH stat technique was transiently reduced by mucosal EIPA (5–(N-ethyl-N-isopropyl)-amiloride) by 12%, which was interpreted as NHE-mediated acid secretion present in the apical membrane [1]. Similarly, in pH stat recordings of acid secretion by primary human airway epithelia, addition of amiloride to the mucosal bath transiently inhibited acid secretion by 34% in a fraction of investigated cultures [31]. In intact distal airways isolated from pigs, mucosal acidification was slightly reduced by mucosal dimethylamiloride [53], and using pH-sensitive microelectrodes intracellular pH slightly and transiently acidified when human airway epithelia were treated with mucosal amiloride [105]. In most of these studies, such effects were considered insignificant and not NHE-mediated [31, 53, 105] and the transient nature of the effects is inconsistent with a block of apical NHE activity. However, these surprisingly similar observations support an alternative notion: acid secretion may have been blocked by amiloride (or its analogue) owing to its effect on apical amiloride-sensitive Na^+ channels and the concomitant hyperpolarization of the apical membrane potential. This is expected to inhibit H^+ channels in the apical membrane (which are blocked by hyperpolarization and activated by intracellular acidity, *see* below) and reduce H^+ channel-mediated acid secretion. Block of H^+ channels, in turn, acidifies intracellular pH resulting in the activation of H^+ channels, and thus, a transient blocker effect on H^+ secretion. This explanation, although hypothetical, may explain the transient effects of mucosal amiloride on H^+ secretion observed in a number of studies in absence of an apical NHE. However, this effect relies on constitutively active apical H^+ channels in airway epithelium. The mixed evidence for such constitutive activity [17, 31, 76, 86] suggests that it may be present only in a fraction of cell cultures, thereby explaining the absence of amiloride effects in many cases [31].

H^+-K^+ ATPase

Activity of the gastric parietal cell H^+-K^+ ATPase is experimentally readily identified by its sensitivity to highly selective probes, such as SCH28080. In cultured bovine tracheal epithelial cells, Poulsen and Machen [80] found variable block of intracellular alkalinization by SCH28080, suggesting the expression of a gastric-type H^+-K^+ ATPase in these cells. However, the localization was not determined in that study. In other studies, addition of SCH28080 to the mucosal side of polarized human airway cultures [17, 31] or intact glands [91] had no effects on acid secretion, arguing against a role of the gastric-type H^+-K^+ ATPase in acid secretion by airways. By contrast, a non-gastric isoform of the H^+-K^+ ATPase, which was initially identified in the apical membrane of rat distal colon epithelium [23], was detected by RT-PCR in cultured bronchial epithelia, and immunocytochemistry localized it to the apical membrane pole [17]. Transport function of the colonic H^+-K^+ ATPase is blocked by ouabain, but not by SCH28080. Mucosal ouabain entirely inhibited ASL acidification of bronchial cultures when treated for several hours [17] and in pH stat experiments in Calu-3 cells mucosal ouabain caused effects consistent with block of an apical H^+-K^+ ATPase [62]. On the other hand, acute effects of mucosal ouabain on H^+ secretion measured in pH stat experiments were small in tracheo-bronchial cultures [31, 86] or in pig tracheal surface epithelium [91].

Vacuolar H^+ ATPase

The vacuolar-type H^+ ATPase (V-ATPase) is an ATP-dependent H^+ pump that is responsible for the acidification of intracellular compartments [32]. In addition, V-ATPases have been shown in the plasma membranes of epithelial cells (such as the sweat duct [38]) where V-ATPases pump H^+ across the plasma membrane and acidify the extracellular space. Identification of V-ATPase activity in functional experiments is aided by the highly selective inhibitor bafilomycin A1 [11]. A role of V-ATPase activity in airway acidification is supported by the work of Inglis et al. [53] in isolated perfused pig bronchi, which acidify their luminal surface at high rates of 2.3 $\mu mole \cdot h^{-1} \cdot cm^2$ [53], of which ~60% is inhibited by addition of luminal bafilomycin A1. In contrast, in a number of other studies, inhibition of acid secretion by bafilomycin A1 showed comparably small effects, including in primary human airway epithelia [31, 86], or no measurable effects in cow tracheal cells [80] or pig airway surface epithelia [91].

H^+ Channels

Voltage-gated, highly selective H^+ channels were first described in snail neurons and since have been found in many other species and cell types, including epithelia [21, 86, 97]. H^+ channels are typically activated by intracellular acidification, extracellular alkalinization, and/or membrane depolarization. These characteristics result functionally in a strong outward rectification of H^+ currents because H^+ currents are both driven and activated by an outwardly directed H^+-motive force, suggesting a general role for H^+ channels as relief valves that permit effective H^+ release from cells during intracellular acidification [21]. In rat alveolar type II cells, for example, the biophysical characteristics have been studied extensively and localization in the apical membrane has been demonstrated using apical patch-clamp recordings [20]. Using intracellular pH measurements in single cells it was found that H^+ channels in alveolar type II cells are active during intracellular acidification [72].

In human primary airway cells or in the JME CF airway cell line [31, 86] we identified a whole-cell H^+ conductance with typical characteristics of H^+ channels as described in alveolar type II cells, including slow current activation at depolarizing potentials, outward rectification, H^+ selectivity, and block by Zn^{2+}. By using Zn^{2+} as a probe for H^+ channel activity in confluent airway epithelia, this conductance was localized to the apical membrane using measurements of H^+ secretion in Ussing chambers. H^+ secretion was blocked 50–70% by $ZnCl_2$ [31, 86], and the remaining unblocked fraction was partially inhibited by mucosal bafilomycin A1 (11%) or ouabain (15%, [86]), suggesting that in these studies the primary mechanism was channel-mediated H^+ secretion. Currently it is unclear why different investigations found different acid-transport mechanisms as the primary mediator of H^+ secretion in airways, such as the H^+-K^+ ATPase in primary airway cultures [17], V-ATPase in intact pig bronchi [53] or H^+ channels in primary airway cultures or an airway cell line [31, 86]. We surmise that this might be affected by the H^+ gradients across the plasma membrane, which will particularly affect the participation of H^+ channels in H^+ secretion.

ATP-driven H^+ transporters such as the V-ATPase or the H^+-K^+ ATPase can transport H^+ against a considerable electrochemical gradient. In contrast, H^+ channel-mediated H^+ secretion occurs only in presence of an outwardly directed electrochemical H^+ gradient owing to the outwardly rectifying characteristics of H^+ currents, which also preclude inward movement of H^+ under most physiological conditions.

H^+ channels are evidently present and active in airway cultures, but what is the mechanism responsible for generating an outwardly directed electrochemical H^+ gradient? For comparison, in professional phagocytes such a transmembrane H^+ gradient is generated by NADPH oxidase activity. NADPH oxidase is a multicomponent enzyme complex that oxidizes intracellular NADPH to $NADP^+ + H^+$ and the resulting electrons are transferred across the plasma membrane where extracellular O_2 is reduced to superoxide. Thus, NADPH oxidase activity both acidifies the cytosol by NADPH oxidation [55] and depolarizes the plasma membrane potential by the outward electron current [22], which creates an outwardly directed H^+ gradient and activates H^+ channels in phagocytes. We hypothesized that a similar process was active in the apical membrane of airway epithelial cells and we set out to investigate the expression and function of NADPH oxidase in airway epithelia.

A number of isoforms of the transmembrane, electron-transporting β subunits of NADPH oxidase have been described. The two large homologs DUOX1 and DUOX2 (for dual oxidase; 83% similarity between isoforms) have been recently identified as the major isoforms and are highly expressed in human airway epithelial cells [37, 44, 86]. By immunocytochemistry DUOX1 and/or DUOX2 were found to be present in the apical membrane region [86]. NADPH oxidase-mediated superoxide production by airway cells was identified [33, 37] and intracellular H^+ production and secretion was correlated with DUOX1 expression in two airway cell types and inhibited by NADPH oxidase blockers [86]. These observations suggested that the airway epithelium expresses a DUOX-based NADPH oxidase that produces superoxide and H^+. Thus, in analogy

to the phagocytic NADPH oxidase, DUOX activity in the apical membrane of airway cells is a candidate to both depolarize the membrane and acidify the cytosol to drive H^+ currents across channels. In cultured human airway epithelia the resting apical membrane potential ranges from −19 mV to −29 mV [16, 19, 105, 106] and the intracellular pH is 7.1, [75, 77, 105], resulting in an inwardly directed driving force for H^+, which essentially rules out a contribution of H^+ channels to H^+ secretion and likely ATP-driven transporters dominate basal H^+ secretion. However, H^+ secretion is greatly increased by mucosal histamine or ATP [31, 91], two agonists that elevate intracellular Ca^{2+}. Both DUOX isozymes express Ca^{2+}-binding EF-hands, and NADPH oxidase activity in airways has been shown to be regulated by the Ca^{2+}-elevating agonists ATP, thapsigargin [33], and ionomycin [37]. Further, DUOX1 expression in airways has been shown to be correlated with intracellular H^+ production [86]. The sum of these observations suggests a role for a DUOX-based NADPH oxidase in apical H^+ production by the airway epithelium, which might support gradient-driven, channel-mediated H^+ secretion. The presence of NADPH oxidase activity in the apical membrane tightly links acid and oxidant production by the airway epithelium. As a corollary to its function in leukocytes, there are indications that NADPH oxidase-derived H^+ and oxidants are part of innate airway defenses [33, 37, 86].

Why does the airway epithelium express at least three different mechanisms to secrete H^+? Although we cannot provide a definitive answer, it appears intuitively clear that the ATP-driven H^+ pumps, which are able to move H^+ against a considerable gradient, are necessary to secrete H^+ when gradients are unfavorable for conductive H^+ exit, possibly under basal conditions. Gradient-driven H^+ secretion is expected when the inside of the apical membrane acidifies, such as during NADPH oxidation. Interestingly, the presence of several parallel H^+-releasing mechanisms supports the notion that continuous H^+ release under various conditions and an acidic ASL pH are critical for normal airway function.

Conclusion

Despite a long history of measurements of airway mucosal pH and its suggested role in airway disease, acid and base transporters expressed by the airway epithelium have been investigated and identified only very recently. Figure 1 summarizes the distribution of currently known transporters to the apical and basolateral poles of airway epithelial cells, and intracellular sources of H^+ and HCO_3^-. Parallel mechanisms for the independent secretion of both H^+ and HCO_3^- result in a sensitive way to tightly regulate ASL pH. There are a number of indications that link ASL pH to airway function and airway disease, including roles of pH in innate defense and airway stress. Changes in ASL pH have been found in airway diseases resulting in epithelial stress and increased susceptibility to bacterial airway infections. Currently, the relations between ASL pH , epithelial integrity and function, and antibacterial activity have not been worked out in detail, however, there are indications that a tightly regulated ASL pH is important for normal airway health.

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