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Review Article

Ion Transport by Pulmonary Epithelia

Monika I. Hollenhorst, Katrin Richter, and Martin Fronius

Institute of Animal Physiology, Justus Liebig University Giessen, Wartweg 95, 35392 Giessen, Germany

Correspondence should be addressed to Martin Fronius, martin.fronius@bio.uni-giessen.de

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The lung surface of air-breathing vertebrates is formed by a continuous epithelium that is covered by a fluid layer. In the airways, this epithelium is largely pseudostratified consisting of diverse cell types such as ciliated cells, goblet cells, and undifferentiated basal cells, whereas the alveolar epithelium consists of alveolar type I and alveolar type II cells. Regulation and maintenance of the volume and viscosity of the fluid layer covering the epithelium is one of the most important functions of the epithelial barrier that forms the outer surface area of the lungs. Therefore, the epithelial cells are equipped with a wide variety of ion transport proteins, among which Na^+, Cl^-, and K^+ channels have been identified to play a role in the regulation of the fluid layer. Malfunctions of pulmonary epithelial ion transport processes and, thus, impairment of the liquid balance in our lungs is associated with severe diseases, such as cystic fibrosis and pulmonary oedema. Due to the important role of pulmonary epithelial ion transport processes for proper lung function, the present paper summarizes the recent findings about composition, function, and ion transport properties of the airway epithelium as well as of the alveolar epithelium.

1. The Airway Epithelium

1.1. Composition of the Airway Epithelium. The airways of mammals can be divided into two parts according to their main function: the conducting airways and the respiratory airways. The conducting airways comprise the nose, the trachea, and the bronchi. They are mainly responsible for transport of the air to the parts of the lung in which the gas exchange takes place. Additionally they warm the air passing them upon breathing in and clean the air from many particles and pathogens that are taken up with the air. The respiratory airways consist of the respiratory bronchi and the alveoli and mediate the gas exchange (see "The alveolar epithelium" for a more detailed description and Figure 1).

All parts of the airways are lined with an epithelium that forms a barrier between the organism and the outside world. Usually the tracheal airway epithelium consists of a layer of columnar or cuboidal cells that originate from the basement membrane and, thus, form a pseudostratified epithelium [1]. These airway epithelia contain various cell types with different morphologies and functions. The following paragraph gives a brief overview of the epithelial cell types in the conducting airways.

In all surface epithelia of the conducting airways, various cell types can be found, which consist mainly of ciliated cells, Clara cells, undifferentiated basal cells, and goblet cells [1, 2]. These cells are expressed in different proportions in the airway epithelia (nasal, tracheal, bronchial), and their local distribution varies [1]. For example, in mouse tracheal epithelium, large numbers of ciliated cells and Clara-like cells have been detected in addition to less distributed goblet cells, serous cells, brush cells, and basal cells [3]. Of the eight different cell types described in rat airway epithelium, the frequency of ciliated cells increases progressively towards the periphery, the number of basal cells decreases progressively more distally, and nonciliated cells are also unequally distributed [1]. Additionally epithelial serous cells are more abundant than goblet cells [1]. The single cell types may also vary in their ultrastructural features between different species, as shown for the microvilli-containing bronchiolar epithelial Clara cells [4]. But the basic functions or the various cell types are similar among different species. Ciliated cells are known to be responsible for the transport of inhaled particles and the mucous layer in the oral direction by beating of their motile cilia. Most airway epithelial cell types such as ciliated cells, Clara cells, and goblet cells secrete ions,

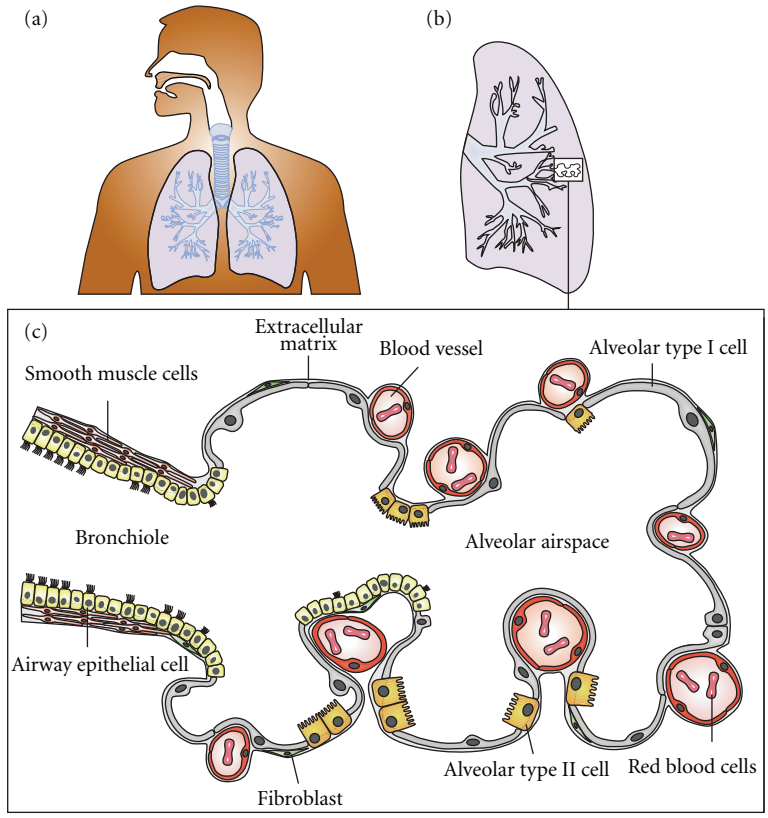

Figure 1: (a) Schematic overview of the lung within the body. (b) Left lung lobe marking the distal part of the lung. (c) Magnification of the distal lung, represented as a cross-section through the distal airways and the alveolar region. The surface of the lung is formed by a continuous epithelial layer consisting of different cell types. In the airways the bulk of epithelial cells are cuboidal cells with cilia. In the alveolar region, the epithelium is formed by alveolar type I and alveolar type II cells.

phospholipids, mucus, surfactant, and immunoprotective proteins such as the Clara cell secretory protein [5, 6]. Basal cells are undifferentiated and serve as stem cells for other airway epithelial cell types like ciliated cells [7]. Yet, the function of other airway epithelial cell types such as the brush cell has recently been newly evaluated and is up to now not fully understood (see below).

In addition to the cell types described decades or even a century ago, some less abundant cell types have been characterised more recently. During the last decade, chemosensory cells have been detected in airway epithelia [8, 9]. These solitary chemosensory cells are present in all airway epithelia (nasal, tracheal, bronchial), but their frequency decreases in the lower airway epithelia [10]. Additionally cells with the morphology of brush cells have been shown to express all components necessary for bitter taste transduction [11], and human ciliated cells also contain functional bitter taste receptors [12]. The function of these chemosensory cells is not completely understood. Yet, recent evidence suggests that solitary chemosensory cells transmit the information about bitter compounds in their environment to sensory nerve endings and by this mechanism are able to take part in vagally mediated breathing control [11]. It has been proposed that these cells could be part of an additional mechanism of the innate immunity of the airways [10]. Thus, chemosensory cells seem to consist of different subtypes that may all be involved in the innate immune response by different mechanisms, like mediating breathing control and protective respiratory reflexes and regulating the mucociliary clearance by increasing the ciliary beat frequency. These findings show that although much has been known about the composition, morphology, and function of the different cell types in airway epithelia for decades, the recent characterisation of new chemosensory functions in several cell types provides new astonishing insights into the airway epithelial function.

1.2. Mucociliary Clearance and Airway Lining Fluid. An intact mucociliary clearance is essential for a healthy lung and is part of the innate immune system. It is responsible for cleaning the airways from inhaled pathogens and particles. Its function is mainly dependent on two parameters: ciliary beat and ion transport. Thus, the ciliated cells occupy an essential role in the mucociliary clearance because of the coordinated beating of their cilia and the set of ion channels they express. The variety of ion-absorbing and ion-secreting mechanisms described in the subsequent paragraph enables

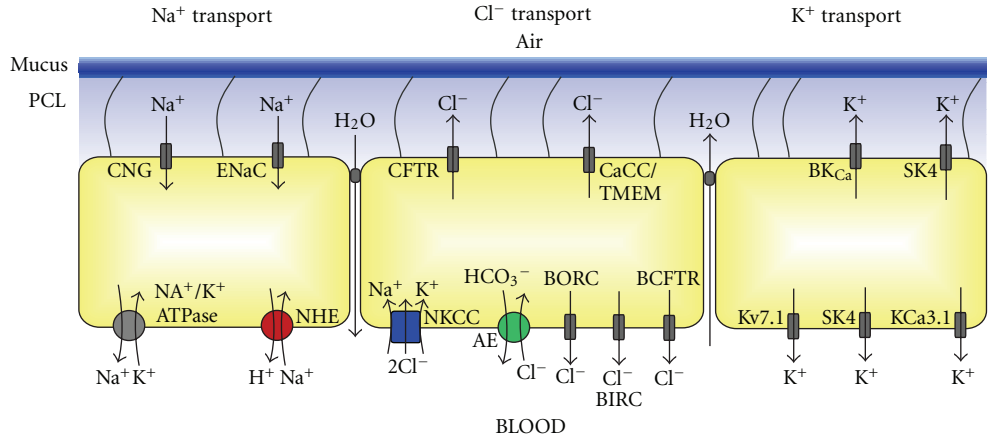

FIGURE 2: Schematic drawing of ciliated airway epithelial cells with Na^+, Cl^-, and K^+ channels and transporters. On the apical side, the airway epithelium is covered by the airway surface liquid that consists of the periciliary liquid (PCL) surrounding the cilia and the mucus layer covering the cilia. The mucus layer with its trapped particles is transported orally by ciliary beat of the ciliated epithelial cells. The composition of the PCL is regulated by ion transport processes, mainly apical Na^+ reabsorption and Cl^- secretion, which H_2O follows passively along the osmotic gradient. Due to transparency reasons, the Na^+, Cl^-, and K^+ channels and transporters have been depicted in different cells (left: Na^+ transport, middle: Cl^- channels, right: K^+ channels), although most of them are usually found in the same cell. The left cell depicts transepithelial Na^+ reabsorption mediated by concerted activity of apical epithelial Na^+ channels (ENaC) and the basolateral Na^+/K^+ ATPase. Apical cyclic nucleotide-gated cation channels (CNG) might also contribute to Na^+ reabsorption. Additionally a Na^+/H^+ exchanger (NHE) has been identified in airway epithelial cells for regulation of intracellular pH. In addition to Na^+ reabsorption airway, epithelia display a prominent apical Cl^- secretion that is mainly mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) in humans and to a lesser extent by Ca^{2+}-dependent Cl^- channels (CaCC) such as the TMEM channels (middle cell). This secretion is kept up by the basolateral $Na^+/K^+/2Cl^-$ cotransporter and the HCO_3^-/Cl^- exchanger (AE). Additionally three basolateral Cl^- channel types have been identified: a basolateral outward rectifying channel (BORC), a basolateral inward rectifying channel (BIRC), and a basolateral CFTR-like channel (BCFTR). These channels have been suggested to be involved in modulation of apical Cl^- secretion. The right cell depicts the K^+ channels so far identified in airway epithelium that are supposed to modulate apical Cl^- secretion. In the basolateral membrane, several voltage-dependent K^+ channels have been identified (Kv7.1–Kv7.5). Ca^{2+}-dependent K^+ channels have been characterized in the apical and the basolateral membrane (SK4, BK_{Ca}, $K_{Ca}3.1$).

the airway epithelial cells to control transepithelial water flow and, thus, to regulate the composition of the periciliary liquid (PCL) surrounding the cilia for optimal ciliary beat [13, 14]. The PCL together with the mucous layer that covers the PCL forms the airway surface liquid (ASL, see Figure 2, [14]). The mucous layer with all its trapped particles and pathogens is transported orally by the ciliary beat and by that forms an important part of the innate immunity of the lung. Thus, severe effects such as respiratory infections are observed when ciliary beat is impaired due to defects in its regulation [15]. Additionally the ASL of the conducting airways represents an important part of the innate immunity, because it contains immunoreactive proteins, such as the Clara cell secretory protein as well as the surfactant proteins A (SP-A) and D (SP-D) that are secreted by the airway epithelial cells [5, 6]. The endogenous function of Clara cell protein is not fully understood, but it is thought to have immunomodulatory functions [5]. SP-A and SP-D play an important role in recognising inhaled pathogens and the innate host defence, and SP-A- and SP-D-deficient mice are more susceptible to death induced by respiratory pathogens [6, 16] like the fungus *Aspergillus fumigatus* [16].

As mentioned above, ion transport processes regulating composition and height of the PCL are important for optimal mucociliary clearance. PCL composition is mainly regulated by Na^+ reabsorption due to the concerted activity of the basolaterally located Na^+/K^+-ATPase and apically located epithelial Na^+ channels (ENaCs) and by Cl^- secretion involving apically located Cl^- channels [17]. Severe respiratory impairment due to malfunction of these ion transport processes is associated with cystic fibrosis (CF) lung disease, where Cl^- secretion is reduced and Na^+ reabsorption is increased due to defective cystic fibrosis transmembrane conductance regulator (CFTR) channels [18, 19]. This was generally assumed to lead to a reduced height of the PCL, impairment of ciliary beating, plugging of the airways with mucus, and inflammation caused by inhaled pathogens, such as *Pseudomonas* bacteria [14]. Yet, the general view of the effects of CF, being caused by a reduced ASL, might have to be reconsidered. A recent study in pig trachea detected no difference in the height of the ASL under basal conditions and when CFTR was inhibited, indicating that the effects of CF might be due to a defect in stimulating a transient increase in ASL induced by Cl^- secretion [13]. Therefore, it is of particular interest to understand the mechanisms, which are involved in the control of transepithelial ion transport and water content of the ASL in the airways.

1.3. Ion Transport Processes That Regulate the ASL. In agreement with the general dogma that all airway epithelia (nasal, tracheal, bronchial) are Na^+ absorptive, an amiloride-sensitive, ENaC-mediated component of baseline ion transport

has been detected in airway epithelia of human and a variety of mammalian species such as rabbit, dog, and mouse [18, 20–23]. In addition to that, Cl^- secretion on the apical side is an important parameter for airway epithelial function, because Na^+ absorption and Cl^- secretion regulate passive transepithelial H_2O flow and, thus, the height of the ASL (see above [13]). In this way, these ion transport processes are mainly responsible for providing optimal environment for ciliary beat. Human nasal epithelial cells have been shown to display a predominant Na^+ absorption consisting of an amiloride-sensitive ENaC-mediated and an amiloride-insensitive component, whereas Cl^- secretion plays only a minor role [24]. However, functional Cl^- secretion mediated by the CFTR is essential to regulate PCL viscosity and, thus, maintain functional ciliary beat as visible from cystic fibrosis (CF) patients with defective CFTR [14]. Additionally, there is increasing evidence for K^+ transport playing an essential role in maintaining and regulating airway epithelial membrane potential and ASL [25].

In addition to Na^+, Cl^-, and K^+ channels, a variety of other ion channels has been detected in the airway epithelium. One example is the acid-sensing ion channel 2 (ASIC2), which belongs to the ENaC/degenerin family of ion channels and has been found in ciliated tracheal cells of rats and ciliated cells in the embryonic rat nasal septum epithelium [26, 27]. These channels might be important for pH sensing at birth and for detection of pathogens due to altered pH in the environment and by this may contribute to the function of the innate immune system of the lung [26, 27]. Another example is the truncated variant of the transient receptor potential melastatin 8 (TRPM8) channel that has been characterized in human bronchial epithelial cells [28]. Since this channel is permeable for Ca^{2+} and sensitive to cold stimuli such as cold air, a role in cold-induced alterations of lung physiology has been suggested for this channel by the authors [28].

The following paragraphs and Figure 2 give a short overview of Na^+, Cl^-, and K^+ transport and channels in the airway epithelium, since these are all involved in ASL regulation.

1.3.1. Sodium Transport. The amiloride-sensitive, Na^+-absorptive ENaC has been characterised in human airway epithelium [19, 20]. It has been well established that ENaC forms a trimer usually consisting of the $\alpha\beta\gamma$-subunits [29]. This isoform is widely distributed in human superficial airway and nasal epithelium [19, 20]. However, this is only part of the story, because, during recent years, two different isoforms ($\delta 1$ and $\delta 2$) of a fourth subunit, the δ-subunit, have been detected [30]. This subunit is able to form an amiloride-sensitive functional Na^+ channel together with the β- and the γ-subunit [30, 31]. This functional $\delta\beta\gamma$-ENaC, containing the $\delta 1$ isoform, is similar abundant in human nasal epithelial cells, as the α subunit [32]. Beside ENaC, a nonselective nucleotide-gated cation channel contributes to apical Na^+ absorption in rat airway epithelium (trachea, bronchi, and bronchioles) [33]. On the basolateral side, Na^+ is secreted from the cells by the Na^+/K^+ ATPase that is ubiquitously expressed in airway epithelia and provides the driving force for apical Na^+ reabsorption [34, 35]. Thus, the Na^+/K^+ ATPase together with the ENaC mediate transcellular Na^+ reabsorption. Additionally an amiloride-sensitive, electroneutral Na^+/H^+ exchanger has been identified in ciliated human nasal epithelial cells, although its exact location remains to be determined [36].

1.3.2. Chloride Transport. A large part of the airway chloride secretion in humans is mediated by the apically located cAMP-dependent cystic fibrosis transmembrane conductance regulator (CFTR) channel [37]. In addition to its Cl^- channel function, the CFTR has been proposed to be able to regulate other ion channels, such as ENaC, since CFTR-deficient epithelia as obtained from CF patients often show Na^+ hyperabsorption [18, 19]. But since this could not be confirmed by recent studies [38, 39], the proposed ENaC regulating property of CFTR needs new evaluation. As mentioned above, the deleterious effects of malfunctioning of the CFTR channel are clearly visible in CF lung disease, as summarized by O'Sullivan and Freedman [40]. Thus, a large proportion of studies that investigate airway ion transport processes deal with characterisation of the function and regulation of this channel in normal and CF airway epithelia.

In addition to the CFTR, Ca^{2+}-activated ion channels (CaCCs) are present on the apical side of airway epithelia. The molecular identity of these channels is so far not completely elucidated. However, the transmembrane protein TMEM16A, also termed anoctamin 1 (ANO1), has recently been identified as being a CaCC in the airways [41]. But this channel mediates only a small proportion of the CaCC-induced current [42]. Although other members of the TMEM/anoctamin family like ANO5, ANO6, ANO8, ANO9, and ANO10 have been detected in mouse tracheal epithelium and seem partially to be involved in Cl^- transport [43], their exact role and contribution to Ca^{2+}-activated Cl^- secretion remains to be elucidated.

Additionally, three different basolateral Cl^- channels were detected in human and bovine tracheal epithelium and human nasal ciliated epithelial cells: (1) a basolateral outwardly rectifying Cl^- channel (BORC) that was voltage dependent, activated upon swelling, and DIDS sensitive [44], (2) a basolateral inwardly rectifying Cl^- channel (BIRC) [44], and (3) a low-conductance linear CFTR-like Cl^- channel (BCFTR) that was activated by cAMP [44]. These channels are suggested to be involved in transcellular Cl^- transport processes and in the regulation of apical Cl^- secretion [44]. Additionally electroneutral cotransporters are involved in basolateral chloride transport, which are less easy to characterize by electrophysiological methods. In airway epithelia, $Na^+/K^+/2Cl^-$ cotransporters (NKCC) exist, which support apical Cl^- secretion and assure the cellular supply of Cl^- [45]. Additionally the presence of a HCO_3^-/Cl^- exchanger has been detected in airway epithelial cells [46]. This exchanger has been suggested to play a role in intracellular pH regulation [46] as well as in Cl^- secretion [47].

1.3.3. Potassium Transport. A wide variety of K^+ channels is known to be expressed in airway epithelial cells (for a recent

review on airway epithelial K^+ channels, see [48]). The different K^+ channels are divided into several subgroups according to their number of transmembrane domains, activation mechanisms, and conductance properties. Several K^+ channels have been identified basolaterally in the airway epithelium. These K^+ channels are important for regulating the membrane potential and for maintaining the electrochemical gradient for apical Cl^- secretion. For example, basolateral Kv7.1 channels in human bronchial epithelium have been identified as playing an important role for maintaining cAMP-dependent Cl^- secretion [49], and luminal UTP-induced Cl^- secretion was dependent on basolateral K^+ channel activity in human nasal epithelium [50], confirming the dependence of Cl^- secretion on K^+ channel activity.

Members of all three Ca^{2+}-dependent K^+ channel classes (BK_{Ca} (large conductance), IK/K_{Ca}3.1 (intermediate conductance) and SK (small conductance)) have been found basolaterally in airway epithelia: in human nasal epithelium SK4, K_{Ca}3.1, and BK_{Ca} channels have been identified along with the voltage-sensitive K^+ channels hKvLQT1 (Kv7.1) [50, 51], and in rat tracheal and bronchial epithelial cells, IK channels are present [52]. Additionally basolateral SK4 K^+ channels have been shown to play a role in Ca^{2+}-dependent Cl^- secretion of cultured human bronchial epithelial cells [53]. In addition to Ca^{2+}-sensitive K^+ channels, several voltage-dependent K^+ channels have been identified in human bronchial epithelium: Kv7.1–Kv7.5 [54]. In mouse tracheal epithelium, the predominant K^+ channel subtype is the Kv7.1 channel along with the β-subunit KCNE3, which supposedly modulates epithelial Na^+ reabsorption and Cl^- secretion [55].

In addition to basolateral K^+ channels, the existence of apical K^+ channels has been postulated over a long period of time, and some apical K^+ channels have been identified in airway epithelium. In cultured human bronchial epithelial cells, SK4 K^+ channels contribute to Ca^{2+}-dependent Cl^- secretion [53]. Additionally apical BK_{Ca} channels have recently been identified as being important for regulating the ASL volume in human bronchial epithelium [25]. However, in mouse tracheal epithelium, it has so far not been possible to identify apical K^+ channels by electrophysiological methods [56].

1.4. Modulators of Airway Epithelial Ion Transport. Airway epithelial ion transport processes can be modulated by various signalling molecules that might, for example, interact directly with ion channels or bind to epithelial cell receptors and then modulate ion transport via second messengers after activating intracellular signalling cascades. Thus, many ion channels are sensitive to an increase of the intracellular Ca^{2+} or cAMP level. As mentioned above, several Ca^{2+}-activated Cl^- and K^+ channels are expressed in airway epithelia, and the CFTR channel in airway epithelia might be the most prominent example for a cAMP-regulated ion channel [25, 41]. Additionally it has been shown more recently, for lung and airway epithelial cells, that the gaseous molecules nitric oxide (NO) and carbon monoxide (CO) are able to modulate ion transport, especially Na^+ transport [57–60]. But this is apparently highly model and species dependent, because in human nasal epithelium no influence of NO on transepithelial ion transport could be detected, although NO increased the intracellular Ca^{2+} level [61]. Yet the mechanisms by which gaseous molecules influence airway epithelial ion transport are so far not fully understood.

Prominent examples for modulators of ion channels that act after binding to membrane receptors are the purinergic nucleotides ATP and UTP. The role for purinergic receptor agonists in acting as secretagogues when present extracellularly in the airway epithelium has been well investigated during the last decade. An UTP-mediated apical Cl^- secretion has been shown in human nasal epithelium [50]. Additionally in mouse tracheal epithelium, luminal ATP or UTP is able to induce transient Cl^- secretion and a sustained inhibition of Na^+ absorption [23]. Activation of different purinergic receptors induces Cl^- secretion in this tissue that involves the CFTR channel and Ca^{2+}-activated Cl^- channels [62, 63]. As reviewed below, ACh represents another example for modulators of ion channels binding to receptors in the membranes of airway epithelial cells.

1.5. ACh as a Modulator of Airway Epithelial Ion Transport. Acetylcholine (ACh) is able to act on airway epithelial ion transport after binding to muscarinic and nicotinic receptors [23, 64]. The role of the cholinergic system of the airways has recently been thoroughly reviewed by Kummer et al. [65]; thus, the following paragraph will focus only on the effect of ACh on airway epithelial ion transport processes.

On the one hand, ACh is able to act on airway epithelia as a neurotransmitter, released from cholinergic nerve endings. This neuronal ACh has been shown to transiently act on epithelial ion transport processes via basolaterally ACh receptors. Cholinergic stimulation on the basolateral side of the epithelium leads to an initial transient apical Cl^- and increased basolateral K^+ secretion and subsequently to a decreased Na^+ absorption in sheep tracheal epithelium [66]. Along with these observations, a nicotine-induced decrease of amiloride-sensitive Na^+ absorption was observed in human nasal epithelium [64]. A cholinergically induced current stimulation has also been observed in monkey bronchial epithelium [67]. Similar observations have been made by Kunzelmann and coworkers in mouse tracheal epithelium, by detecting an increased cholinergically induced current that was supposedly due to a cholinergically mediated stimulation of basolateral SK4 K^+ channels increasing the driving force for an apical Cl^- secretion [23].

On the other hand, it has been well established during the last years that ACh can additionally be synthesised and released by different nonnervous cells such as airway epithelial cells themselves [68, 69, 69, 70]. Evidence for this is derived from detection of the ACh-synthesising enzyme choline acetyl transferase as well as ACh in airway epithelial cells and by identifying organic cation transporters as possible molecules for mediating ACh release on the apical and basolateral side of the airway epithelium [70, 71]. In this context, the terms nonneuronal cholinergic system and nonneuronal ACh have been introduced to distinguish it from

the cholinergic system and ACh in the nervous system [68]. Considering the above-described effect of neuronal ACh on airway epithelial ion transport, nonneuronal ACh released from epithelial cells represents an emerging new field as a modulator of airway epithelial ion transport. It is tempting to speculate that nonneuronal ACh might act as a luminal secretagogue similar to extracellular ATP or UTP.

1.6. Models for Studying Airway Epithelial Ion Transport. Over the last decades, a wide variety of different models for studying the airway epithelium and especially airway epithelial ion transport have been established. Mostly electrophysiological measurements such as patch clamp and the Ussing chamber techniques have been used to assess epithelial ion transport properties and to investigate the function of defined ion channels. Among these techniques, the Ussing chamber measurements have up to now been proven to be a useful tool. Along with this, one recent study describes the Ussing chamber measurements of bronchial epithelial cells as being more sensitive than radioactive flux measurements for investigating Cl^- transport [72].

The use of airway epithelial cell cultures grown in monolayers is quite common to investigate epithelial ion transport in vitro, although the ion conductance properties have been shown to depend on culture conditions [73], and there are some minor genetic differences between in vitro cultured airway epithelial cells and freshly isolated cells [74]. Airway epithelial cell cultures exist from humans and many mammalian species. Cultured human cells of all three airway epithelia types have been investigated: bronchial epithelial cell cultures [53, 72], tracheal epithelium [44], and human nasal epithelial cells [32, 36, 64]. Additionally cultured cells from many mammalian species have been used over time such as monkey bronchial epithelial cells [67] and dog tracheal epithelial cells [22]. Cultures of one single epithelial cell type, such as rabbit bronchiolar Clara cells, have also been used [21]. Besides cultured cells, freshly isolated intact tissues are used to study airway epithelial ion transport such as murine trachea [23], equine trachea [45], pig trachea [13], guinea pig trachea [35], and sheep trachea [66]. Thus, considering the variety of available models from different species and airway epithelial types for characterising airway epithelial ion transport, it is important to carefully choose the model according to the problem that needs to be investigated.

2. The Alveolar Epithelium

Air-breathing mammals realise their demand of oxygen by a huge gas exchanging area represented by the alveolar surface of the lungs. Maximisation of this area is achieved by miniaturisation of the lung structures from the upper airways down into a large number of small-sized subunits, the alveoli. The human lung is made of ~480 million of these small bubble-like structures [75]. This special lung design maintains a large surface area of contact between air and blood [76] with a minimum requirement of place. During inspiration this area comprises ~120 m^2 (as big as the expanse of a tennis-curt), and this equals 99% of the surface area of the lung [76–78]. Therefore, the lung alveolar system represents the largest surface area of the body that is exposed to the outer environment [77].

The most important requirements for an efficient air-exchanging structure are first, it must have a vast surface area, and this is achieved by the miniaturisation. Second, it must be thin to facilitate gas exchange between the environment and the organism. This has been realized by an anatomical characteristic that is referred to as the three-ply design and is accomplished by the alveolar epithelial barrier, the basal lamina, and the endothelial barrier [79].

The alveoli are composed of a continuous layer of epithelial cells referred to as the alveolar epithelium (AE). The AE is very thin (0.1-0.2 μm) [80] and close to the vascular endothelium, which facilitates efficient gas exchange due to the relative short diffusion distance for the breathing gases. The surface of the alveolar epithelium is separated from the gas phase by a fluid layer (alveolar lining fluid, ALF), which covers the entire alveolar epithelium [48, 81]. The ALF originates primarily from fluid infiltration into the alveolar airspace, and this is the product of a pressure gradient between the blood capillaries and the alveolar airspace. The amount and volume of ALF in the alveoli affects gas exchange, because it impairs the diffusion distance for the gases. Therefore, one of the most important functions of the AE is the control and regulation of the volume and electrolyte composition of the ALF [48, 82]. In order to avoid excessive fluid infiltration into the alveolar airspace, the AE must exhibit an impermeable barrier to limit solute diffusion to keep the alveoli relatively dry. This is mainly achieved by tight junctions (*zonula occludens*), which form a continuous, gasket-like seal near the apical surfaces of adjacent cells in the AE [48, 83]. Thus, tight junctions confine the paracellular passage of lipid-insoluble molecules between the alveolar and the interstitial space [83, 84], and their dynamic permeability is physiologically regulated, for example, by the intracellular calcium concentration [85–87]. Therefore, tight junctions play a crucial role for the regulation of the transepithelial paracellular transport. But the bunch of transepithelial transport processes is mediated via transcellular pathways through the alveolar epithelial cells by ion-transporting proteins.

2.1. Characteristics of Alveolar Type I (ATI) and Type II (ATII) Cells. The AE consists of two different epithelial cell types, alveolar type I (ATI) and alveolar type II (ATII) cells [80, 88–90], which differ in their morphology and function.

ATI cells are large and squamous with a diameter ranging from 50–100 μm and a volume of ~2,000 to 3,000 μm^3 [78, 90]. Although they constitute only 1/3 of the epithelial cells in number, they cover more than 95% of the alveolar surface [91–93]. The precise functions of ATI cells are still discussed and remain largely speculative. For a long time period, it was thought that these cells play solely an important role in gas exchanging processes because of their low metabolic activity [94]. Recent studies demonstrated that ATI cells express transcripts of proteins, that participate in ion transport processes and exhibit the highest known water permeability of any mammalian cell type, indicating an involvement of ATI cells

in ion and water transport processes as well [92, 95–97]. The detection of vesicles and caveolin in ATI cells further suggests that these cells may additionally be involved in the transport of macromolecules in and out of the cells [90, 98, 99].

In contrast to ATI cells, ATII cells are much smaller. They are cuboidal with a diameter of ~10 μm, have a volume of ~450–900 μm^3, and cover the remaining ~5% of the alveolar surface [78, 90, 92]. Although they cover only a relatively small area of the alveolar surface, their number is much higher compared with the number of ATI cells [91, 92]. In contrast to ATI cells, ATII cells have many cell organelles and exhibit a high metabolic activity [94]. The main function of ATII cells is characterized by their ability to synthesize, secrete, and recycle components of the lung surfactant [77, 90, 92, 100]. The surface-active substances of the surfactant reduce the surface tension and prevent the alveoli from collapsing in order to enable an efficient gas exchange. Surfactant components are stored in and secreted via lamellar bodies, and quantification revealed that every ATII cell possesses approximately 120–180 of these special organelles [77].

Further, ATII cells are known to play a crucial role in immune defence responses in the lung. It has been demonstrated that they deliver stimulatory signals for T cells. This provides evidence that ATII cells are able to act as antigen-presenting and, thus, as immune-regulatory cells in the lung [101, 102]. They are also modulating the innate immune response by the production of cytokines [103, 104], participation in inflammatory cell recruitment via the production of monocyte chemoattractant protein 1 (MCP-1) [105], and express different Toll-like receptors [106].

ATII cells also play an important role in tissue repair processes after lung injury, by proliferation and migrating to injured areas. This was first indicated in an autoradiographic study on rats, in which it has been observed that tissue repair after NO_2 damage was only done by ATII cells [107]. ATII cells were, therefore, assumed to be the stem cells of ATI cells [108]. This idea was further supported by findings that under certain conditions cultured ATII cells lost their characteristics and became "ATI-like" cells [109, 110]. Up to date mesenchymal stem cells (MSCs) were detected in the human distal airways, and it seems that these cells are alveolar epithelial progenitor cells and play a crucial role in lung repair processes [111–113]. It is hypothesized that injured lungs produce soluble factors, which stimulate MSCs to proliferate and migrate to sites of injured tissue [113]. Nevertheless, a recent study of Fujino et al. showed for the first time stem cells in adult human lungs that were able to differentiate in ATII cells [114]. In that study undifferentiated progenitor cells from adult human lungs were isolated that expressed surface markers characteristic for MSCs, as well as proteins that are characteristic for ATII cells (e.g., pro-surfactant protein C and CD90) [114]. The possibility that these MSCs may also differentiate into ATI cells is recently discussed but yet unproven [112, 114].

2.2. Alveolar Ion Transport. The ability of alveolar epithelial cells to mediate transepithelial ion transport is very important for the regulation of the ALF, in order to guarantee proper gas exchange. Until the year 1982, there was no information on how lung fluid balance was regulated across the distal airways, and it had been generally believed that differences in hydrostatic and protein osmotic pressures (Starling forces) accounted for the removal of excess fluid from the airspaces of the lung [80]. The first evidence that fluid balance in the lung was regulated by active ion transport mechanisms was given in 1982 by experiments on lungs of anesthetized, ventilated sheep [80, 115, 116]. This hypothesis was supported by later findings that the use of amiloride, an inhibitor of sodium uptake, in AE inhibited 40 to 70% of basal fluid clearance in sheep, rabbits, rats, guinea pigs, mice, and in the human lung [85]. Up to date a variety of different ion channels and transport proteins have been detected in ATI and ATII cells. The following paragraphs and Figure 3 give a short overview of known Na^+, Cl^-, and K^+ transport mechanisms and channels in alveolar epithelial cells, all together maintaining alveolar fluid balance.

2.2.1. Transepithelial Sodium Transport and Fluid Reabsorption. Like in the upper airways, it is generally accepted that the major driving force for fluid reabsorption in AE is provided by passive apical sodium uptake via the amiloride-sensitive epithelial Na^+ channels (ENaCs) (for details see "mucociliary clearance and airway lining fluid") and amiloride-insensitive nonselective cyclic nucleotide-gated (CNG) cation channels [96, 117]. Sodium follows the electrochemical gradient that is maintained by the basolateral ouabain-sensitive Na^+/K^+ ATPase [82, 85, 118]. This process generates a transepithelial osmotic gradient, which facilitates the osmotic removal of water out of the alveoli into the interstitium.

Initially, vectorial ion transport processes were only described for ATII cells [90, 119–122]. Different studies demonstrated the localization of ENaCs and Na^+/K^+ ATPase in ATII cells [123–126]. During this time period, ATI cells were thought not to be involved in transepithelial ion transport. They were only suggested to play a role in water reabsorption due to their high expression of aquaporins and because evidences about the expression of ion transport proteins were missing [90, 119, 120]. More recent data provide evidence that this hypothesis must be reconsidered. In 2002 Borok and colleagues provided evidence for the expression of Na^+/K^+ ATPase and ENaC subunits in ATI cells [97]. Subsequent studies detected an amilorid-sensitive Na^+ uptake as well as an ouabain-sensitive Na^+/K^+-ATPase current in freshly isolated ATI cells [96, 127]. Thus, the ability of ATI cells for active Na^+ reabsorption has been identified [96, 127]. ENaC was also detected in ATI cells by patch clamp single-channel recordings in lung slice preparations of adult rats [128]. These findings indicate that ATI cells participate in transepithelial ion transport processes beside their crucial role in mediating water permeability due to the expression of aquaporin 5 (AQP5) [129].

Although amiloride-sensitive Na^+ uptake via ENaCs is suggested to represent the bulk of transepithelial Na^+ reabsorption, also amiloride-insensitive pathways were detected in ATI and ATII cells [96]. This amiloride-insensitive Na^+

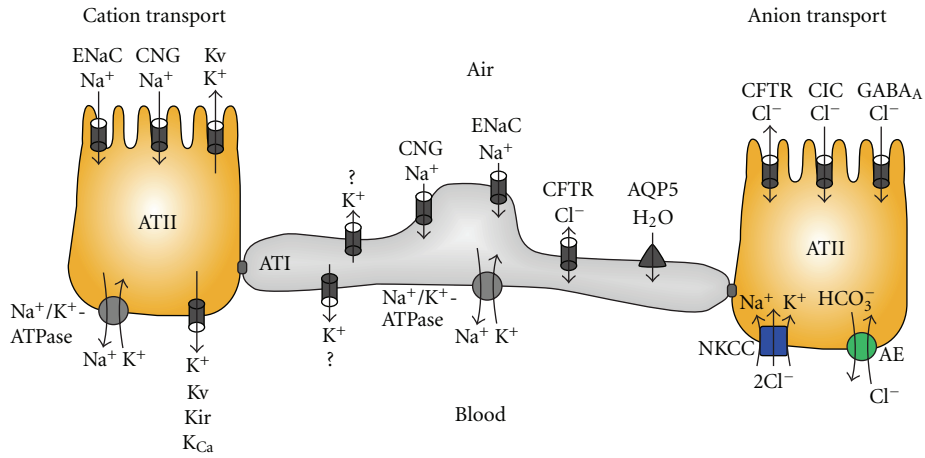

Figure 3: Ion transport proteins identified in alveolar type I (ATI) and alveolar type II cells (ATII). In ATII cells a variety of ion transporting proteins have been identified (ENaC: epithelial Na^+ channel; CNG: cyclic-nucleotide-gated channel; Kv: voltage-gated potassium channels; Na^+/K^+-ATPase: sodium/potassium ATPase; Kir: inward rectifying K^+ channel; K_{Ca} : calcium-activated potassium channel; CFTR: cystic fibrosis transmembrane conductance regulator; ClC: voltage-sensitive Cl^- channels; $GABA_A$: γ-aminobutyric acid type A Cl^- channel; NKCC: sodium/potassium two chloride cotransporter; AE: anion exchanger). For clarity of the scheme, the subtypes of the different K^+ channels were omitted. ATI cells are similarly equipped with ion transporting proteins. In addition these cells express aquaporin 5 (AQP5). The molecular identity of the K^+ channel described is not known yet.

reabsorption is suggested to be mediated by nonselective cyclic nucleotide-gated (CNG) cation channels [117, 130, 131]. These channels can be activated by micromolar concentrations of cGMP, are inhibited by di- and trivalent cations, and show no preference for Na^+ over K^+ as permeating ions [131]. It has been proposed that CNG channels also play a role in alveolar fluid reabsorption, since exogenous cGMP stimulates lung liquid absorption [117].

2.2.2. Chloride Transport by the Alveolar Epithelium. Beside proteins that participate in Na^+ reabsorption, different Cl^- channels were identified in AE. For example, the CFTR Cl^- channel has been identified to participate in alveolar fluid balance [132–135]. But the function of CFTR in AE is controversial since some studies indicate that CFTR is involved in Cl^- absorption [136–138], while others report that the CFTR is involved in Cl^- secretion [132, 135]. Similar to the proteins facilitating Na^+ reabsorption, primarily the CFTR was suggested to be present solely in ATII cells [136]. Meanwhile, CFTR function and protein expression have been confirmed in freshly isolated ATI cells by patch clamp experiments and immunohistochemistry [134]. Beside the CFTR, some other Cl^- channels have been detected in the alveolar epithelial cells. There is evidence concerning the presence of the ionotropic γ-aminobutyric acid type A ($GABA_A$) Cl^- channel [139] and different types of voltage-gated chloride channels (CLC5 and CLC2) [140]. Knowledge about Cl^- transport in alveolar epithelial cells is scarce, and the role of Cl^- channels in the AE is still discussed. One reason for this problem may be due to the problems of using isolated and cultured alveolar epithelial cells. There is sufficient evidence demonstrating that the ion transport properties of the cells vary depending on the culture conditions and cultivation time [141, 142] (more details below). Therefore, deciphering the role as well as the molecular identity of Cl^- channels in the AE remains a challenge for future studies.

Usually Cl^- channels are suggested to be localized in the apical membrane of pulmonary epithelial cells. In a recent study from our group, we were able to identify the presence and function of a Cl^- channel in the basolateral membrane of AE cells [47]. In this study freshly dissected lungs from *Xenopus laevis* were used for electrophysiological Ussing chamber recordings, and inhibition of basolateral Cl^- channels was observed to influence apical Cl^- secretion [47].

Last but not least, the participation of electroneutral cotransporters should be mentioned as important components of transepithelial Cl^- transport. For example, $Na^+/K^+/2Cl^-$ cotransporters (NKCC), K^+-Cl^- cotransporters (KCC), and HCO_3^-/Cl^- exchangers have been identified in AE [143–147]. These proteins, as known from the airway epithelium, are involved in cellular Cl^- uptake against the electrochemical gradient. This uptake is crucial to facilitate the passive diffusion of Cl^- along its electrochemical gradient.

2.2.3. Alveolar Potassium Transport. One of the main functions of K^+ channels in epithelia is to control the membrane potential and, thus, to maintain an electrochemical gradient that is required for ion and fluid transport [48]. In addition there is evidence that they are able to sense changes of oxygen levels [48]. Thus, there has been a growing interest in the research of K^+ channels in the AE, and up to date a wide variety of K^+ channel subtypes has been detected.

K^+ channels are classified in three main groups, according to their predictive number of transmembrane domains (TMD): (1) six TMD K^+ channels: consisting of voltage-dependent K^+ channels (Kv) and Ca^{2+}-activated K^+ channels (K_{Ca}), (2) four TMD K^+ channels represented by the two-pore domain K^+ channels (K_2P), and (3) two TMD K^+

channels also known as inward-rectifying K^+ channels (Kir) [48]. Up to date in alveolar epithelial cells, no members belonging to the group of K_2P channels have been detected. In contrast to this, members of the six TMD K^+ channel family and the Kir channel family have been found in alveolar epithelial cells. For example, the expression of different Kv channel subtypes (i.e., KvLQT1, Kv1.1, 1.3, 1.4, 2.2, 4.1, 4.2, 4.3, and 9.3) was reported in ATII cells [48, 81, 148, 149]. In addition members of the K_{Ca} channels like the large-conductance K_{Ca} channel (BK_{Ca}) or high-conductance K_{Ca} channels (maxi-K_{Ca}, Slo1) have been detected in ATII cells [149]. In particular the BK_{Ca} channels are suggested to act as oxygen sensors, since their open time is reduced in response to decreased oxygen levels [150].

Among the proteins belonging to the Kir channel family, transcripts encoding Kir2.1 (IRK1) have been detected in ATII cells isolated from fetal guinea pig lung [151], although the function of this channel is unknown. Another member of the Kir family has been found in adult alveolar cells, Kir6.1, which is able to assemble the ATP-sensitive K^+ channel (K_{ATP} channel), when coexpressed with the sulfonylurea receptor (SUR) subunits [152, 153]. The K_{ATP} channels are known to act as metabolic sensors, since their activity depends on the concentration of intracellular ATP. Changes of the metabolic state of the cell and, thus, changes of intracellular ATP levels influence the activity of the channel, and this affects the membrane potential [154–156]. In AE there is evidence that K^+ channels may be involved in alveolar epithelial cell repair processes [48], since the inhibition of K_{ATP} and KvLQT1 K^+ channels reduces wound healing, cell migration, and proliferation in a model of mechanical injury of primary cultured rat ATII cells [153]. Other studies hypothesise that modulation of K^+ channel activity exerts sustained control in Na^+, Cl^-, and fluid transport, by regulating the expression of ENaC as well as CFTR [149].

In summary, both types of alveolar epithelial cells express the following cation channels: (a) ENaCs (nonselective and highly selective channels) [134, 157], (b) cyclic nucleotide-gated channels (CNGs) [134], and (c) a variety of K^+ channels [48, 148, 158]. As anion channels, the CFTR channel, the $GABA_A$ channel [139], different voltage-gated chloride channels (CLC5 and CLC2) [140], and yet unidentified basolateral Cl^- channels [47] have been detected (Figure 3).

2.3. Clinical Relevance of Alveolar Fluid Balance. Maintaining alveolar fluid clearance by active ion transport mechanisms is one of the main functions of the AE. Alveolar fluid clearance seems to depend mainly on Na^+ channel activity, since an impaired Na^+ reabsorption is associated with the formation of pulmonary oedema due to impaired fluid reabsorption. This was shown in an experiment with α-ENaC knock-out mice, which leads to the death of newborn mice due to their inability to clear their lungs of fluid in adaption to air breathing [159]. Although the formation of pulmonary oedema could have different causes, a decreased alveolar fluid clearance is a hallmark of pulmonary oedema.

Hydrostatic pulmonary oedema, for example, are caused by an acute elevation of left heart atrial pressure, which results in an increased pressure in the pulmonary vein and, thus, in increased fluid flux from the pulmonary capillaries into the alveolar airspace [78]. As a consequence, the gas exchange across the AE is decreased. This leads to local hypoxia, which then results in the decreased expression of ENaC and Na^+/K^+ ATPase and, thus, insufficient clearance of the fluid from the airspace [160–163].

Other lung diseases, like acute lung injury (ALI) or its more severe manifestation the acute respiratory distress syndrome (ARDS), are also related to pulmonary oedema formation associated with damages of the alveolar-capillary barrier [78, 164, 165] caused by bacterial sepsis, acid aspiration, smoke inhalation, and reperfusion injury after lung transplantation [78].

Another incident for the formation of pulmonary oedema is represented by artificial ventilation. This ventilator-induced lung injury (VILI) is reasoned by over-distention of the alveoli leading to an increased alveolar-capillary permeability and, thus, influx of oedema fluid into the alveoli [164, 166]. Although modified ventilation strategies proved to be beneficial to avoid VILI [167, 168], targeting alveolar fluid absorption mechanism is still a major therapeutic option for the resolution of the oedema fluid in these patients [169, 170].

2.4. Model Systems Used for Alveolar Transepithelial Ion Transport Studies. Due to the miniaturization of the mammalian lung structures, studies of alveolar ion transport processes using native tissue are difficult. Pioneer studies used to instil fluid into the lungs (in vivo or isolated lungs) and determined the rate at which water and solutes were resolved from the lung lumen [82, 115].

Other approaches have been performed by the Ussing chamber measurements using amphibian lungs, which depending on species either secrete Cl^- or absorb Na^+ and, thus, exhibit more or less the situation known from mammalian fetal respiratory epithelium (Cl^- secretion) or adult alveolar epithelium (Na^+ absorption) [82, 171]. In this context, the African clawed frog *Xenopus laevis* should be mentioned. *X. laevis* lungs seem to be a useful model for studying transepithelial ion transport processes since using it enables the use of a native alveolar epithelium. The main benefit of the *Xenopus* lung is its relative simple, sac-like lung anatomy [172]. This enables the dissection of the organ to a flat preparation suitable for transepithelial Ussing chamber measurements. A morphological similarity to the mammals is the three-ply design of the blood-air barrier [79]. Instead of the mammalian AE, the pulmonary epithelium of *X. laevis* consists of only one cell type [172, 173]. But these cells exhibit morphological properties of ATI cells [172], with functional properties that are characteristic for ATII cells (surfactant secretion) [173]. In addition, *Xenopus* AE is classified as a sodium-absorbing epithelium in which the function of ENaCs and the Na^+/K^+ ATPase has been observed [173, 174]. Further, the CFTR Cl^- channel was detected in the apical membrane [135], as well as Cl^- uptake via a basolaterally located anion exchanger [47, 147]. By using a modified Ussing chamber, it was also possible to investigate

the impact of mechanical stress on transepithelial ion transport processes [175].

A lot of the studies focusing on alveolar epithelial ion transport processes were done on isolated and cultured ATI and ATII cells. ATII cells have been intensively investigated for more than 30 years [109, 176] and were early isolated from a wide range of species, as, for example, from rat, mice, rabbit, cow, hamster, and human [127, 141]. The ion transport mechanisms of ATII cells were investigated by electrophysiological measurement techniques [127, 177]. Further patch clamp measurements are used for ion transport investigations on cultured and freshly isolated ATII cells [119]. A problem of isolated and cultivated AE cells is evident from several studies. Their ion transport properties vary depending on the culture conditions [141, 157]. For example, when ATII cells were grown on plates submerged in culture-media that lacked steroids, the predominantly detected Na^+ channel was a nonselective cation channel (NSC), while, in cultured cells in the presence of steroids and air interface, a low-conductance highly Na^+-selective channel (HSC) was predominantly found [157]. In a recent study, this phenomenon has also been shown in a cell line [57].

In contrast to ATII cells, isolation and culture techniques of ATI cells have only recently been developed and are continually evolving [88, 141, 142]. Up to date, isolation of ATI cells was solely successful from rat lungs [92, 142, 178, 179]. However, isolation of ATI cells was a milestone enabling the identification of ion transport mechanisms in these cells by patch clamp measurements [127, 134].

Another possibility for studying alveolar ion transport is represented by electrophysiological recordings using lung slice preparations. For this approach, mammalian lungs are inflated with agarose or gelatine and are then cut in sections of 250–300 μM [128, 158, 180]. This preparation procedure preserves the alveolar architecture and allows access to intact AE cells. Identification and discrimination of ATI and ATII cells are enabled by fluorescence microscopy using ATI- and ATII-selective markers [128, 180]. The lung slices can then be used for patch clamp measurements enabling single-channel recordings as demonstrated for the detection of amiloride-sensitive ENaCs in ATI cells [128].

From our perspective, it might be beneficial to use different techniques in combination with different models to reveal the mechanisms of how transepithelial ion transport is accomplished. Presently, the perfect model for alveolar ion transport studies is not available, but it might be considered that every model has some advantages as well as disadvantages. Therefore, choosing a particular model depends on the question that should be addressed.

3. Concluding Remarks

Ion transport accomplished by the pulmonary epithelial cells is imperative for proper lung function. Although the basic mechanisms of transepithelial ion transport are defined, it is obvious that a detailed knowledge concerning the underlying processes and the interaction of the different ion transporting proteins in particular is poorly understood. The situation becomes even more complicated when considering that each epithelium (airway and alveolar epithelium) consists of different cell types and that these different cells are differentially equipped with ion transporting proteins. In addition, a huge variety of different ion channels have been identified in these epithelial cells, although their function is unknown. This is at least evident when one considers that up to 40 different types of K^+ channels were detected in pulmonary epithelial cells [48], but their particular function is still obscure. Regarding the identity as well as the function of Cl^- channels and Cl^- transporters, the situation is also far from being understood.

Therefore, let us roll up our sleeves, and let us rise to accomplish this particular challenge!

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