Contribution of CFTR to apical-basolateral fluid transport in cultured human alveolar epithelial type II cells

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Fang, Xiaohui, Yuanlin Song, Jan Hirsch, Luis J. V. Galietta, Nicoletta Pedemonte, Rachel L. Zemans, Gregory Dolganov, A. S. Verkman, and Michael A. Matthay. Contribution of CFTR to apical-basolateral fluid transport in cultured human alveolar epithelial type II cells. Am J Physiol Lung Cell Mol Physiol 290: L242–L249, 2006. First published September 2, 2005; doi:10.1152/ajplung.00178.2005.—Previous studies in intact lung suggest that CFTR may play a role in cAMP-regulated fluid transport from the distal air spaces of the lung. However, the potential contribution of different epithelial cells (alveolar epithelial type I, type II, or bronchial epithelial cells) to CFTR-regulated fluid transport is unknown. In this study we determined whether the CFTR gene is expressed in human lung alveolar epithelial type II (AT II) cells and whether the CFTR chloride channel contributes to cAMP-regulated fluid transport in cultured human AT II cells. Human AT II cells were isolated and cultured on collagen I-coated Transwell membranes for 120–144 h with an air-liquid interface. The cultured cells retained typical AT II-like features based on morphologic studies. Basal fluid transport was 0.9 ± 0.1 μl·cm⁻²·h⁻¹ and increased to 1.35 ± 0.11 μl·cm⁻²·h⁻¹ (mean ± SE, n = 18, P < 0.05) by stimulation with cAMP agonists. The CFTR inhibitor, CFTRinh-172, inhibited cAMP stimulated but not basal fluid transport. In short-circuit current (Isc) studies with an apical-to-basolateral transepithelial Cl⁻ gradient, apical application of CFTRinh-172 reversed the forskolin-induced decrease in Isc. Real time RT-PCR demonstrated CFTR transcript expression in human AT II cells at a level similar to that in airway epithelial cells. We conclude that CFTR is expressed in cultured human AT II cells and may contribute to cAMP-regulated apical-basolateral fluid transport.

MATERIALS AND METHODS

Isolation of human lung alveolar epithelial cells. Human alveolar AT II cells were isolated by a modification of methods previously described (2, 8, 11). In brief, AT II cells were isolated from cadaveric human lungs that were declined by the Northern California Transplant Donor Network. We selected the lobe of the lung that had no obvious consolidation or hemorrhage by gross inspection. Previous studies described (2, 8, 11). In brief, AT II cells were isolated from cadaveric human lungs that were declined by the Northern California Transplant Donor Network. We selected the lobe of the lung that had no obvious consolidation or hemorrhage by gross inspection. Previous studies indicate that these lungs are generally in a relatively normal condition and whether the CFTR chloride channel contributes to cAMP-regulated fluid transport. The primary objective of this study was to determine whether CFTR is expressed in human lung AT II cells and whether the CFTR chloride channel contributes to cAMP-regulated fluid transport in human AT II cells cultured with an air-liquid interface.

NEW MATERIALS AND METHODS

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D.Nase (500 μg/ml). The cell-rich fraction was filtered by sequential filtration through one layer of sterile gauze, two layers of gauze, and 150-μm and 30-μm nylon mesh. The solution was then layered onto a discontinuous Percoll density gradient 1.04–1.09 g/ml solution and centrifuged at 400 g (1,500 rpm) for 20 min. The upper band containing a mixture of type II pneumocytes and alveolar macrophages was collected and centrifuged at 800 rpm for 10 min. The cell pellet was washed and resuspended in Ca²⁺/Mg²⁺-free PBS containing 5% FBS. The cells were then incubated with magnetic beads coated with anti-CD-14 antibodies at 4°C for 40 min under constant mixing. Then the beads were depleted with a Dynal magnet (Dynal Biotech, Oslo, Norway). The remaining cell suspension was incubated in human IgG-coated tissue culture-treated Petri dishes in a humidified incubator (5% CO₂, 37°C) for 90 min. Unattached cells were collected and counted. Cell viability was assessed by the trypan-blue exclusion method. The purity of isolated human AT II cells was examined by Papanicolaou staining.

**Immunostaining of zonula occludens protein-1 of human AT II cell monolayers.** For detection of intercellular tight junctions, human AT II cells were seeded on Lab-Tek II chamber slides at a density of 2× 10⁶ cells/cm². At 120 h after seeding, the cell monolayer was washed twice with 4°C PBS and fixed in 4% paraformaldehyde for 30 min. The slides were then washed with PBS three times for 10 min each in a gently shaking chamber at room temperature. The cell preparations were subsequently quenched with NH₄Cl and glycine for 10 min and permeabilized with PBS/fish skin gelatin (FSG)/saponin (SAP)/RNase for 30 min at 37°C. After being incubated with primary rabbit polyclonal anti-zonula occludens protein-1 (ZO-1; Chemicon, Temecula, CA) for 1 h at 37°C, the slides were again washed four times with PBS/FSG/SAP/RNase for 10 min and incubated with a mixture of secondary rhodamine-labeled anti-rabbit-IgG and 4’,6-diamidino-2-phenylindole (Molecular Probes) for 45 min. Then the slides were mounted with Vectashield mounting medium and covered with cover glass. Images were obtained by a Bio-Rad MRC-1024 laser scanning confocal microscopy system.

**Cell markers for human AT II and AT I phenotypes.** To evaluate the phenotype of human AT II cells, we examined the morphological characteristics of these human AT type II cells grown on Transwell membranes cultured with an air-liquid interface. Cells grown on Transwell membranes at 120 h were preincubated at 37°C in DMEM with Lysotrack green DND-26 (150 nmol/l, 30 min; Molecular Probes), a fluorescent dye that selectively stains lamellar bodies in primary culture of AT II cells (1, 16). Images were obtained with a Nikon Inverted Microscope (TE 2000-E) and Simple PCI Advance Image Capture system. Cell monolayers at 120 h were fixed in 4% paraformaldehyde and stained with aquaporin 5 antibodies (AQP5, Chemicon) to determine the relative numbers of AT I-like cells. Monolayers on Transwell membranes were also fixed in 4% paraformaldehyde for 20 min and then incubated in a small volume of primary antibody (mouse anti-human AT II cell antibody provided by Dr. L. G. Dobbs, University of California, San Francisco) for 20 min. After washing the cells with RPMI 1640 five times, we incubated the cells with a secondary antibody (goat anti-mouse IgG). The cells were washed, coverslipped, and viewed under fluorescence microscope.

**Ultrastructure of cultured human AT II cells.** Human AT II cell monolayers grown on Transwell membranes were fixed with 3% (wt/vol) Karnovsky fixative for 1 h at 0°C, and the membranes were removed. The cells were postfixed for 1 h in 1% veronal buffered osmic acid and then were dehydrated in graded ethanols and/or propylene oxide. The cell preparations were then embedded in Epon or Araldite resins cured at 60°C. Thin sections were contrasted with saturated aqueous uranyl acetate and Reynolds lead citrate. The sections were then imaged with a JEOL 1200 EX transmission electron microscope operating at 80 kV.

**Measurement of fluid transport across human AT II cell monolayers.** The rate of fluid transport by human AT II cells was measured as described for rat AT II cells (14). Transwells (pore size 0.4 μm, collagen I coated) with protein permeability >3% over 24 h were discarded, as described before (14). With this criterion, 20% of the Transwells were discarded. Measurement of fluid transport from the apical to basolateral membranes of the human epithelial cell monolayer was done at 120 h after the isolation and plating of the cells, 48 h after an air-liquid interface had been achieved. We pipetted 150 μl of serum-free medium containing 0.3 μCi/ml ¹³¹I-albumin into the apical chamber of the Transwell in a humidified tent. Five minutes after the ¹³¹I-albumin was added, 20 μl of the medium were aspirated as the initial sample. After 24 h, 20 μl were aspirated from the upper compartment of the Transwell as the final sample. The samples were weighted and counted in a gamma counter (Packard MINAXI 5000 series). Fluid transport was calculated as before (14). All fluid in the lower compartment at the end of experiments was also collected to determine the protein permeability by measuring the unidirectional flux of labeled ¹³¹I-albumin from the apical (upper compartment) to the basolateral side (lower compartment). Forskolin and IBMX (10 μM each), amiloride (10 μM), and ouabain (10 μM) were added to the apical culture medium. CFTR inh-172 (10 μM) (26) was added to both upper and lower compartment in selected studies. The experiments were done in eight different human lung preparations.

**Short-circuit current study of human AT II cell monolayers.** Freshly isolated human AT II cells were seeded in collagen I-coated Snapwell membranes (pore size 0.4 μm, surface area 1.13 cm²). The culture medium was changed daily. Hydrocortisone (10⁻⁷M) and insulin-transferrin-Sel-G were added to the culture medium. The cells were grown in an air-liquid interface 72 h after seeding. At 120–144 h, the Snapwell inserts were mounted in an Ussing chamber system (World Precision Instrument, Sarasota, FL). For experiments without a chloride gradient, we used symmetrical bathing solution in both hichemicals. The bathing solution contained 130 mM NaCl, 2.7 mM KCl, 15.5 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, 10 mM Na-HEPES, and 10 mM glucose, pH 7.3. For the chloride gradient experiments, the bathing solution in the basolateral chamber was modified by substituting all NaCl with sodium gluconate and increasing the CaCl₂ concentration to 2 mM to compensate for calcium buffering caused by gluconate. The basolateral membrane of the cells was permeabilized with 250 mg/ml amphotericin B. Both hichemicals were bubbled with 5% CO₂, and measurements were performed at 37°C. The hichemicals were connected to a DVC-1000 voltage clamp (World Precision Instrument) via Ag/AgCl electrodes and 1 M KCl agar bridges. Short-circuit current (Iₛ) was digitized and recorded. The experiments were done in seven different human lung preparations.

Iₛ studies were also done in rat AT II cells that were isolated as described previously (14) and seeded on polycarbonate Snapwell membranes (pore size 0.4 μm, surface area 1.13 cm²). The cells were grown on an air-liquid interface 72 h after seeding as described above for human AT II cells. At 120 h, the Snapwell inserts were mounted in the same Ussing chamber system. Experiments were done both in the presence and absence of chloride gradient using the same bathing solution as for human AT II cells. Ten different lung preparations were used in experiments.

**Real-Time RT-PCR.** Gene expression in human AT II and bronchial epithelial cells was measured by a two-step multiplex real-time RT-PCR method previously described in detail (10). Human bronchial epithelial cells were a gift from Dr. W. F. Finkbeiner’s laboratory (San Francisco General Hospital, University of California, San Francisco). Primer and probe sets for genes of interest were designed with “Primer Express” software (Perkin Elmer, Foster City, CA) based on sequencing data from National Center for Biotechnology Information databases and purchased from Biosearch Technologies (Novato, CA). The primer and probe sequences employed are provided in: http://asthmagenomics.ucsf.edu/pubs/publications.htm. Total RNA (~10–20 ng) was reverse transcribed with random hexamers, and the resulting cDNA prod-
uct was amplified using a mix of gene-specific primers and hot-start PCR for 25 cycles. Transcript quantifications were run along with -RT cDNA controls in a 384-well format on an ABI Prizm 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Data were collected as PCR cycle threshold values for each gene and then converted to relative gene copy numbers (GCN) based on linear regression as previously described (10). Transcript copy was normalized using a panel of 10 housekeeping genes. The geometric mean value of the housekeeping genes most stably expressed across the samples was used for normalization using GeNorm software, a recently published approach (Vandesompele).

Normalized GCN in bronchial vs. alveolar epithelial cells were compared by the Student’s unpaired t-test.

RESULTS

Phenotype and electrophysiological properties of cultured human AT II cells. AT II cells were isolated from human lungs that had been rejected for transplantation. Overall, 95% of the electron microscopy sections from those lungs (30 sections from eight patients) that were selected for ion and fluid transport studies showed normal morphology. Characteristic ultrastructural images demonstrated normal alveolar epithelial and endothelial structure, and a segmental bronchus with gland secretion (Fig. 1). Cell yield just after isolation was $2.3 \pm 0.7 \times 10^6$ cells/g of lung tissue. Purity of freshly isolated alveolar type II cells was $90 \pm 3\%$ assessed by modified Papanicolaou staining and staining with a type II cell antibody. Cells grown on collagen I-coated Transwell membranes at 120 h after seeding were stained with Lysotrack green DND-26, which selectively stains lamellar bodies in primary cultures of AT II cells (1, 244).
The monolayer of cells showed green granules representative of lamellar bodies (Fig. 2). Immunostaining of cell monolayers with a type II cell antibody was positive (Fig. 3). Immunostaining of the cell monolayer with AQP5 antibody was negative. Electron microscopy of the monolayer demonstrated that the cells had ultrastructural features that are characteristic of AT II cells, including a cuboidal shape, lamellar bodies, and microvilli (Fig. 2).

Freshly isolated cells grown on collagen I-coated Transwell membranes developed a transmembrane electrical resistance (TER) at 48–72 h, with a peak TER of 700 Ω·cm² at 144 h (Fig. 4A). Epithelial tight junctions were seen after 72 h of culturing by the presence of ZO-1 protein in the cultured monolayers (Fig. 4B). At this time point, the cells showed characteristic AT II cell morphological features as described above. For fluid transport and I_{sc} experiments, cells were used 120 and 144 h after initial plating.

Fluid transport across cultured human AT II cells. The human AT II cells grown on a collagen I-coated Transwell membrane with an air-liquid interface had a protein permeability of 2.3 ± 0.6% over 24 h. Basal fluid transport in these human AT II cell monolayers was 0.9 ± 0.1 μl·cm⁻²·h⁻¹. Amiloride inhibited basal fluid transport by 43%, similar to the effect of amiloride in the intact human lung (39). Fluid transport was stimulated by forskolin and IBMX; amiloride inhibited cAMP-stimulated fluid transport. CFTRinh-172 had no effect on basal fluid transport but inhibited cAMP-stimulated fluid transport to a level not significantly different from basal
After pretreatment with amiloride, forskolin (10 μM) produced no significant change of $I_{sc}$ (Fig. 6A). However, application of apical CFTRinh-172 (10 μM) reduced the $I_{sc}$ by 31% following forskolin ($P < 0.05$). To further evaluate CFTR function, $I_{sc}$ was measured in the presence of a Cl⁻ gradient (130 mM apical, 0 mM basolateral) after permeabilization of the basolateral membrane with amphotericin B (Fig. 6B). In the absence of a chloride gradient, amiloride had no significant effect on basal $I_{sc}$. Apical forskolin (10 μM) produced a downward and sustained change of $I_{sc}$, reflecting Cl⁻ influx through the apical membrane. This current was inhibited by CFTRinh-172 in a dose-dependent manner.

We found a similar pattern in rat AT II cells. Basal $I_{sc}$ in rat AT II was $10.2 ± 2.1$ μA ($n = 6$, mean ± SE) (Fig. 7A). Addition of 10 μM amiloride to the apical chamber inhibited basal $I_{sc}$ by 79%. Stimulation by forskolin after amiloride pretreatment produced a 12% increase in $I_{sc}$ ($P < 0.05$). Subsequent addition of CFTRinh-172 completely blocked this increase in $I_{sc}$ (Fig. 7A). After permeabilization of the basolateral membrane with amphotericin B, forskolin induced a 24% downward and sustained deflection of $I_{sc}$, suggesting a Cl⁻ absorptive current, which was blocked by CFTRinh-172 (Fig. 7B).

**Gene expression profiling.** By real-time RT-PCR, gene expression was compared in human AT II cells and in human bronchial epithelial cells from the same lung (Table 1). The expression of surfactant proteins A, B, C, and D was limited to human AT II cells, and the expression of Muc2 was limited to human bronchial epithelial cells, demonstrating the purity of the isolated human AT II cells. CFTR transcript was detected in the human AT II cells. The ratio of CFTR transcript copy number in human AT II cells compared with human bronchial epithelial cells was close to 1, indicating that CFTR gene expression was similar in the human airway and alveolar epithelial cells. Also, the expression profiles of genes encoding the epithelial sodium channels, ENaC-α and ENaC-β, were similar in the AT II cells and the bronchial epithelial cells (Table 1).
In this study, we found that CFTR is present in human AT II cells and may play a role in cAMP-mediated apical-basolateral ion and fluid transport. Using real-time RT-PCR, we found that CFTR transcript expression in human AT II cells was comparable to the expression in bronchial epithelial cells. Functional evidence for CFTR activity in ion and fluid transport was obtained from measurements of apical-to-basolateral vectorial fluid transport across human AT II cell monolayers in which CFTRinh-172 inhibited fluid transport. Also, in I_sc studies with an apical-to-basolateral chloride gradient, CFTRinh-172 inhibited the forskolin-induced change in I_sc.

These results support our earlier hypothesis that CFTR may play a role in cAMP-upregulated fluid clearance from the distal air spaces of the human lung (13, 42). In the lung, CFTR is generally thought to function in airway submucosal glands and surface epithelial cells. The function of CFTR has been mostly studied in primary cultures of bronchial epithelial cells or airway cell lines (5, 9, 26). However, there is less information regarding CFTR and its possible role in fluid transport from the distal air spaces and alveolar epithelia. In 1994, one study reported expression of CFTR by in situ hybridization in the alveoli of human lungs (12). More recent work indicates that there is CFTR mRNA and protein expression in rat AT II cells (4, 20). However, studies of CFTR and its transport function in human AT II cells have not been reported.

Apical-basolateral fluid transport was measured in human AT II cell monolayers cultured with an air-liquid interface. Under basal conditions, these cultured human AT II cells showed net fluid absorption (apical to basal direction) that was sensitive to amiloride. Amiloride inhibited 43% of basal fluid transport, which was a little less than that reported by other authors in rat AT II cells probably because human AT II cells have more amiloride-insensitive sodium channels. The effect of amiloride in this intracellular study was similar to previous intact human lung study from our group (39). CFTRinh-172 had no effect on basal fluid transport, indicating that basal fluid transport is dependent in part on ENaC (18, 31), but not on CFTR. This finding is in agreement with our prior results in the intact mouse and human lung (13). However, when the epithelium was stimulated with cAMP agonists, the increase in fluid absorption was inhibited by CFTRinh-172, thus demonstrating the involvement of CFTR-mediated Cl^- influx in stimulated fluid transport.

In I_sc studies under physiological conditions (equal Cl^- concentration in apical and basal chambers), amiloride inhibited I_sc in human AT II cells. When the basolateral membrane was permeabilized with amphotericin B and a Cl^- gradient was applied (130 mM apical, 0 mM basolateral), stimulation with forskolin elicited a large and sustained change in I_sc. The direction of the changes caused by forskolin (downward) and subsequently by CFTRinh-172 (upward) was consistent with activation and inhibition of Cl^- influx through CFTR (Fig. 6B). I_sc studies in rat AT II cells showed a similar pattern. How do these I_sc studies relate to the possible contribution of

**DISCUSSION**

**Table 1. Relative transcript copy number for nine genes in human bronchial epithelial cells vs. human alveolar epithelial cells**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Transcript Number × 10^6</th>
<th>Ratio, Alveolar Cells/bronchial Cells</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC2</td>
<td>0.9 ± 0.2</td>
<td>0.003 ± 0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFTPA1</td>
<td>&lt;0.0001</td>
<td>14.2 ± 0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFTPB</td>
<td>0.07 ± 0.01</td>
<td>15.8 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFTPC</td>
<td>0.003 ± 0.0005</td>
<td>9.6 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFTPD</td>
<td>0.07 ± 0.01</td>
<td>5.0 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CFTR</td>
<td>1.2 ± 0.4</td>
<td>1.0 ± 0.5</td>
<td>0.8 0.72</td>
</tr>
<tr>
<td>ENaC-α</td>
<td>4.2 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>0.8 0.7</td>
</tr>
<tr>
<td>ENaC-β</td>
<td>0.003 ± 0.0003</td>
<td>&lt;0.001</td>
<td>0.3 &lt;0.01</td>
</tr>
<tr>
<td>ENaC-γ</td>
<td>1.3 ± 0.08</td>
<td>0.6 ± 0.1</td>
<td>0.5 0.03</td>
</tr>
</tbody>
</table>

Data are means ± SE.
CFTR to fluid transport in human AT II cells? As expected for an anion channel, CFTR may transport Cl\(^-\) in both directions, and thus net ion flux depends on the electrochemical potential for Cl\(^-\) and the transmembrane electrical potential difference. Under symmetrical Cl\(^-\) conditions, the response to cAMP-elevating agents was insignificant. This finding is probably not due to a low activity or expression of CFTR since relatively large currents are observed when a Cl\(^-\) gradient is applied. Therefore, under resting conditions, and after application of amiloride, there is little driving force for transepithelial Cl\(^-\) transport (Fig. 6A). This behavior contrasts with airway epithelial cells, which display large secretory responses under the same conditions (28). It is probable that in human AT II cells, the apical membrane potential and/or the activity of cation/Cl\(^-\) cotransporters is different from that of airway epithelial cells so that Cl\(^-\) across the apical membrane is close to equilibrium when ENaC is inhibited (Fig. 5A). However, when ENaC is allowed to function without amiloride, the influx of Na\(^+\) across the apical membrane probably generates depolarization that favors the absorption of Cl\(^-\) through CFTR. This coordinated NaCl absorption would be the basis for cAMP-stimulated fluid absorption. Recent studies also revealed interdependency between CFTR and the β-adrenergic receptor that is essential for upregulation of active Na\(^+\) transport and fluid clearance in intact mouse lung (35).

We propose that, under basal conditions, active fluid transport is mainly driven by ENaC, although Cl\(^-\) must follow for electroneutrality through an as yet unidentified pathway, possibly another chloride channel. With cAMP stimulation, CFTR opens and more ENaC channels are recruited to the apical membrane (6, 37), so that both Na\(^+\) and Cl\(^-\) absorption are increased. Under such stimulated conditions, Cl\(^-\) permeability provided by CFTR channels is now a limiting factor for fluid transport, accounting for the inhibitory effect of CFTR

Chloride secretion by fetal AT II cells is well accepted as important for establishing the osmotic driving force necessary for fluid secretion into the lumen of the developing lung (15, 32, 34). The role of Cl\(^-\) channels and transcellular Cl\(^-\) absorption in the adult lung has been uncertain. Although a direct link between Cl\(^-\) channel function and CFTR expression was not fully established by these prior studies, previous in situ hybridization and immunolocalization studies provide evidence for CFTR in fetal alveolar epithelial cells (33). After the original report of high-conductance, voltage-dependent Cl\(^-\) channels in adult rat AT II cells (23, 40), there have been conflicting results and conclusions regarding the expression of cAMP-activated Cl\(^-\) channels in these cells. One study (44) suggests that only a small fraction of adult AT II cells express cAMP-activated Cl\(^-\) channels and that Cl\(^-\) transport occurs passively through a paracellular pathway between alveolar epithelial cells. In contrast, Jiang et al. (21) demonstrated that stimulation of adult rat alveolar epithelial cell monolayers with a selective β2-adrenoreceptor agonist resulted in activation of Cl\(^-\) channels in the apical membrane. Cl\(^-\) channel activation stimulates transepithelial Cl\(^-\) absorption (24, 27, 36). Also, studies from other investigators have shown that β-adrenergic receptor stimulation with isoproterenol produced an apical-to-basolateral Cl\(^-\) flux when measured with Cl\(^-\) -selective microelectrodes (25). Inhibition of Cl\(^-\) absorption (50–70%) by Cl\(^-\) channel blockers indicates that the Cl\(^-\) channel in the apical membrane was responsible for Cl\(^-\) uptake (25).

In the experiments presented here, human AT II cells were isolated from lungs that had relatively normal epithelial features as shown by electron microscopy. Human AT II cells were studied between 120 and 144 h, a time period when cultured rat AT II cells are known to have features of both type I and II cells (7, 31). However, recent work indicates that growth of cultured rat AT II cells in an air-liquid interface preserves the AT II phenotype, including the expression of highly selective sodium channels (18). Therefore, the phenotype of human AT II cells was examined with Lysotracker green DND-26 and electron microscopy in this study. Electron microscopy revealed that the human AT II cells were more cuboidal in shape. The cells had microvilli and lamellar bodies, which are typical characteristics of AT II cells. Therefore, the rates of fluid transport in the air-liquid Transwell system probably reflect primarily the transport properties of human AT II cells.

In conclusion, our experiments demonstrated that CFTR is functionally expressed in human alveolar epithelial type II cells, and may contribute to cAMP-regulated ion and fluid transport across AT II cells in the human lung.

**GRANTS**

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