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Paracellular Permeability of Bronchial Epithelium is Controlled by CFTR

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Key Words

Transepithelial resistance • ^{14}C-mannitol flux • 16HBE14o$^-$ • CFBE41o$^-$ • Cystic fibrosis • ML-7 • MLCK • Tight junctions

Abstract

In normal airway epithelium, the cystic fibrosis transmembrane conductance regulator (CFTR) transports Cl^- ions to the apical surface of the epithelium paralleled by the flow of water through transcellular and paracellular pathways. The hypothesis was tested whether CFTR not only regulates the transcellular but also the paracellular shunt pathway. Therefore, we performed measurements of transepithelial electrical resistance (TER) and paracellular ^{14}C-mannitol permeability in wtCFTR (16HBE14o$^-$) and delF508-CFTR (CFBE41o$^-$) expressing human bronchial epithelial cells. Under resting conditions, CFBE41o$^-$ cell monolayers exhibit a higher paracellular permeability and lower TER as compared to 16HBE14o$^-$ monolayers. Stimulation of CFTR by cAMP induces opposite effects in the two cell lines. 16HBE14o$^-$ monolayers show a sharp decrease of TER, in parallel with a concomitant increase of paracellular permeability. The change in paracellular permeability is mediated by a myosin II dependent mechanism because it can be blocked by the myosin light chain kinase inhibitor ML-7. In contrast, CFBE41o$^-$ cells respond to cAMP stimulation with a decrease of paracellular permeability, paralleled by slight increase of TER. We conclude that stimulation of wtCFTR increases vectorial transcellular salt transport and, simultaneously, the paracellular permeability allowing water to follow through the paracellular pathway. In contrast, in CF epithelium cAMP stimulation increases neither vectorial salt transport nor paracellular permeability which is likely to contribute to the CF pulmonary phenotype. Taken together, our results link CFTR dysfunction to an improper regulation of the paracellular transport route.

Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) gene encodes a cAMP-regulated chloride channel found in the apical membrane of epithelial cells of various tissues (lung, pancreas, testes, and others [1, 2]). The delF508 mutation in the CFTR gene is the most common genetic malformation present in at least

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one allele of ~90% of CF patients [3]. DelF508-CFTR is defective with respect to multiple functions including cAMP-regulated chloride conductance, nucleotide transport and regulatory actions on other ion channels [4-6]. In bronchial epithelia CFTR is crucial for the formation of functional airway surface liquid (ASL). The airway surface layer consists of a two-layer liquid system. The upper phase is a gel-like phase generated by secreted mucins and the lower phase is known as the periciliary liquid (PCL), a poly-anionic watery layer. This two-layer system allows efficient ciliary beating and mucus clearance as a component of lung defense. In normal lung, the physiological height of the PCL is maintained at about seven micrometers and regulated by purinergic signalling [7], coordinated activities of different ion channels [8] and passive osmotically driven water flow [9]. The epithelium forms a continuous barrier that selectively restricts the movement of solutes and water between the lumen and the interstitium. Water, ions and other solutes can move across the epithelium via two pathways, the transcellular and the paracellular route. Transcellular transport is the result of the combined function of various transporters and channels in the apical and basolateral plasma membrane. It can be active, i.e. directly ATP dependent, or passive, driven by electrochemical gradients [10]. Paracellular transport is passive, driven by the transepithelial electrochemical gradient [11] and modulated by the tight junction (TJ) permeability [12, 13]. A limiting factor for fluid transport in bronchial epithelium is the plasma membrane protein CFTR [14] CFTR dysfunction results in impaired water secretion and subsequently depletion of PCL height [15] causing accumulation of dehydrated mucus, impaired mucociliary clearance [16] and chronic bacterial infection. Commonly, the role of CFTR in epithelial water secretion is described as providing the driving force for Na^+ towards the lumen followed by osmotically driven water flow. The presence of aquaporins (water channels; APQs) in bronchial epithelial cells suggests that AQPs provide the principal route for osmotically driven water transport between airspace and capillary compartments. Matsui et al. state that for human bronchial epithelial cells the osmotic water flow is mainly transcellular and that paracellular flow will be small [9]. However, alveolar fluid clearance in the neonatal and adult lungs of mice is not affected by the deletion of APQ1 and APQ5, nor is lung fluid accumulation in experimental models of lung injury. In the airways, though AQP3 and AQP4 facilitate osmotic water transport, their deletion does not impair airway hydration, regulation of airway surface liquid or fluid absorption [17]. Therefore, a participation of paracellular water transport in airway secretion is likely to complement the transcellular pathway. The permeability and ion selectivity of the paracellular pathway is critical for establishing or dissipating ion concentration gradients and hence for determining the ionic composition of the apical compartment and net volume flow. Thus, transcellular and paracellular pathways must work in concert, being functionally matched to meet the transport requirements of a specific tissue [18]. It is likely that the paracellular pathway is regulated in parallel to the transcellular pathway so that both pathways "in concert" determine net fluid transport. CFTR as the crucial factor of epithelial fluid secretion and absorption is assumed to be involved in the regulation of the paracellular permeability [19-21]. The first description of coupling between apical and paracellular transport came from observations made in isolated rodent small intestine. Pappenheimer and coworkers demonstrated that glucose-stimulated liquid absorption through SGLT1 is accompanied by an increase in the paracellular permeability due to impaired tight-junctions [22, 23]. The mechanisms whereby apical transporters alter the paracellular pathways are yet unknown.

In the present study we used polarized human bronchial epithelial cell lines expressing wtCFTR (16HBE14o$^-$) and ΔF508-CFTR (CFBE41o$^-$) to investigate whether CFTR influences the paracellular permeability. We found that the paracellular transport route in bronchial epithelium is under the control of CFTR.

Materials and Methods

Cell Culture

16HBE14o$^-$, a wt-CFTR expressing cell-line, and CFBE41o$^-$ cells (homozygous for the delF508-CFTR mutation) were grown in Eagle's Minimal Essential Medium with 10% fetal bovine serum, 2 mM L-glutamine, 50U/ml penicillin and 50mg/ml streptomycin. Both cell lines were cultured at 37°C in a 5% CO_2 incubator. Cells were seeded at a density of $1.6x10^5$ on ThinCert™ cell culture inserts (Greiner Bio-One, Frickenhausen, Germany). Culture flasks and ThinCert™ have been coated over night with a solution containing 0.01% collagen type I from calf skin and 1mg/ml bovine plasma fibronectin (Invitrogen, Karlsruhe, Germany). Both cell lines were a generous gift from Dr. D. Gruenert (Cardiovascular Research Institute, University of California, San Francisco, CA, USA).

Transepithelial electrical resistance (TER)

In order to perform TER measurements cells were seeded on 6-well plate ThinCert™ inserts (area 4.5 cm², pore diameter

0.4 µm). TER was measured with an EVOM (epithelial volt-ohm-meter;WPI, Berlin, Germany) equipped with STX2 chopstick electrodes by applying a 20 µA square wave alternating current at 12.5 Hz. The resistance of cell-free ThinCert™ inserts (128 Ω·cm²) was subtracted from the TER raw data.

Experiments were performed between day 7 to 10 after seeding when TER of 16HBE14o$^-$ and CFBE41o$^-$ monolayers were about 800 Ω·cm² and 600 Ω·cm², respectively. Activators and/or inhibitors were added simultaneously to both compartments. Cells were stimulated with 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate sodium salt (8cpt-cAMP) at a concentration of 100 µM. The blocker cocktail was used to inhibit the majority of plasma membrane ion conductances using the CFTR specific inhibitor $CFTR_{Inh}172$ (10 µM), bumetanide (10 µM) to block the Na-K-2Cl cotransporter (NKCC1), clotrimazole (25 µM) to inhibit calcium activated potassium currents, amiloride (10 µM) to block the epithelial sodium channel (ENaC), NPPB (5-nitro-2- (3phenylpropylamino) benzoic acid) (100 µM) to inhibit anion channels and barium chloride (5 mM) to block potassium channels. The blocker cocktail was added 10 minutes prior to the stimulation with cAMP. Before each experiment stock solutions were prepared containing the three-fold concentration of the inhibitors/activators. Experiments were started by replacing 1ml medium of the apical and basolateral compartment by 1 ml of the pre-warmed stock solution.

TER was measured inside of the incubator at 37°C and 5% CO_2. TER data are expressed as relative values to allow appropriate comparisons between the different series of experiments. The value measured directly before adding any substances was set as 100% (reference value).

Continuous transepithelial electric resistance (cTER) measurements

We developed a system which allows to measure simultaneously the TER of eight filter culture inserts at time intervals of 20 s or 5 min. A cover lid for 12-well ThinCert™ plates (area, 1.13 cm²; pore diameter 0.4 µm) was equipped with six titanium electrodes for each well. Electrodes, four to inject current and two to measure voltage, were arranged in a way that resulted in a fairly homogenous electrical field. This modified cover lid could be easily attached to the ThinCert™ culture plate instead of the standard lid. Electrical current pulses with a frequency of 25 Hz were applied at a given time interval for 1 s. The electrical resistance of cell-free ThinCertTM inserts (128 Ω·cm²) was subtracted from TER raw data. Data acquisition and processing was done by a 2-channel PowerLab system (26 Series, ADInstruments GmbH, Germany). After 7-11 days of culture the cell monolayers exhibited a resistance of about 400 Ω·cm² and continuous recordings of transepithelial electric resistances (cTER) were started. A cAMP stimulation cocktail consisting of 100 µM 8cpt-cAMP, 100 µM 3-Isobutyl-1-methylxanthin (IBMX) and 10µM forskolin or the same volume of standard culture medium without the cocktail (control) was added simultaneously to the apical compartment. All cTER experiments were performed in culture medium at 37°C and 5% CO_2.

Measurement of ^{14}C-mannitol transport

The paracellular permeability was studied by determining the apical-to-basolateral transport of the isotope-labelled ^{14}C-mannitol. MEM medium without phenol red indicator was used for flux measurements. Cells were seeded on coated ThinCert™ cell culture inserts (area 1.13 cm²; pore diameter 0.4 µm; 12-well plate, Greiner Bio-One, Germany). TER was measured prior to experiments to control confluence (TER = 500 Ω cm²) of the cell monolayers. A stock solution of ^{14}C-mannitol was mixed with fresh MEM medium (final concentration: 0.25 µCi/ml) prior to each experiment. The basolateral compartment was filled with 1.4 ml MEM medium and the medium in the apical compartment was replaced by 0.6 ml of pre-warmed ^{14}C-mannitol containing medium. A sample volume of 100 µl was removed from the apical and the basolateral compartment immediately after adding ^{14}C-mannitol (t=0 min) and mixed with 3 ml of a scintillation cocktail. Further samples (100 µl each) were removed from both compartments after 60, 90 and 120 min. Radioactivity was quantified with a Beckmann LS 1801 liquid scintillation counter. The radiation of apical samples at t=0 min was usually about 100,000 CPMs (counts per minute). The amount of transported ^{14}C-mannitol is expressed as fraction of initial radiation, e.g. as the ratio of the CPMs of the basolateral over the apical sample.

Chemicals

All chemicals, unless otherwise mentioned, were purchased from Sigma (Deisenhofen, Germany). Cell culture media and supplements were purchased from Invitrogen-Gibco (Karlsruhe, Germany). Further chemicals were: $CFTR_{Inh}172$ (Calbiochem Darmstadt, Germany), ^{14}C-mannitol (Hartmann Analytical, Braunschweig, Germany) and scintillation cocktail Rotiszint® (Carl Roth GmbH, Karlsruhe, Germany), ML-7 (Calbiochem, La Jolla, CA, USA).

Statistics

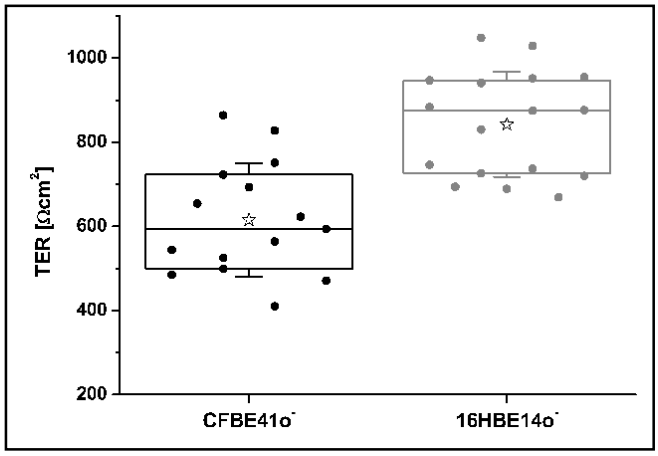
All data are presented as mean ± s.e.m. unless otherwise mentioned. Two-sided t-tests were performed to evaluate statistical significance. A P-value of <0.05 was accepted to indicate a significant difference between the compared data.

Results

Transepithelial electrical resistance

TER of epithelial cell monolayers is composed of the in-series electrical resistances of the apical and basolateral cell membrane, and of the in-parallel resistance of the paracellular shunt pathway. Monolayers of delF508-CFTR expressing CFBE41o$^-$ cells exhibit a 15% lower TER value (615 ± 134.8 Ω·cm², n 15) as compared to wtCFTR expressing 16HBE14o$^-$ cells (842 ± 125.3 Ω·cm², n= 17) (mean ± SD). Standard deviation is rather high reflecting the variance of TER amongst individual filter cultures. Nevertheless, median of TER is comparable to the mean values for CFBE41o$^-$

Fig. 1. Transepithelial electrical resistance (TER) [Ω·cm²] across 16HBE14o⁻ (n=17), CFBE41o⁻ (n=15) monolayers. Data are presented as box-plot showing raw data (circles), arithmetic mean (open star), standard deviation (whiskers), 25 and 75 percentile (box) and the median (horizontal line).



(594 Ω·cm²) and 16HBE14o⁻ cells (875 Ω·cm²) (Fig. 1). For better comparison of the experimental results, data are subsequently presented as relative values (% of the initial value).

Under control conditions both cell types show stable TERs whereas cAMP stimulation induces opposite effects (Fig. 2). Within six minutes, electrical resistance of 16HBE14o⁻ cells decreases by 52.7 ± 2.88 % (n= 27) in response to 100 µM of the membrane permeable cAMP analogue 8cpt-cAMP. In contrast, TER of CFBE41o⁻ cells rises by 19.0 ± 3.63 % (n= 24). The reliability of these results obtained with chopstick electrodes were tested using the continuous TER (cTER) method. Fig. 3 shows a representative measurement using the six-electrode configuration of cTER. The time course of these resistance measurements are identical to the data obtained with chopstick electrodes. After adding cyclic AMP, resistances of 16HBE14o⁻ cell monolayers decrease by 55% while TERs of CFBE41o⁻ cells increase by 22%.

To distinguish between the paracellular and transcellular transport route of 16HBE14o⁻ monolayers, the majority of the plasma membrane ion conductances were blocked with a cocktail containing $CFTR_{Inh}172$ (10 µM), NPPB, (100 µM), bumetanide (10 µM), clotrimazole (25 µM), amiloride (10 µM) and Ba^{2+} (5 mM). TER of ion conductance blocked monolayers decreases in response to cAMP by 28.4 ± 2.43 % (n=8) (Fig. 4). The breakdown in electrical resitance is about 30 % smaller as compared to the cAMP-effect observed in untreated 16HBE14o⁻ monolayers (52.7 ± 2.88 %; n= 27). This indicates that about 50% of the cAMP-induced electrical conductance most likely originates from the paracellular route.

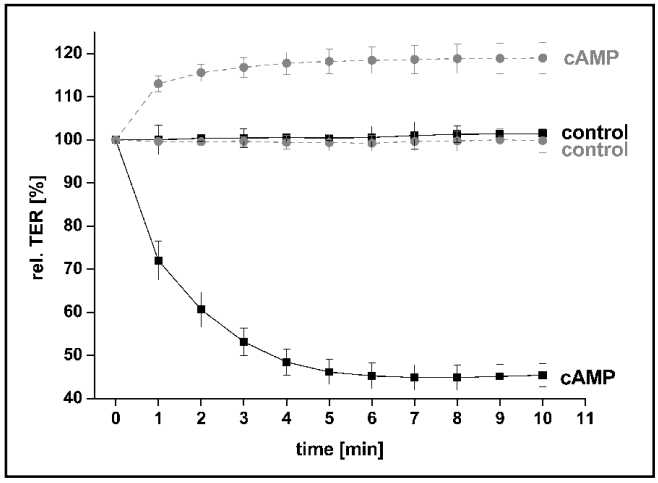


Fig. 2. Cyclic AMP induced change of TER. Black squares represent wtCFTR expressing 16HBE14o⁻ cells, grey circles delF508-CFTR expressing CFBE41o⁻ cells.

Transepithelial ^{14}C-mannitol transport

The electrical resistance of the paracellular shunt pathway, mainly due to the tight junction complex limits the movement of marker molecules (i.e. mannitol), large peptides and macromolecules [24, 25]. To characterize the permeability of the paracellular shunt pathway the flux of the isotopic molecule ^{14}C-mannitol was measured across confluent monolayers of 16HBE14o⁻ and CFBE41o⁻ cells. ^{14}C-mannitol was added to one com-

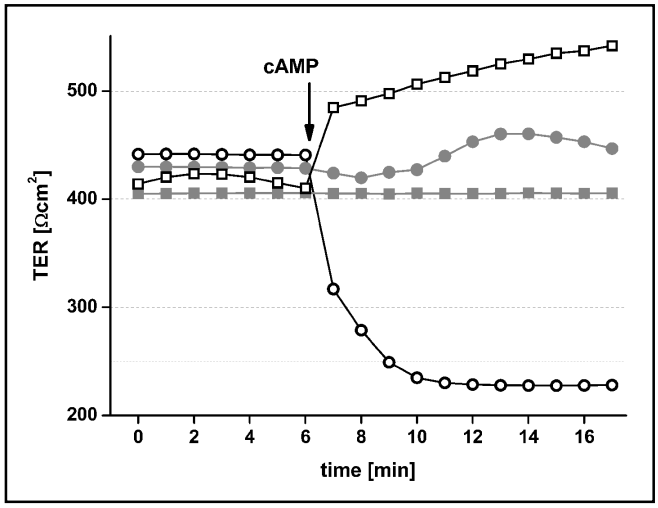


Fig. 3. Continuous measurement of transepithelial electrical resistance (cTER) across 16HBE14o⁻ monolayer (grey circles) and CFBE41o⁻ monolayer (gray squares). Stimulation of CFTR induces a decrease of TER for 16HBE14o⁻ cells (open circles) and an increase of TER for CFBE41o⁻ cells (open squares).

partment (initial radiation) and, then, the accumulation of radioactive labeled mannitol was measured in the other compartment. The fraction of initial radiation reflects the permeability of the paracellular pathway to ^{14}C-mannitol. Importantly, no difference was observed between basolateral-to-apical and apical-to-basolateral mannitol flux (data not shown). The fraction of initial radiation under control conditions was significantly larger in CFBE41o$^-$ cultures (Fig. 5B, black squares) as compared to 16HBE14o$^-$ monolayers (Fig. 5A, black squares). This indicates that the paracellular permeability of the delF508-CFTR expressing epithelium is higher as compared to the wtCFTR expressing epithelium. Adding 8cpt-cAMP increases the mannitol permeability of 16HBE14o$^-$ monolayers while, in contrast, reduces the paracellular permeability of the CFBE41o$^-$ monolayers (Fig. 5A, B, grey circles).

Myosin II activity is supposed to be involved in the regulation of the paracellular permeability [26]. This argument was tested by measuring the ^{14}C-mannitol flux upon cAMP stimulation after a preincubation period of 20 min with 10 µM ML-7 (1-(5-iodonaphthalene-1-sulphonyl) 1H-hexahydro 1,4-diazepine hydrochloride), an inhibitor of the myosin light chain kinase (MLCK). ML-7 blocks the cAMP-effect on the paracellular permeability of 16HBE14o$^-$ monolayers approaching values clearly below corresponding controls (Fig. 5A, open stars). In contrast, the effects of cAMP stimulation alone and ML-7 + cAMP stimulation on paracellular permeability of CFBE41o$^-$ cells are hardly distinguishable from each other (Fig. 5B, open stars).

Discussion

In this paper we provide evidence that CFTR regulates the paracellular permeability of the wtCFTR (16HBE14o$^-$) and the delF508-CFTR (CFBE41o$^-$) expressing human bronchial epithelium. We used TER- and ^{14}C-mannitol measurements to analyze the effect of CFTR activation on epithelial barrier function. Transepithelial electrical resistance of polarized airway epithelial cells show a considerable intraindividual variability. 16HBE14o$^-$ and CFBE41o$^-$ monolayers exhibited TER values in the range of 672 to 1052 Ω·cm² and 411 to 865 Ω·cm², respectively. Data show a non-gaussian distribution indicating an individual variation even under well-controlled cell culture conditions. Small variations of cell culture parameters (e.g. cell density, proliferation rate etc.) among individual filters are

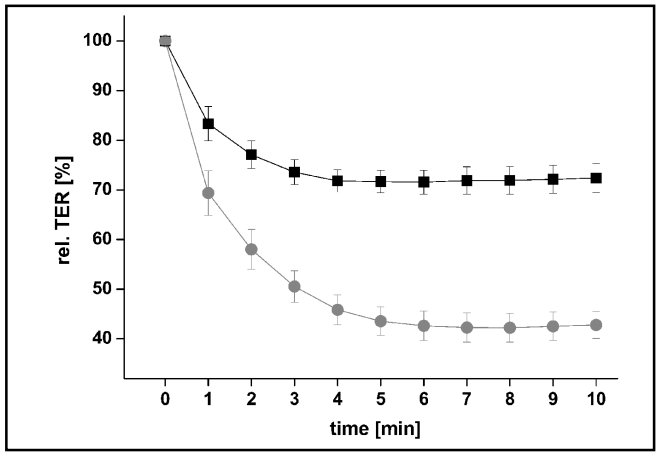


Fig. 4. Effect of cAMP on TER of 16HBE14o- cells without (grey circles) and after preincubation (black squares) with a blocker cocktail containing CFTRInh172 (10 µM), NPPB (100 µM), bumetanide (10 µM), clotrimazole (25 µM), amiloride (10 µM), Ba^{2+} (5 mM).

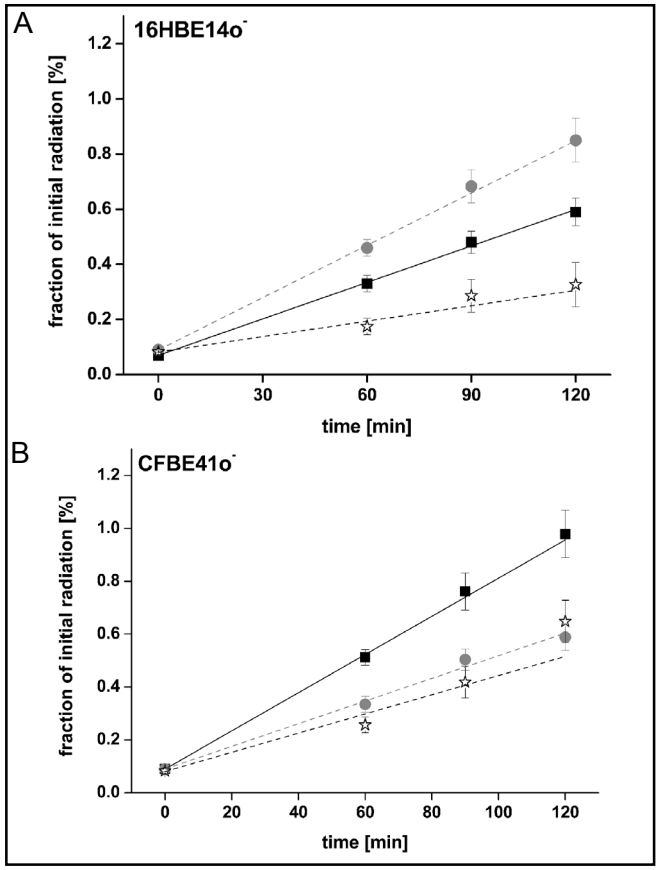


Fig. 5. Permeability of 16HBE14o$^-$ (A) and CFBE41o$^-$ (B) cell monolayers for ^{14}C-mannitol. The mannitol permeability is significantly larger under control conditions (black squares) for CFBE41o$^-$ cells as compared to 16HBE14o$^-$ cells. Cyclic AMP (grey circles) evoke an increase of mannitol permeability in 16HBE14o$^-$ cells and a decrease in CFBE41o$^-$ cells. Preincubation with ML-7 (open stars) reduces the cAMP-induced permeability for both cell types (n = 11 - 19).

unavoidable and it is likely that this contributes to the variance of the TER values. This observation suggests to use relative TER values (% of the initial value) for comparing experimental results. Therefore data are presented as relative TER in the following. Nevertheless, arithmetic mean as well as median revealed that monolayers of 16HBE14o$^-$ cells generate significant larger TERs (by 20%) than CFBE41o$^-$ monolayers.

Application of cAMP causes a sharp decrease of TER in 16HBE14o$^-$ monolayers reflecting the activation of CFTR and other CFTR dependent electrical membrane conductances. Comparable changes in TER values were observed by Nilsson et al. in CFBE41o$^-$ cells stably transfected with CFTR and 16HBE14o$^-$ cells after addition of the CFTR activators IBMX and forskolin [27]. We show that only about 50% of the cAMP-induced TER-breakdown was sensitive to a cocktail of inhibitors which should block the majority of plasma membrane ion conductances. This indicates that about half of the permeability of wtCFTR expressing epithelium can be attributed to the paracellular pathway which is in good agreement with previous observations [28]. This assumption is supported by the present finding that the decrease of TER was paralleled by a considerable increase of the paracellular permeability for the marker molecule ^{14}C-mannitol.

The present data suggest that non-stimulated bronchial epithelium exhibits a considerable paracellular permeability allowing passive (osmotically driven) water flow to replenish the evaporative water losses from airway surfaces during tidal breathing. Stimulating epithelial secretion of healthy epithelium by increasing cAMP concentration leads to an increase of transcellular vectorial chloride transport (i.e. decrease of TER) and, simultaneously, to an increase of gradient-driven paracellular transport of sodium and water (indicated by an increase of the paracellular permeability for mannitol). The decrease of TER is caused by an increase of the plasma membrane ion conductance due to the activation of CFTR chloride channels and other CFTR dependent membrane transport processes [29], and electrically compensated by the in-parallel ion flux through the paracellular shunt.

CFTR is a key player in epithelial transport. Therefore, it was not surprising that the transepithelial permeability and the response to cAMP were altered in delF508-CFTR expressing bronchial epithelial cells, CFBE41o$^-$. Confluent monolayers of CFBE41o$^-$ cells exhibit a significantly lower TER as compared to 16HBE14o$^-$ monolayers. This corresponds well to the elevated paracellular permeability of the CF cell monolayer (Fig. 5B). In agreement with this finding, an increased permeability of the paracellular shunt pathway has been reported for enterocytes of the small intestine of CF patients [30] implying that an impaired barrier function is characteristic for CF epithelium. As shown in the present study, stimulating CFBE41o$^-$ monolayers with cAMP results in an increase of TER and a reduction of the paracellular permeability. In CF epithelium, the epithelial sodium channel (ENaC) is hyperactive [31, 32] leading to enhanced sodium-driven fluid absorption. The (more permeable) paracellular shunt may contribute to this pathophysiological effect by providing the pathway for an accompanying flux of chloride and water [33]. Cyclic AMP is not only unable to increase transcellular vectorial chloride transport but rather increases transepithelial electrical resistance which is paralleled, and most likely caused, by a decrease of the paracellular shunt permeability.

Paracellular permeability is regulated by tight junctions which are structures localized at the sites of direct contact between neighbouring cells [34]. Tight junctions are composed of several different components including transmembrane, peripheral and cytoskeletal proteins which act in concert to control the paracellular permeability. Crucial for tight junction regulation is the contraction of the perijunctional actin, a dense ring structure of actin and myosin II that encircles the cell at the level of the adherens and tight junctions [13]. Contraction of the perijunctional actin ring causes an increase of the paracellular permeability when myosin II regulatory light chain (MLC) phosphorylation by the myosin light chain kinase (MLCK) occurs [35, 36]. Two major pathways that regulate MLC phosphorylation have been identified. The first is through the classical Ca^{2+}-calmodulin-dependent MLCK phosphorylating MLC. The second is a Ca^{2+} independent signal pathway that involves the small GTPase RhoA, a major cytoskeletalon-regulating small GTPase and the ensuing Rho kinase (ROK). ROK inhibits MLC dephosphorylation by phosphorylating, and thereby inactivating, MLC phosphatase [10]. In addition, in some cells ROK can directly phosphorylate MLC [37]. As shown in the present study, the paracellular permeability of 16HBE14o$^-$ monolayers is highly sensitive to the MLCK inhibitor ML-7. Blocking MLC phosphorylation (and therefore blocking perijunctional actin ring contraction) results in an increase of the tight junction barrier function. Since the paracellular permeability of CFBE41o$^-$ monolayers was already low upon cAMP application, no additional decrease by applying ML-7 could be detected. Thus, it is obvious that MLCK-driven MLC phosphorylation is involved in reducing the barrier func-

tion upon cAMP application similar as reported for other epithelia [38].

From the present data it can be derived that in bronchial epithelium the regulation of the paracellular permeability and, thus, the control of the tight junctions depend on (functional) wtCFTR. These findings are in good correlation to the results of Nielson et al., who suggest an interaction between CFTR activity and the TJ protein complex via the cytoskeleton [39].

Activation of CFTR causes a transmembrane efflux of chloride which is paralleled by a depolarization of the apical cell membrane. Membrane depolarization was found to be a physiological stimulus for MLC phosphorylation, either in a Ca^{2+} dependent or Ca^{2+} independent way. Depolarization-induced changes of MLC phosphorylation are mostly due to influx of Ca^{2+} through voltage-sensitive channels in neurons [40], whereas the Ca^{2+} independent pathway (RhoA/ROK pathway) was found in epithelial cells [41].

Taken together, it can be assumed that CFTR activation causes plasma membrane depolarization which activates the Rho/ROK pathway and thereby enhances myosin light chain (MLC) phosphorylation; this in turn increases paracellular permeability. In CF airway epithelium ENaC is hyperactive leading to excessive sodium uptake which depolarizes the apical plasma membrane. This view is consistent with the finding that the CFBE41o$^-$ monolayer exhibits, under "resting" conditions, a paracellular permeability which is comparable to that observed in cAMP-stimulated 16HBE14o$^-$ monolayers. It was reported recently that CFTR trafficking is correlated with an increase in TER but independent of its anion channel function [42]. The results suggest that CFTR trafficking is required for structural/functional organisation of tight junctions and, consequently, that a reduction in barrier function can be caused by endoplasmic reticulum retention of ΔF508-CFTR. These findings are different from our results in which we found a clear correlation of epithelial barrier function and CFTR chloride channel activity. One explanation could be that in the former study air–liquid interface cultures of CFBE41o$^-$ cells, stably transfected with wtCFTR or the mutant delF508-CFTR, were used. Both the different cell culture method (air-liquid / liquid-liquid) as well as the use of transfected and CFTR (wildtype and mutant) overexpression could be responsible for the difference in the results. Additionally, barrier function was not tested under CFTR stimulating conditions.

Here we show that lack of functional CFTR in CF cells abolishes their ability to properly regulate paracellular permeability. The mechanism by which CFBE41o$^-$ epithelium reduces its paracellular permeability upon cAMP application is yet unclear. It is important to note that contraction of the perijunctional actin ring is only one of several possible parameters determining the paracellular permeability. The tight junction permeability is closely related to protein kinase C (PKC) activity [43, 44] and the function of claudins [45, 46] which are crucial components of the barrier forming protein complex [47, 48].

In conclusion, the depolarization-induced MLC phosphorylation is likely to be a central mechanism for transepithelial transport whereby electrogenic transmembrane transport processes control paracellular transport [26]. So far, the upstream mechanisms that couple membrane depolarization to Rho activation are yet unknown. However, it is conceivable that membrane depolarization could serve as a functional link that triggers the cross-talk between electrogenic transmembrane transport and the paracellular shunt pathway [10].

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