Cystic Fibrosis Transmembrane Conductance Regulator Does Not Affect Neutrophil Migration across Cystic Fibrosis Airway Epithelial Monolayers

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Recent studies have shown that airway inflammation dominated by neutrophils, i.e., polymorphonuclear cells (PMN) was observed in infants and children with cystic fibrosis (CF) even in the absence of detectable infection. To assess whether there is a CF-related anomaly of PMN migration across airway epithelial cells, we developed an in vitro model of chemotactic migration across tight and polarized CF15 cells, a CF human nasal epithelial cell line, seeded on porous filters. To compare PMN migration across a pair of CF and control monolayers in the physiological direction, inverted CF15 cells were infected with increasing concentrations of recombinant adenoviruses containing either the normal cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, the ΔF508 CFTR cDNA, or the β-galactosidase gene. The number of PMN migrating in response to N-formyl-Met-Leu-Phe across inverted CF15 monolayers expressing β-galactosidase was similar to that seen across CF15 monolayers rescued with CFTR, whatever the proportion of cells expressing the transgene. Moreover, PMN migration across monolayers expressing various amounts of mutated CFTR was not different from that observed across matched counterparts expressing normal CFTR. Finally, PMN migration in response to adherent or Pseudomonas aeruginosa was equivalent across CF and corrected monolayers. The possibility that mutated CFTR may exert indirect effects on PMN recruitment, via an abnormal production of the chemotactic cytokine interleukin-8, was also explored. Apical and basolateral production of interleukin-8 by polarized CF cells expressing mutated CFTR was not different from that observed with rescued cells, either in baseline or stimulated conditions. CF15 cells displayed a CF phenotype that could be corrected by CFTR-containing adenoviruses, because two known CF defects, Cl− secretion and increased P. aeruginosa adherence, were normalized after infection with those viruses. Thus, we conclude that the presence of a mutated CFTR does not per se lead to an exaggerated inflammatory response of CF surface epithelial cells in the absence or presence of a bacterial infection. (Am J Pathol 2000, 156:1407–1416)

Cystic fibrosis (CF), a hereditary disease caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is associated with chronic airway inflammation dominated by neutrophils (PMN) that release large amounts of factors contributing to tissue destruction. The mechanisms involved in PMN immigration into the airway lumen are still poorly understood. Although the inflammatory response is thought to be maintained by the presence of characteristic bacterial pathogens and the release of factors chemotactic for PMN, recent observations suggest that the initiation of PMN immigration into CF Airways may result, at least in part, from a different mechanism. Indeed, airway inflammation was observed in infants and children with CF even in the absence of detectable bacterial, viral, or fungal colonization or infection. Because PMN and the chemotactic cytokine interleukin-8 (IL-8) can be found in CF bronchial secretions in the absence of a detectable infection, the question arises as to whether there is a CF-related anomaly of PMN migration across the airway epithelium. Some manifestations of the CF phenotype may be due to the absence of functional CFTR at the plasma membrane, whereas others may be related to the presence of certain amounts of mutated CFTR in the endoplasmic reticulum. Thus, we addressed two related questions: 1) Does correction of CF cells with CFTR lead to a difference in PMN migration across CF airway epithelia; and 2) Does the presence of different amounts of mutated CFTR lead to an increase in PMN migration and/or an aberrant IL-8 production?

To explore these questions, we developed an in vitro model to measure PMN migration in the physiological direction, across a tight-polarized human CF airway ep-
ithelial cell line, the CF15 cells,8 corrected or not with CFTR. Matched CF and non-CF monolayers were created by infecting the CF epithelial sheets with adenoviral vectors containing either the wild-type or ΔF508 CFTR cDNA, or a β-galactosidase gene reporter. Our data show that a similar number of PMN migrated across the different types of monolayers, and that no difference in production of IL-8 was observed whatever the proportion of cells expressing the transgenes.

**Materials and Methods**

**Cell Culture and Seeding on Permeable Filters**

The CF15 human nasal airway epithelial cell line, derived from a CF patient homozygous for the ΔF508 mutation, was transformed and characterized by Jefferson et al.8 CF15 cells were passaged once a week, plated in flasks coated with human placental collagen IV (50 μg/ml) and cultured in Dulbecco’s minimal essential medium/Ham F-12 (3:1) supplemented with 10% fetal calf serum (FCS) and seven growth factors.8 For culture on permeable filters, the cells were seeded at a density of 0.6 × 10⁶ on 1-cm² polycarbonate 3-μm-pore filters (Transwell inserts, Costar, Badhoevedorp, The Netherlands). When airway cells were seeded on inverted inserts, 200 μl of medium containing the cells were disposed on the lower surface of the filter and the cells were allowed to attach overnight at 37°C, before turning the inserts again. PMN migration and transepithelial electrical measurements were performed on day 8 after seeding. The cells were regularly tested for the presence of mycoplasma, and only pathogen-free cells were used for this study. All tissue culture supplies were obtained from Life Technologies, Inc. (Basel, Switzerland), FCS was from SeraTech (Griesbach, Germany), epidermal growth factor was from Collagen (Basel, Switzerland), and all other reagents were purchased from Sigma Chemical Co. (Buchs, Switzerland).

**Recombinant Adenoviruses**

**Transgenes Driven by Rous Sarcoma Virus (RSV) or Cytome Galovirus (CMV) Promoters**

The replication-defective adenoviruses were derived from the human adenovirus serotype 5, and contained either the CFTR cDNA controlled by the RSV promoter (AdTG 6429) or the CMV promoter (AdTG 6418), the CMV promoter-driven eGFP (enhanced green fluorescent protein) gene (AdTG 6297), or the RSV promoter-driven lacZ gene.9 All vectors were constructed as infectious plasmids by homologous recombination in E. coli as described.10,11 The vectors contain a deletion in E1 (Δ nucleotides 459-3327) and in E3 (Δ nucleotides 28592–30470). All vectors have the transgene incorporated in place of the viral E1 gene. For the generation of viruses, the viral genomes were released from their respective plasmids by Plac digestion and transfected into E1-complementing 293 cells as described.10 Viral stocks were prepared from the transfected cells, purified, and stored in viral storage buffer (1 mol/L sucrose; 10 mmol/L Tris-HCl, pH 8.5; 1 mmol/L MgCl₂, 150 mmol/L NaCl; and 0.005% Tween 80).

**Viral Infections and 5-Bromo-4-Chloro-3-Indolyl-β-d-Galactopyranoside (X-Gal) Staining**

Confluent CF15 monolayers on inserts were infected with adenoviruses on the day 6 of culture for 16 hours (unless otherwise indicated) with 200 μl of OptiMeM (Life Technologies, Inc.) supplemented with 2.5% FCS and containing or not the viruses at a multiplicity of infection (MOI) of 1 to 1000 (for RSV- and CMV-based viruses, a MOI of 1 = 1 infectious unit/cell (~1/2 plaque-forming unit/cell), whereas for β-actin-based viruses, a MOI of 1 = 1 plaque-forming unit/cell). For infection of inverted monolayers, the inserts were placed directly on a 200-μl drop of medium containing the viruses. The cells were then rinsed and cultured for an additional 24 hours in normal medium before the experiment. Expression of β-galactosidase was detected by light microscopy as nuclei-localized blue staining using the X-Gal substrate. Infected cells were rinsed with phosphate-buffered saline (PBS), fixed with 0.5% glutaraldehyde for 10 minutes, and incubated for 6 hours at 37°C with 1 mg/ml X-Gal, 5 mmol/L K⁺ ferricyanide, 5 mmol/L K⁺ ferrocyanide, and 1 mmol/L MgCl₂ in PBS.

**Transepithelial Electrical Measurements**

Right-side-up or inverted CF15 monolayers on inserts were placed in a modified and thermostatized Ussing chamber (manufactured by J. Pahud, CHUV, Lausanne, Switzerland) containing Hanks’ balanced salt solution (HBSS) supplemented with 1.3 mmol/L Ca²⁺, 1 mmol/L Mg²⁺, and 10 mmol/L (N-[Hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]) (HEPES), pH 7.4 (HBSS⁺), heated to 37°C. Transepithelial potential difference (ΔV) and short circuit current (Iₛ𝑐) were measured with apical and basolateral agar bridges connected to Ag/AgCl electrodes of a current/voltage clamp apparatus (VCC 600, Physiological Instruments, San Diego, CA). Transepithelial electrical resistance (TER) was calculated from Ohm’s law, after measuring the difference in current induced by voltage pulses of 1 mV. The sequential electrical responses to various drugs were determined: 100 μmol/L amiloride, added to the mucosal side of the epithelium, mucosal Cl⁻ replacement with HBSS⁺ containing 3.6
were then placed in new 12-well plates with 1 ml HBSS
P. aeruginosa (cfu)

Whole-Cell Patch-Clamp Recordings of CFTR-Dependent Cl⁻ Currents

CF₁₅ cells seeded on Petri dishes were infected for 16 hours with either Ad RSV CFTR or Ad RSV β-galactosidase at a MOI of 500. Whole-cell patch-clamp experiments were performed as described. briefly, the cells were superfused with a solution containing 136 mmol/L NaCl; 4 mmol/L KCl; 1 mmol/L CaCl₂; 1 mmol/L MgCl₂; 2.5 mmol/L glucose; and 10 mmol/L HEPES, pH 7.4, supplemented with 100 μmol/L amiloride. Patch electrodes were filled with a solution containing 1 mmol/L NaCl; 138 mmol/L KCl; 2.9 mmol/L CaCl₂; 5.5 mmol/L MgCl₂; 3 mmol/L MgATP; 0.1 mmol/L GTP; and 10 mmol/L HEPES, pH 7.2. Cells were held at 0 mV and membrane capacitance was determined using the automatic compensation procedure of an EPC-9 patch-clamp amplifier (Heka Elektronik, Lambrecht, Germany). Every 5 seconds, cells were depolarized from the holding potential to −70 mV for 600 milliseconds. CFTR-dependent currents were then stimulated with 50 μmol/L forskolin (or the solvent ethanol) added to the superfusing solution.

IL-8 Production by Polarized Monolayers

To determine IL-8 production at the apical or basolateral side of the CF₁₅ monolayers, the cells were seeded on 1-cm² inserts and infected for 16 hours on day 6. Ten hours after the end of infection, the monolayers were incubated overnight with culture medium without FCS and with 0.1% BSA, before being challenged on their mucosal side with or without tumor necrosis factor-α (TNF-α) for 1 or 4 hours. Apical and basolateral supernatants (500 μl each) were collected after 4 hours or 16 hours. For IL-8 production in response to adherent P. aeruginosa, the monolayers were exposed on their apical side to 5 × 10⁷ cfu PAO1 for 2 hours. After rinsing four times with HBSS⁺, the monolayers were incubated for 4 hours with culture medium without FCS or antibiotics, supplemented with 100 μg/ml cycloserine and 0.1% BSA, before supernatant collection. IL-8 was measured using an ELISA kit (CLB, Amsterdam, The Netherlands).

Pseudomonas aeruginosa Adherence

Confluent CF₁₅ cells in 24-well plates were infected with adenoviruses for 16 hours on day 6, and bacteria were allowed to adhere 1 day after the end of infection. P. aeruginosa strain PAO1 was grown to a density of 5 × 10⁸ cfu/ml and labeled with ³⁵S-methionine (Amersham, Zürich, Switzerland) to a specific activity of ~5000 cfu/cpm for 15 minutes. After being washed in PBS, the bacteria were suspended in CF₁₅ culture medium without FCS or antibiotics, supplemented with 100 μg/ml cycloserine. PAO1 was then added to confluent CF₁₅ cells (5 × 10⁷ cfu/well) for 2 hours at 37°C. Unbound bacteria were removed by rinsing the monolayers three times with PBS. CF₁₅ cells and bacteria were solubilized in 0.5 ml sodium dodecyl sulfate 2% by shaking at 110 rpm and scintilla-
**Results**

**Neutrophil Migration across CF<sub>15</sub> Monolayers in the Physiologically Relevant Direction**

To determine whether PMN migration across electrically-tight CF airway monolayers is affected by the orientation of the epithelium, CF<sub>15</sub> Cells were seeded on either side of the permeable inserts, before being exposed to PMN that were allowed to cross the monolayers in response to fMLP. PMN migration across right-side-up monolayers was expressed as a percentage of that obtained across inverted monolayers. Values are means ± SEM and compared in quadruplicates. A two-tailed paired Student’s t-test was used.

Expression of Adenoviral-Derived Transgenes by Inverted Polarized CF<sub>15</sub> Monolayers and by Single Cells

To compare PMN migration in the physiologically relevant direction, a pair of matched CF and non-CF airway monolayers was created. For this purpose, inverted CF<sub>15</sub> monolayers were infected with adenoviruses containing the CFTR (Ad RSV CFTR) or β-galactosidase (Ad RSV βgal) gene. We determined whether cyclic adenosine monophosphate (cAMP)-mediated Cl⁻ secretion was restored in the group of cells infected with Ad CFTR using various drugs (Figure 2A). This typical recording shows the correction of defective transepithelial Cl⁻ secretion in a monolayer exposed to Ad CFTR, with an important increase in I<sub>sc</sub> in the presence of a low Cl⁻-containing solution. The cAMP-stimulating drug forskolin promoted a further increase of the response. In contrast, the cells infected with Ad βgal displayed only a small increase of I<sub>sc</sub> in response to low Cl⁻, without any change when challenged with forskolin (Figure 2B). The fact that inverted monolayers maintained their polarity when seeded on the lower side of the filter is shown by comparing Figure 2A to 2C. The latter shows the transepithelial Cl⁻ current obtained with a CF<sub>15</sub> right-side-up monolayer infected with Ad RSV CFTR. The trace mirrored that obtained with inverted cells, because I<sub>sc</sub> was of opposite
Comparison of PMN Migration across Inverted CF\textsubscript{15} Monolayers Corrected or Not with CFTR

We determined whether the presence of a functional CFTR affects cellular processes involved in PMN migration. Inverted CF\textsubscript{15} monolayers were infected with various MOI of two different pairs of viruses containing either the RSV (Figure 4A and B) or the CMV (Figure 4, C and D) promoter, and assayed for chemotactic migration. Figure 4A shows that a similar number of PMN migrated across monolayers of cells infected with Ad RSV CFTR or Ad RSV βgal at all of the viral MOI tested. Moreover, no significant difference of migration could be observed between the monolayers infected with increasing MOI of a given virus. Ad RSV CFTR-infected cells exhibited a response to low Cl\textsuperscript{−} and forskolin that increased with the virus load (Figure 4B), whereas this response remained almost undetectable in monolayers infected with Ad βgal. The level of transgene expression in the latter group also varied with the MOI, because the number of blue cells increased from ~20% to 90%. PMN migration experiments were also performed with monolayers expressing higher levels of transgenes (Figure 4, C and D). Likewise, the number of PMN having migrated across monolayers infected with Ad CMV CFTR was different from that having crossed their counterpart infected with Ad CMV eGFP. The group of cells expressing CFTR displayed a MOI-dependent increase of response to low Cl\textsuperscript{−} and forskolin ~2.5- to 3-fold higher than that seen with the same MOI of Ad RSV CFTR (Figure 4, D and B, respectively). Monolayers expressing eGFP did not respond to those challenges, but their fluorescence increased with the viral load (data not shown).

Other experiments, performed with adenoviruses containing the RSV promoter (with MOI ranging from 0 to 1000), confirmed the lack of difference of PMN migration between inverted CF and corrected monolayers, all groups displaying similar results. Moreover, the data were identical to those obtained across parental noninfected monolayers, indicating that the adenoviral infection per se does not influence this process.

Comparison of PMN Migration across CF\textsubscript{15} Monolayers Expressing Various Amounts of Normal or Mutated CFTR

To determine whether the amount of mutated CFTR plays a role in cellular events involved in the migratory process, inverted CF\textsubscript{15} epithelia were infected with increasing MOI of matched adenoviruses containing either the normal or ΔF508 CFTR cDNA (Ad CB CFTR and Ad CB ΔF508, respectively). As shown in Figure 5, no significant difference in the number of transmigrated PMN was observed across monolayers expressing various quantities of either normal or mutated CFTR. The data were also similar to those obtained across uninfected monolayers (n = 2 experiments, data not shown). These adenoviruses also displayed a very efficient level of infection, because the response to low Cl\textsuperscript{−} and forskolin showed a dose-dependent increase when the Ad CB CFTR viral load was
increased; indeed, over a MOI range of 25 to 500, $\Delta I_{sc}$ increased from 22 to 243 $\mu A/cm^2$.

**Comparison of PMN Migration in Response to P. aeruginosa across Inverted CF$_{15}$ Monolayers Corrected or Not with CFTR**

To determine whether PMN migration in the physiological direction in response to adherent *P. aeruginosa* is influenced by the presence of a functional CFTR, experiments were done with monolayers rescued or not with CFTR and challenged with PAO1 (Figure 6). Our data show that the presence of adherent bacteria on the apical surface of monolayers expressing CFTR does not induce a difference in PMN migration, as compared to that seen across monolayers either uninfected or expressing $\beta$gal.

**IL-8 Secretion by Polarized CF$_{15}$ Cells Expressing Various Amounts of Normal or Mutated CFTR**

Polarized CF$_{15}$ monolayers corrected or not with CFTR were tested for IL-8 secretion from their apical or basolateral side (Figure 7). IL-8 present in the supernatant collected from monolayers infected or not with Ad RSV CFTR or Ad RSV $\beta$gal showed a dose-dependent increase in response to TNF-$\alpha$, in both the apical (Figure 7A) and basolateral (Figure 7B) compartment. However, no significant difference in IL-8 secretion was detected between cells expressing CFTR or $\beta$-galactosidase, in the presence or absence of TNF-$\alpha$, in either compartment. The presence of the adenoviral vector did not significantly affect IL-8 production in cells challenged with 10 nM TNF-$\alpha$, as compared to parental uninfected cells. The adenoviruses did not either affect baseline IL-8 production (data not shown). Other experiments of IL-8 secretion done with monolayers infected with adenoviral MOI ranging from 0 to 1000 demonstrated that the production of this cytokine in Ad CFTR-infected cells was similar to that observed in Ad $\beta$gal-infected cells, with no increase paralleling the increasing adenoviral load (data not shown). The profile of IL-8 secretion by CF$_{15}$ cells infected with either Ad CB CFTR or Ad CB $\Delta$F508 was comparable, demonstrating that the produc-
tion of this cytokine is not affected by the presence or amount of either type of CFTR. To measure IL-8 production in response to a bacterial stimulus, polarized monolayers infected with Ad RSV CFTR or Ad RSV β-galactosidase (MOI 500) were challenged with PAO1 on their apical surface. The absolute values of three experiments done in quadruplicate were pooled. Data are means ± SEM of the 12 samples pooled. In parallel, monolayers that were neither infected with viruses nor challenged with PAO1 were assessed for migration in response to 50 nmol/L fMLP as a means of comparison. In the Ad β-gal-infected groups, X-Gal staining revealed between ~40% and ~80% of blue cells and, in the Ad CFTR-infected groups, the response to low Cl⁻ and forskolin varied between 28 and 57 μA/cm².

P. aeruginosa Adherence to CF15 Cells Corrected or Not with CFTR

To confirm that CF15 cells indeed displayed a CF phenotype, experiments were done to test the adherence of P. aeruginosa to the plasma membrane, a function reported to be increased in CF.16–18 Figure 8 shows that there was no difference of adherence between uninfected cells or cells infected with Ad RSV β-gal. However, a significant decrease of adherence was observed with the cells rescued with CFTR, in each of the three experiments performed. This demonstrates that the CF15 cells display a CF phenotype that can be corrected by CFTR.

Discussion

Research on PMN migration across CF airway epithelium and its comparison to that occurring across non-CF epithelia has been hampered by the lack of adequate models of human tight airway epithelial monolayers displaying a TER characteristic of epithelial barriers. Although tight primary cultures of human tracheal epithelium can be successfully obtained,19 their use is limited by the difficulty in obtaining simultaneously CF and non-CF monolayers of similar tightness to compare PMN migration. Several CF transformed human airway epithelial cell lines have been isolated and characterized20 but, until now, no CF cell line has been described to retain differentiated features after several passages in culture. Here we describe a model of PMN migration in the physiologically relevant direction, across tight human CF airway epithelial monolayers, that has been developed with possible use in long-term culture. The CF15 cell line is, to our knowledge, the only CF airway epithelial cell line that could be induced to maintain tight junctions and vectorial ion trans-
port, without undergoing a well-known process of dedifferentiation.\(^2\) CF\(_{15}\) cells did not lose their CF characteristics either, because the presence of the homozygous \(H9004/F508\) mutation, the production of endogenous mutated CFTR mRNA, as well as the lack of response to forskolin measured on \(I_{sc}\), have been reconfirmed (data not shown).

In our model, PMN migration was shown to be greater in the physiological direction than in the apical-to-basolateral direction. This is in agreement with other studies suggesting that the polarity of the epithelium plays a role in PMN migration across intestinal monolayers such as T84 epithelial cells \(^2\)–\(^4\) and airway epithelial barriers.\(^5\)–\(^7\) However, no comparisons of the initial TER of inverted and right-side-up monolayers were done in the former studies, whereas only qualitative TER measures were provided in the latter ones. To address this issue, we compared PMN migration across right-side-up and inverted CF\(_{15}\) monolayers with similar initial TER and showed that the increase of migration observed in the physiological direction still persisted. This increase may be related to a difference in the polarity of epithelial receptors. Alternatively, CF\(_{15}\) cells may also have features similar to those of T84 intestinal epithelial cells, which were shown to display a luminal retention signal influencing PMN migration asymmetrically by cytoskeletal reorganization.\(^8\)

The fact that airway inflammation was observed in infants and children with CF even in the absence of

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**Figure 7.** IL-8 production by polarized CF\(_{15}\) cells expressing normal or mutated CFTR. CF\(_{15}\) monolayers on inserts were infected or not with Ad RSV CFTR or Ad RSV \(\beta\)gal (MOI 500) (A, B) or Ad CB CFTR or Ad CB \(\Delta F508\) (MOI 250) (C, D) before incubation with various concentrations of TNF-\(\alpha\). The apical (A, C) and basolateral (B, D) supernatants were collected after 4 hours and assayed for IL-8 production. A and B: Data are means \pm SEM (\(n = 3\)) of a typical experiment. C and D: The data of three experiments were pooled and presented as a fold increase over controls (monolayers corrected with CFTR and not submitted to TNF-\(\alpha\)) and are means \pm SEM of the eight to nine samples.

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**Figure 8.** PAO1 adherence to CF\(_{15}\) cells rescued or not with CFTR. Confluent CF\(_{15}\) cells were infected or not with Ad RSV CFTR or Ad RSV \(\beta\)gal (MOI 500), before being incubated with PAO1. The data of three experiments were pooled and presented as a percentage of bacteria adhering to uninfected CF\(_{15}\) cells (means \pm SEM of the 12 samples pooled). The number of bacteria adhering to uninfected cells, determined in the third experiment, was of 13 ± 1 PAO1/CF15 cell (mean \pm SEM, \(n = 4\) wells). ***\(^P < 0.001\) versus uninfected CF\(_{15}\) cells, \^\(^P < 0.005\) versus cells infected with Ad \(\beta\)gal.
detectable pathogens\textsuperscript{4–6} has led to the suggestion that the CFTR defect may play a direct role in the initiation of PMN immigration into CF airways. To compare PMN migration across CF and control monolayers, the use of ex vivo primary CF airway epithelial cultures makes it difficult to distinguish between the responses due to the primary defect or secondary to the microenvironment to which the cells were formerly exposed. To address this issue, we set up an in vitro model of a matched pair of CF and control monolayers differing only by the presence or absence of a normal CFTR. With this model, it was also possible to create matched pairs expressing increasing amounts of transgene by varying the adenoviral load. Moreover, within a given experiment, the monolayers displayed a comparable tightness, because they originated from the same source. Our data demonstrated that a similar number of PMN migrated across inverted monolayers of CF\textsubscript{15} cells expressing wild-type CFTR or \(\beta\)-galactosidase, whatever the level of transgene expression. In the group of monolayers infected with Ad RSV \(\beta\)gal, a MOI of 500 generally yielded ~50% of blue cells when detected by X-Gal staining. This value is certainly underestimated, because the sensitivity of detection with this method was reported to be relatively low.\textsuperscript{27} In the group of monolayers infected with Ad RSV CFTR, the defective Cl\textsuperscript{−} current was corrected to levels observed in non-CF airway monolayers at a MOI of ~100 to 500, depending on the experiment. PMN migration experiments were therefore always performed with various MOI. This allowed us to confirm the lack of difference in PMN migration across monolayers displaying from 0 to >90% of transgenic cells. We therefore conclude that correction with CFTR does not lead to a difference in PMN migration across CF airway epithelial cells.

Although part of the cellular manifestations of the CF phenotype has been attributed to the absence of a functional CFTR in airway epithelial cells, other ones have been suggested to be the consequence of the presence of a mutated CFTR. For example, an abnormal activation of nuclear factor-\(\kappa\)B in CF bronchial epithelial cells has been reported by DiMango et al\textsuperscript{7} and suggested to be a consequence of cell stress caused by the accumulation of mutant CFTR in the endoplasmic reticulum. To determine whether the amount of \(\Delta F508\) CFTR affects intracellular pathways involved in the interactions of epithelial cells with PMN, the migratory process was done across CF\textsubscript{15} monolayers expressing various amounts of either normal or \(\Delta F508\) CFTR. Because no significant difference was observed in the number of PMN having crossed the different types of monolayers, we suggest that there is no direct link between the CF genetic defect and the process of PMN migration across airway epithelial barriers, in the absence of pathogens.

The fact that adherent \(P.\) aeruginosa promoted no difference in PMN migration across monolayers rescued or not with CFTR further strengthens the results of migration we obtained in response to fMLP. Indeed, those bacteria were chosen as a specific stimulus encountered in CF patients. The data acquired with our model suggest that the combined presence of a mutated CFTR and of \(P.\) aeruginosa is not enough to explain the excessive amount of PMN found in CF airways colonized by these bacteria. This also further underlines the complexity of the CF inflammatory response \textit{in vivo}.

We then explored the possibility that CFTR may exert indirect effects on PMN recruitment, via an abnormal production of the potent chemotactic cytokine IL-8. Whereas some studies have reported high concentrations of this cytokine in bronchoalveolar lavage fluid from uninfected infants and children with CF, as compared to controls,\textsuperscript{4–6} another study found no difference of IL-8 in bronchoalveolar lavage from uninfected infants with or without CF, both levels being very low.\textsuperscript{28} Contradictory data were also published with \textit{in vitro} experiments. Indeed, subcultures of primary human CF bronchial gland cells were recently shown to spontaneously release much higher levels of IL-8 than non-CF ones,\textsuperscript{29} although no difference of baseline or stimulated IL-8 production was observed in primary or immortalized airway epithelial cells, in other studies.\textsuperscript{30–32} Massengale et al\textsuperscript{33} recently even reported a defective IL-8 secretion by CF airway cells. We addressed this issue with polarized CF\textsubscript{15} cells expressing various amounts of either normal or mutated CFTR cDNA, or the \(\beta\)-galactosidase transgene. The cells were grown as tight monolayers on filters because differentiation of epithelial cells was shown to regulate CFTR expression,\textsuperscript{34} and because it is possible that part of the effects of CFTR dysfunction on intracellular processes are not revealed unless cell polarization occurs. Our data demonstrate that correction of CF\textsubscript{15} monolayers with CFTR did not induce a change in the pattern of IL-8 secretion in either the basolateral or apical compartments. The fact that monolayers overexpressing \(\Delta F508\) CFTR displayed a similar profile of IL-8 production as matched counterparts rescued with normal CFTR provides hitherto unreported evidence that the accumulation of mutated CFTR in the ER does not lead to an aberrant synthesis of this cytokine in polarized airway surface epithelial cells.

To confirm the validity of our model, in particular the fact that CF\textsubscript{15} cells display a biological dysfunction (other than the Cl\textsuperscript{−} secretion defect) that can be corrected by CFTR, parallel experiments were done as a positive control. We tested the adherence of \(P.\) aeruginosa, which has been shown to be increased at the surface of epithelial CF cells,\textsuperscript{16–18} and observed that the number of bacteria bound to CF\textsubscript{15} cells was indeed corrected by CFTR-containing adenoviruses.

In summary, our data suggest that there is no intrinsic defect of the airway surface epithelial cells predisposing to PMN infiltration into the lung. Although these results are consistent with the studies of Armstrong et al,\textsuperscript{28} they do not exclude other possibilities. For example, the inflammation observed in CF airways\textsuperscript{4–6} may have been initiated by poorly cleared airborne particles and/or members of the respiratory flora. Alternatively, CF cells other than surface epithelial cells may deliver a signal mediating PMN transmigration.\textsuperscript{29} We conclude that the presence of a mutated CFTR does not per se lead to an exaggerated inflammatory response of CF surface epithelial cells.
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