Proinflammatory Effect of Sodium 4-Phenylbutyrate in ΔF508-Cystic Fibrosis Transmembrane Conductance Regulator Lung Epithelial Cells: Involvement of Extracellular Signal-Regulated Protein Kinase 1/2 and c-Jun-NH₂-Terminal Kinase Signaling

Telma Roque, Emilie Boncoeur, Vinciane Saint-Criq, Elise Bonvin, Annick Clement, Olivier Tabary, and Jacky Jacquot

Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche-S 893, Paris, France (T.R., E.B., V.S.-C., E.B., A.C., O.T., J.J.); Université Pierre et Marie Curie, Université Paris 06, Paris, France (T.R., E.B., V.S.-C., E.B., A.C., O.T., J.J.); and Assistance Publique-Hôpitaux de Paris, Hôpital Trousseau, Pediatric Pulmonary Department, Paris, France (A.C.)

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ABSTRACT

Sodium 4-phenylbutyrate (4-PBA) has attracted a great deal of attention in cystic fibrosis (CF) pathology due to its capacity to traffic ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) to the cell membrane and restore CFTR chloride function at the plasma membrane of CF lung cells in vitro and in vivo. Using two different ΔF508-CFTR lung epithelial cell lines (CFBE410- and IB3-1 cells, characterized with ΔF508-homozygous and heterozygous genotype, respectively) in vitro, 4-PBA induced an increase of proinflammatory cytokine interleukin (IL)-8 production in a concentration-dependent manner. This 4-PBA-induced IL-8 production was associated with a strong reduction of proteasome and nuclear factor-κB transcriptional activities in the two ΔF508-CFTR lung cells either in a resting state or after tumor necrosis factor-α stimulation. In contrast, a strong increase of activator protein-1 transcriptional activity was observed. The inhibition of extracellular signal-regulated protein kinase 1/2 (ERK1/2) by 1,4-diamino-2,3-di-cyano-1,4-bis[2-amino-phenylthio] butadiene (U0126) and 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98059) and c-Jun-NH₂-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) by anthra[1,9-cd] pyrazol-6 (2H)-one (SP600125), respectively, was associated with a reduction (2–3.5-fold) of IL-8 production in both ΔF508-CFTR lung cell lines treated with 4-PBA. No significant change of IL-8 production was observed after an inhibition of p38 MAPK with 4-[4-(4-fluorophenyl)-5-(4-pyridyl)]-1H-imidazol-2-yl phenol (SB202190). Therefore, we suggest that inhibition of both ERK1/2 and JNK signaling may be a means to strongly reduce 4-PBA-induced IL-8 production in combination with 4-PBA treatment to restore CFTR Cl⁻ channel function in lung epithelial cells of patients with CF.

Cystic fibrosis (CF) is a lethal disease caused by defective function of the cftr gene product, the CF transmembrane conductance regulator (CFTR) that leads to abnormal chloride transport in lung epithelium. The major cause of mortality and morbidity in CF patients is lung disease with the development of a progressive chronic respiratory insufficiency, characterized by a neutrophil-dominated lung inflammation (Chmiel and Davis, 2003). Sodium 4-phenylbutyrate (4-PBA) restores chloride conductance by promoting trafficking of mature ΔF508-CFTR to the cell membrane in the IB3-1 lung epithelial cell line (expressing the heterozygous ΔF508/F508 mutation), and CFBE410- lung epithelial cell line (expressing the homozygous ΔF508/ΔF508 mutation), and

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ABBRévIATiONS: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; 4-PBA, sodium 4-phenylbutyrate; HSC, heat shock cognate; NF, nuclear factor; AP-1, activator protein 1; IL, interleukin; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated protein kinase 1/2; JNK, c-Jun-NH₂-terminal kinase; U0126, 1,4-diamino-2,3-dicyano-1,4-bis[2-amino-phenylthio] butadiene; PD98059, 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; SB202190, 4-[4-(4-fluorophenyl)-5-(4-pyridyl)]-1H-imidazol-2-yl phenol; SP600125, anthra[1,9-cd] pyrazol-6 (2H)-one; TNF-α, tumor necrosis factor-α; PBS, phosphate-buffered saline; XTT, sodium 3′-[1(phenylami-nocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; HSP, heat shock protein; HDACi, histone deacetylase inhibitor; MG132, N-benzoyloxycarbonyl (Z)-Leu-Leu-leucinal.
primary cultures of nasal epithelial cells from CF patients (Rubenstein et al., 1997; Rubenstein and Zeitlin, 1998; Andersson and Roomans, 2000). Correction of CFTR chloride function in the IB3-1 cells treated with 4-PBA may be the result of increased transcription of CFTR, resulting in more mutated CFTR escaping ubiquitination in the cytoplasm and being released to the cell membrane (Rubenstein et al., 1997). In a recent study, it has also been reported that 4-PBA could markedly stimulate amiloride-sensitive Na+ channel activity in primary cultures of human CF nasal epithelial cells. This stimulation was related to increased abundance of α-, β-, and γ-epithelial sodium channel subunits in the apical membrane (Prulière-Escabasse et al., 2007). In vivo, phase I and phase II clinical trials with 4-PBA showed improved nasal potential differences in ΔF508-CFTR patients, consistent with increased apical CFTR activity (Rubenstein and Zeitlin, 1998; Zeitlin et al., 2002).

To date, the molecular actions of 4-PBA are not well defined, and several reports have shown that 4-PBA modulates the transcription of many genes in different cell types, an effect that is mainly attributed to the inhibition of histone deacetylases (Wade, 2001; Monneret, 2005). Other effects of 4-PBA have not been clearly related to inhibition of deacetylase enzyme activity in IB3-1 cells (Rubenstein and Lyons, 2001). Genomic and pharmacoproteomic reports have demonstrated that 4-PBA-treated IB3-1 cells also up-regulated many proteins involved in the trafficking activity, protein folding, and ion transport (Wright et al., 2004; Singh et al., 2006). Furthermore, 4-PBA down-regulated proteins involved in the ubiquitin-proteasome pathway responsible for cleaving regulatory proteins such as heat shock cognate (HSC)70 in IB3-1 cells (Singh et al., 2006). Whereas the therapeutic value of 4-PBA that increases CFTR expression in cell membrane is potentially important in CF, the precise targets of action of 4-PBA on CFTR-deficient lung cells have not been well defined and need to be clarified.

We and other groups have demonstrated constitutive activation of two major nuclear transcription factors [NF-κB and activator protein-1 (AP-1)] in CF lung epithelial cells that play a central role in the onset and persistence of early inflammatory processes in the lungs of patients with CF (Tabary et al., 1999, 2006; Verhaeghe et al., 2007). Elevated constitutive activation of NF-κB and AP-1 is associated with exaggerated proinflammatory cytokine interleukin (IL)-6 and IL-8 production in CF human adult primary bronchial epithelial cells and lung epithelial cell lines compared with corrected and normal lung epithelial cells (Tabary et al., 1999; Weber et al., 2001).

In lung epithelial cells, both activation of NF-κB and mitogen-activated protein kinases (MAPKs) leading to the activation of AP-1 play a pivotal role in a rapid amplification of the inflammatory response (Roebuck, 1999; Adcock et al., 2006). Using a series of chemical inhibitors, we recently found a crucial role for extracellular signal-regulated protein kinase 1/2 (ERK1/2) MAPK and NF-κB in enhanced IL-1β-induced IL-8 production in IB3-1 lung cells when exposed to oxidative stress (Boncoeur et al., 2008). Another study reported an important contribution of the c-Jun-NH2-terminal kinase (JNK) pathway in ozone-induced inflammatory cell recruitment, modulation of inflammatory gene expression, and airway hyper-responsiveness in mice (Williams et al., 2007). Gaining a mechanistic understanding of the cross-talk between NF-κB, AP-1, p38, ERK1/2 and JNK MAPK activities in ΔF508-CFTR lung cells after a treatment with 4-PBA may be a key to developing new therapies to modulate lung inflammation in patients with CF.

The goal of the present study was to gain insight into the effect of 4-PBA treatment within a therapeutically relevant range (6–10 mM) on the ΔF508-CFTR localization to apical membrane, production of IL-8, activities of nuclear transcription factors (NF-κB and AP-1), and MAPK signaling in two different CFTR-deficient lung epithelial cell lines, IB3-1 and CFBE410-. Our results show an increase of IL-8 production in two ΔF508-CFTR lung cell lines treated with 4-PBA in a concentration-dependent manner. This elevated IL-8 production is associated with a strong activation of AP-1 and correlated with the activation of both ERK1/2 and JNK, but not p38 MAPK.

Materials and Methods

Materials. U0126 and PD98059, two specific inhibitors of the ERK1/2 MAPK pathway, and SB202190, a specific inhibitor of the p38 MAPK pathway, were purchased from Calbiochem (VWR International, Fontenay-sous-Bois, France). U0126, PD98059, and SB202190 were dissolved in dimethyl sulfoxide and used at final concentration of 10 μM, as described previously (Muselet-Charlier et al., 2007). SP600125, a selective inhibitor of JNK catalytic activity (Bennett et al., 2001), and recombinant human tumor necrosis factor α (TNF-α) were purchased from Sigma-Aldrich (Saint-Quentin-Falavier, France) and used at a final concentration of 3 μM and 10 ng/ml, respectively. Eagle’s minimum essential medium with Earle’s salts, penicillin-streptomycin, and 1-glutamine were purchased from Invitrogen SARL (Cergy Pontoise, France). Fetal bovine serum was purchased from Eurobio (Courtaboeuf, France).

Cell Culture. The human CF bronchial epithelial cell lines IB3-1 and CFBE410- were used. IB3-1 was a bronchial epithelial cell line derived from a CF patient (CFTR genotype ΔF508/W1282X), and it was purchased from American Type Culture Collection (LGC Promochem SARL, Strasbourg, France). CFBE410- was generated by the transformation of bronchial epithelial cells obtained from a CF patient homozygous for the ΔF508 allele (CFTR genotype ΔF508/ΔF508), which were generous gifts from Dr. D. C. Gruenert (California Pacific Medical Center Research Institute, San Francisco, CA). CF cell lines were maintained in minimum essential medium with Earle’s salts and 1-glutamine, supplemented by 10% fetal bovine serum and 100 U/ml penicillin-streptomycin in a humidified CO2 incubator (37°C, 5% CO2). 4-PBA (a generous gift from Orphan Europe, Paris, France) was added from a 0.2 M solution dissolved in sterile phosphate-buffered saline (PBS). IB3-1 and CFBE410- were then grown for 24 h in the presence of either 6, 8, or 10 mM 4-PBA as demonstrated in previous studies (Andersson and Roomans, 2000; McGrath-Morrow and Stahl, 2000; Wright et al., 2004; Singh et al., 2006).

XTT Cell Viability Assay. Cells were grown in a 96-well microplate in a final volume of 100 μl of culture medium per well. After incubation with 4-PBA, the XTT-labeling mixture (Roche Diagnostics, Meylan, France) (prepared with 50 μl of XTT-labeling reagent and 1 μl of electron-coupling reagent) was added to each well. The plate was incubated for 4 h at 37°C in a humidified atmosphere. The tetrazolium salt (XTT) is cleaved to orange formazan by a complex of cellular mechanism. This bioreduction occurs in viable cells only and is primarily related to glycolytic NAD(P)/H production. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture. Orange formazan is quantified using a spectrophotometer at 450 nm.

Enzyme-Linked Immunosorbent Assay IL-8. IB3-1 and CFBE410- cells were treated with 4-PBA, with or without specific
inhibitor for a 24-h period. Growth media were collected, and IL-8 protein concentration was determined using the human IL-8 Duo Set kit (R&D Systems, Lille, France), following the manufacturer’s instructions.

**CFTR** Cell Enzyme-Linked Immunosorbent Assay. **CFTR** cell enzyme-linked immunosorbent assay (ELISA) was performed on cultured cell monolayers according to the recently published method of Medjane et al. (2005). After three washes with PBS, nonspecific binding was blocked with 1× Tris-buffered saline and 5% milk for 1 h at 4°C. All incubations were followed by three washes with PBS. **CFTR** was detected by incubation with specific CFTR antibody CF-3 at 4°C. All incubations were followed by three washes with PBS. The results were read at 450 nm (OD450) in a plate reader. To normalize the luciferase activity, Renilla luciferase activity was monitored to normalize firefly luciferase activity.

**Proteasome Activity.** Cells were grown in a black 96-well plate in a final volume of 100 µl of culture medium per well. After treatment with 4-PBA, the plate was equilibrated to room temperature before adding the Proteasome-Glo Cell-Based Assay Reagent (100 µl/well) (Promega, Lyon, France). A value of p < 0.05 was considered statistically significant.

**Results**

The effect of 4-PBA was investigated on two cultured CFB4E410- and IB3-1 lung epithelial cell lines (expressing the homozygous ΔF508/ΔF508 mutation and heterozygous ΔF508/W1282X, respectively). The choice of 4-PBA concentration was based on previous observations obtained by other groups who used the concentrations between 5 and 10 mM 4-PBA in CF lung cells for 12 to 48 h (Andersson and Roomans, 2000; McGrath-Morrow and Stahl, 2000; Wright et al., 2004; Singh et al., 2006). In the present study, we used 4-PBA at the concentrations of 6, 8, and 10 mM for a 24-h period.

**Effect of 4-PBA Treatment on Cell Viability and Morphology.** Treatment of CFB4E410- cells with 6, 8, and 10 mM concentrations of 4-PBA for 24 h showed no significant effect on cell viability. Only a slight but significant decrease of 20% in cell viability was observed in IB3-1 cells treated with 8 and 10 mM concentrations of 4-PBA (Fig. 1A). When we treated the two cell lines with different concentrations of 4-PBA, we only observed a little histological difference between 10 mM 4-PBA and control with long spindle connection (Fig. 1B), as previously reported in 4-PBA-treated IB3-1 cells (McGrath-Morrow and Stahl, 2000).

**ΔF508-CFTR Localization after 4-PBA Treatment.** To investigate whether 4-PBA affects ΔF508-CFTR localization, apical membrane ΔF508-CFTR protein was quantified by CFTR cell ELISA. In CFB4E410- and IB3-1 cells treated with 4-PBA (6, 8, and 10 mM for 24 h), we observed a significant increase of the ΔF508-CFTR protein in cell membranes. After 8 mM 4-PBA treatment, the increase of ΔF508-CFTR protein in CFB4E410- and IB3-1 cells was 2.5- and 8.0-fold compared with untreated control cells, respectively (Fig. 2A). Vij et al. (2006) have shown that inhibition of proteasome in IB3-1 cells may be involved in ΔF508-CFTR localization to the cell surface. Thus, we investigated whether the increase of ΔF508-CFTR localization in apical membrane by 4-PBA was correlated with changes in proteasomal activity in two CFB4E410- and IB3-1 cells. We observed a 60% reduction of proteasome activity in CFB4E410- treated with 6 mM 4-PBA for 24 h. In IB3-1 cells, an 80% reduction of proteasome activity was observed after treatment with 10 mM 4-PBA for 24 h (Fig. 2B).

**Effect of 4-PBA on the Production of Chemokine IL-8.** Exposure of both CFB4E410- and IB3-1 cells to 4-PBA (6, 8, and 10 mM for 24 h) induced an increase of the IL-8 production compared with untreated control cells. The IL-8
release in both cell lines was increased up to 10-fold with 10 mM 4-PBA treatment in a concentration-dependent response (Fig. 3A). Reverse transcriptase-PCR analyses revealed that 4-PBA treatment also induced an increase of the mRNA IL-8 level in both cell lines. We showed that 4-PBA induced an increase of the IL-8 mRNA level in a concentration-dependent response in CFBE41o- and IB3-1 cells (Fig. 3, B and C).

Effect of 4-PBA on the NF-κB and AP-1-Dependent Transcriptional Activities. The increase of IL-8 production observed after 4-PBA treatment prompted us to explore the NF-κB and AP-1-dependent transcriptional activities. To this end, both CFBE41o- and IB3-1 cells were transiently transfected with a NF-κB transcription reporter gene in resting and TNF-α-stimulated conditions (Fig. 4). As shown in Fig. 4, A and B, we first observed that stimulation of both cell lines by TNF-α (10 ng/ml) resulted in a strong increase of NF-κB-dependent transcriptional activity, by 3.0- and 8.2-fold in CFBE41o- and IB3-1 cells, respectively. Exposure to 4-PBA (6, 8, and 10 mM for 24 h) of two cell lines resulted in a strong reduction of NF-κB-dependent transcriptional activity in both basal and TNF-α-stimulated conditions (Fig. 4, A and B). The CFBE41o- and IB3-1 cells were then transiently transfected with an AP-1 transcription reporter gene. We showed that 4-PBA treatment of two cell lines resulted in a significant increase of AP-1 activity, using various 4-PBA concentrations (Fig. 4C).

Effect of 4-PBA on p38, ERK1/2, and JNK Signaling. Our data prompted us to investigate the MAPKs signaling, which are upstream molecules activating AP-1. To determine the respective role of p38, ERK1/2, and JNK MAPK in the 4-PBA-induced IL-8 production, specific inhibitors (SB202190, U0126, PD98059, and SP600125) were tested on the IL-8 release in both CFBE41o- and IB3-1 cells. The inhibitor of p38 MAPK (SB202190, 10 μM for 24 h) did not significantly affect 4-PBA-mediated IL-8 induction in CFBE41o- (Fig. 5A) and IB3-1 (Fig. 5B) cells compared with untreated control cells, respectively. In contrast, a significant reduction (2-fold) in IL-8 production of both 4-PBA-treated CFBE41o- and IB3-1 cells was observed in the presence of either U0126 or PD98059 (10 mM for 24 h, respectively), two ERK1/2-specific inhibitors (Fig. 6, A and B). Interestingly, we also observed a strong reduction (by a 2–3.5-fold factor) of the 4-PBA-induced IL-8 release in both 4-PBA-treated CFBE41o- and IB3-1 cells was observed in the presence of the JNK-selective inhibitor SP600125 (3 μM for 24 h). Furthermore, no significant change in the amount of apical membrane ΔF508-CFTR protein was observed after inhibition of both ERK1/2 and JNK signaling in two 4-PBA-treated CFBE41o- and IB3-1 cells (Fig. 8, A and B).
Discussion

In the present study, we analyzed the effects of 4-PBA on the expression level of chemokine IL-8, modulation of nuclear transcription factor (NF-κB and AP-1) activities, and MAPK signaling in two ΔF508-CFTR lung epithelial cell lines. We showed that ΔF508-CFTR lung cells receiving 6 mM of 4-PBA (a clinically relevant concentration) are accompanied with a strong ERK1/2- and JNK-dependent IL-8 activation. Furthermore, we found that 4-PBA treatment blocked the NF-κB transcriptional activity in two resting and TNF-α-stimulated ΔF508-CFTR lung cells. We also demonstrated a strong reduction of proteasomal degradation in two 4-PBA-treated ΔF508-CFTR lung cells, which could explain our observation of an increase of ΔF508-CFTR protein expression in the apical cell membrane. Consistent with our results, Vij et al. (2006) showed that inhibition of proteasome of IB3-1 cells with bortezomib modulated endoplasmic reticulum-associated degradation proteins and therefore may be involved in ΔF508-CFTR rescue to cell surface. Another report (Singh et al., 2006) demonstrated a down-regulation of 26S subunit 9 (PSMD11) of the proteasome complex in 4-PBA-treated IB3-1 cells, which may be a common mechanism by which 4-PBA facilitates processing of the ΔF508-CFTR to the trafficking pathway. Exposure of 4-PBA could facilitate exit of ΔF508-CFTR from the endoplasmic reticulum to plasma membrane by up-regulation of HSP70 and down-regulation of HSC70 in IB3-1 cells (Choo-Kang and Zeitlin, 2001; Rubenstein and Lyons, 2001). It has been well established that modulation of the ratio of HSP70 to HSC70 by cochaperones facilitated rescue of CFTR (Farinha and Amaral, 2005).

4-PBA is a histone deacetylase inhibitor (HDACi) that activates transcription of a variety of genes that are impor-
tant in the regulation of cell development through multiple signaling pathways (Wade, 2001). In general, HDACi have been noted for their ability to induce cell cycle arrest by inducing p21<sup>CDP/WAF</sup>, a cyclin-dependent kinase 2 inhibitor, and occurring at the G<sub>1</sub> phase in 4-PBA-treated IB3-1 cells (McGrath-Morrow and Stahl, 2000). It has been reported that HDACi could modulate the activity of NF-κB in a number of different cancer cells. Several studies have reported that butyrate’s ability to suppress NF-κB activity depends in part on its ability to suppress cellular proteasome activity (Yin et al., 2001). In the present study, ΔF508-CFTR lung cells that were treated with 4-PBA resulted in a reduction of proteasome activity, which is associated with a marked inhibition of NF-κB activity. Our data are in accordance with a report demonstrating that HDACi (butyrate and trichostatin A) suppressed proteasome activity by down-regulating the expression of select proteasome subunits and interfered with the proteasome-dependent activation of NF-κB in the Caco-2 colonic cell line (Place et al., 2005). The question of whether HDACi stimulates or inhibits inflammatory gene expression is still under debate, because studies have shown that many genes are suppressed or induced according to the cell types and stimuli (Adcock, 2007; Ito et al., 2007). Thus, trichostatin A enhanced lipopolysaccharide-stimulated IL-8, but it repressed IL-12 expression in the normal BEAS-2B bronchial cell line (Iwata et al., 2002). A recent study showed that IB3-1 cells that were treated with 5 mM 4-PBA for 24 h down-regulated transcript levels of cyclooxygenase-2 and IL-6 but not IL-8 (Vij et al., 2008).

To the best of our knowledge, the present study is the first to demonstrate that 4-PBA, at the relevant 5 to 6 mM concentration that is widely used to induce mature ΔF508-CFTR and increased Cl<sup>−</sup> secretion in ΔF508-CFTR lung cells (Rubenstein et al., 1997; Andersson and Roomans, 2000), markedly increases the expression and production of IL-8 in an ERK1/2- and JNK-dependent manner. Proteasome inhibitors were reported to stimulate many pathways that activate stress kinases, including JNK, ERK1/2, and p38 MAPK. AP-1 is mobilized through activation of JNK, ERK1/2, and p38 MAPK (Karin, 1995). A recent study showed that the JNK pathway modulates ozone-induced airway hyper-responsiveness and inflammation, lung neutrophil accumulation, and controlled the expression of 29 of 400 pulmonary genes induced by ozone, including IL-6 and CXCL1 (KC) in the lungs of mice (Williams et al., 2007). Another study showed that ERK1/2 regulates IL-8 expression in an AP-1-dependent and NF-κB-independent manner in the normal 16HBE14o- lung cell line (Li et al., 2002). The same group also reported that activation of inhibitor of κB kinase, NF-κB-inducing kinase, and ERK1/2 was increased in IB3-1 cells that were stimulated with asialoGM1 (Li et al., 2003). Although ERK1/2 activation was required for maximal IL-8 expression, inhibition of ERK1/2 signaling had no effect on NF-κB activation, suggesting that ERK1/2 regulated IL-8 expression in an NF-κB-independent manner. More recently, it has been shown that most of the proinflammatory genes (IL-1β, IL-6, and IL-8) that are overexpressed in a CF fetal tracheal cell line (CFT-2, carrying the ΔF508ΔF508 muta-
tion), compared with a normal fetal tracheal cell line (NT-1), were found to be ERK1/2-dependent (Verhaeghe et al., 2007).

Whether all of the effects that we describe in the present study are mainly caused by inhibition of proteasomal degradation of \( \Delta F508\)-CFTR lung cells after 4-PBA treatment awaits further investigation. Previous reports showed a strong IL-8 induction in normal primary lung epithelial cells in response to proteasome inhibition (Wu et al., 2002; Gerber et al., 2004). They clearly demonstrated that the proteasome inhibitors MG132 and lactacystin induced IL-8 through mitogen-activated protein kinase kinase- and JNK-dependent AP-1 stimulation and suppressed NF-\( \kappa \)B activation. The enhanced IL-8 production was accompanied by increased IL-8 mRNA levels and increased RNA stability. Therefore, it is tempting to think that 4-PBA could also act through its proteasome inhibitor function to increase the IL-8 expression and secretion in the \( \Delta F508\)-CFTR lung cells.

Relationships between activation of MAPK and trafficking of \( \Delta F508\)-CFTR protein in apical membrane of 4-PBA-\( \Delta F508\)-CFTR lung cells have not been investigated yet. The current study demonstrates that inhibition of ERK1/2 and JNK MAPK did not affect the amount of apical membrane \( \Delta F508\)-CFTR protein in two 4-PBA-treated \( \Delta F508\)-CFTR lung cell types. Our data are in good agreement with a report showing that the activation of ERK1/2 in Cos7 cells augmented the biogenesis and trafficking of wild-type-CFTR, but not \( \Delta F508\)-CFTR protein (Sugita et al., 2004). Reports have identified more than 100 CFTR-interacting proteins required for folding and function of CFTR and considered CFTR as a molecule involved in signal transduction through specific interactions between its intracytoplasmic domain and various proteins such as calnexin, EBP50, and NHERF2 (Wang et al., 2006). Therefore, we propose that such interactions could possibly change in \( \Delta F508\)-CFTR lung cells after a 4-PBA treatment, thus leading to the increase of ERK1/2 and JNK-dependent IL-8 expression. We recommend caution in interpreting our observations obtained with \( \Delta F508\)-CFTR lung cell lines for therapy in CF patients. We suggest that the present data will therefore serve as a reference database for analyses on human primary normal and \( \Delta F508\)-CFTR lung cells. In additional investigations, we will include primary epithelial cells (collected by brushing the nasal mucosa of CF patients) to examine the ERK1/2 and JNK-dependent IL-8 production in 4-PBA-treated \( \Delta F508\)-CFTR nasal epithelial cells, to confirm our results.

In conclusion, the present study has demonstrated that 4-PBA leads (in addition to the well known increase of apical CFTR activity) to an AP-1-dependent up-regulation of IL-8 gene expression in two \( \Delta F508\)-CFTR lung cell types, and therefore it raises the question about 4-PBA's potential in the treatment of CF lung disease. Thus, we propose that targeting both the ERK1/2 and JNK pathway...
with selective molecule kinase inhibitors for reducing damaging lung inflammation, in combination with a treatment with 4-PBA to improve CFTR chloride function in CF lung epithelium, is certainly a promising therapeutic strategy for treatment of cystic fibrosis.

References

Address correspondence to: Dr. Jacky Jacquet, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche-S 893, Hôpital Saint-Antoine, 184, rue du Fg Saint-Antoine, 75012 Paris, France. E-mail: jacky.jacquet@inserm.fr