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The NFκB Signaling in Cystic Fibrosis Lung Disease: Pathophysiology and Therapeutic Potential

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Abstract

Lung disease is the major cause of morbidity and mortality of cystic fibrosis (CF), an autosomal recessive disease caused by mutations in CF transmembrane-conductance regulator (CFTR) gene. In CF, elevated levels of interleukin-8 (IL-8) signaling mediated by the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) result in chronic infection, neutrophilic inflammation, and progressive airway destruction. The most frequent mutation in the CFTR gene is the deletion of phenylalanine 508 (ΔF508), which results in its endoplasmic reticulum associated degradation (ERAD) by the ubiquitin-proteasome system. The inability of ΔF508-CFTR to reach cell surface leads to inherently high levels of NFκB. Severity of CF lung disease depends on the levels of functional CFTR on cell surface that control its chloride transport and NFκB mediated innate immune response functions. NFκB mediated chronic inflammation is a prominent feature of CF lung disease and the mechanism(s) by which CFTR regulates these inflammatory signaling pathways is becoming apparent. Recent data suggest that CFTR localization to lipid-rafts is critical for regulating NFκB mediated innate immune response and chronic CF lung disease. We anticipate that targeting the pathways, which facilitates CFTR's rescue to the cell surface and lipid-rafts, will not only restore CFTR channel function but also control NFκB mediated chronic inflammation, although the level of correction may be a critical factor for therapeutic efficacy. We discuss here the mechanisms of NFκB induction in CF, pathogenesis of CF lung disease, and novel therapeutic strategies that may help in reversing the chronic CF lung disease.

Introduction

Cystic fibrosis (CF) is a disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a cAMP dependent and ATP-gated chloride channel that regulates epithelial surface fluid secretion in respiratory and gastrointestinal tracts. The mutations in CFTR gene result in different levels of defects in the lungs, pancreas, liver, sweat glands, and vas deferens indicating that both the level of CFTR expression and organ or tissue environment determine the underlying phenotype (Trezise and Buchwald, 1991). The major cause of morbidity and mortality in CF is the chronic lung disease that is considered to be a result of recurring infections and pathophysiological defect caused by the lack of CFTR protein on the cell surface (Jacquot et al., 2008; Vij et al., 2009). Although the clinical significance is unclear, patients with CF have also been noted to have increased colonization rates of bacterial species including *Lactobacillus*, *Pseudomonas*, *Staphylococcus*, and *Enterococcus* in GI tract (Wilschanski and Durie, 2007).

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In addition, the role of CFTR-dependent lipid-rafts in the immune privileged cornea due to *P. aeruginosa* induced keratitis is well documented (Zaidi et al., 2008). These studies clearly indicate that CFTR is critical for inflammatory response. The lack of a similar immune defect in other organs of CF patients indicates the importance of tissue specific CFTR expression levels and function as well as exposure to pathogenic microorganism(s).

Out of more than 1,500 known mutations in the CFTR gene, those that lead to deletion of phenylalanine at position 508 (ΔF508) in CFTR are the most common CF causing mutations that result in a temperature sensitive folding defect, retention of the protein in the endoplasmic reticulum (ER), and its subsequent degradation by the proteasome (Thibodeau et al., 2005). CF patients exhibit a typical phenotype that is characterized by thick mucus secretions clogging the airways and persistent pulmonary infections leading to the pulmonary failure and death. Bronchoalveolar fluid (BALF) of CF patients contains increased levels of pro-inflammatory cytokines and neutrophils. CF cells have increased basal levels of pro-inflammatory C-X-C chemokine, interleukin-8 (IL-8), attributed to activated NFκB (nuclear factor kappa light chain enhancer of activated B cells) (Jacquot et al., 2008; Zaman et al., 2004). In addition to the significant increase in IL-8 (keratinocyte chemoattractant, KC) mediated neutrophil chemotaxis, the potent anti-inflammatory cytokine IL-10, which suppresses NFκB activation, is reduced in BALF of human CF subjects and *uninfected* $CFTR^{-/-}$ mice (Soltys et al., 2002). Several studies have clearly demonstrated over past two decades that the innate immune response in CF is predisposed towards a hyper-inflammatory state. We have recently demonstrated that expression of wild-type-CFTR (wt-CFTR) downregulates NFκB and IL-8 promoter activities in HEK293 and CFBE4lo- cells (Vij et al., 2009). Data from our and other groups have also shown the ability of lipid-raft localized CFTR to modulate NFκB mediated inflammatory signaling and innate immune response (Dudez et al., 2008; Grassme et al., 2008; Kowalski and Pier, 2004; Reiniger et al., 2007; Schroeder et al., 2002; Vij et al., 2009). Although the key players in the innate and adaptive immune responses are the immune cells, how CFTR deficiency affects the functions of inflammatory cells is scarcely investigated. Macrophages from $CFTR^{-/-}$ mice are shown to secrete higher levels of pro-inflammatory cytokines after lipopolysaccharide (LPS) stimulation (Bruscia et al., 2009). In contrast, neutrophil infiltration is present quite early in CF airways, even before the onset of bacterial infection (Khan et al., 1995).

These studies have shown that the role of CFTR in NFκB mediated inflammatory signaling is very critical but the exact mechanisms are relatively less studied. The present review tries to contemplate on the direct link between expression of functional lipid-raft localized CFTR and the NFκB driven pro-inflammatory immune response in CF. We discuss here the significance of understanding the mechanisms of NFκB induction and CF pathogenesis in relevance to designing a novel therapeutic regime that may help in reversing the chronic lung disease.

CFTR Mediated NFκB Signaling, Innate Immune Response, and Chronic Lung Disease

It is well documented that NFκB mediated IL-8 chemokine secretion and neutrophil influx is a prominent early feature of CF (Nakamura et al., 1992). IL-8, the C-X-C chemokine, is a potent chemoattractant for neutrophils (Yoshimura et al., 1987). The airway epithelium is one of several sources of IL-8 in the airway (Standiford et al., 1990) that serves as the first line of host defense against invading microorganisms. In case of the ΔF508-CFTR mutation, constitutive NFκB activation results in IL-8 mediated chronic neutrophilic lung disease. Some in the field believe that airway inflammation in CF is secondary to the persistent bacterial infection resulting from impaired mucociliary clearance; however, new evidence

supports the idea that dysregulation of the inflammatory response is an intrinsic component of the CF phenotype, and therefore, airway inflammation may occur before or in the absence of bacterial infection (Tirouvanziam et al., 2000). To list a few, several reports have verified that lung epithelial cells expressing mutant CFTR have increased production of pro-inflammatory cytokines and exaggerated NFκB-activation (Blackwell et al., 2001; Joseph et al., 2005). In addition, other studies have also described the presence of neutrophils and elevated levels of IL-8, in the absence of any detected pulmonary pathogen in BALF of CF newborns as compared to the healthy individuals (Lyczak et al., 2002; Noah et al., 1997).

As discussed above, one school of thought believes that excessive inflammation in CF is a result of underlying bacterial infection(s) in the lungs. In contrast, another school believes that CFTR dysfunction in CF results in exaggerated NFκB signaling leading to the pathogenesis of chronic lung disease. In support of the second school of thought, recent data shows a significant increase in the expression of a number of inflammatory markers in the sterile environment of CF fetuses prior to any direct exposure to the pathogens. The study demonstrates that there is a significant increase in activation of NFκB driven genes in CF fetus as compared to the non-CF fetus (Verhaeghe et al., 2007), and also shows that fetal lungs from CF have enhanced NFκB activation. Moreover, others have shown that when CF and non-CF human fetal tracheal grafts were explanted under the skin of immunodeficient SCID mice, there was increased intra-luminal IL-8 and leukocyte levels in the sub-epithelial region of the CF grafts as compared to the non-CF grafts (Tirouvanziam et al., 2000). Although this recent data is convincing, there is little consensus on the mechanism that links CFTR and its inherited mutant forms to chronic lung inflammation. Another interesting observation by Pier and colleagues demonstrates the key role for CFTR in bacterial ingestion and lung clearance of *P. aeruginosa*. They proposed that CFTR is a pattern recognition molecule that extracts *P. aeruginosa* LPS from outer membrane into epithelial cells and activates NFκB signaling (Schroeder et al., 2002). They hypothesized that the lack of this initial IL1-β-NFκB pro-inflammatory signaling (Reiniger et al., 2007) in ΔF508- CF patients results in chronic airway inflammation. They explained that significantly higher NFκB and IL-8 chemokine levels in chronic stages of CF lung disease in ΔF508- human subjects are a consequence of the lack of initial pro-inflammatory response.

Although it has been almost two decades since the identification of the CFTR gene, it remains enigmatic as to how abnormalities in CFTR can cause chronic inflammation that leads to bronchiectasis and end-stage lung disease with lung transplant as the only option to save the patient's life (Rubin, 2007). The excessive inflammation in the CF airways is largely responsible for the development of bronchiectasis, but it has not been clearly understood whether this hyperinflammatory milieu is a result of the chronic infection or it is the primary outcome of the CFTR dysfunction (Machen, 2006). We recently tested the hypothesis that functional CFTR on the cell surface is required for controlling both NFκB activity and downstream inflammatory signaling. Our data showed that the expression of functional CFTR on the cell surface regulates NFκB mediated inflammatory signaling (Figure 1) (Vij et al., 2009). Although it is not completely clear how mutations in CFTR lead to abnormalities of the NFκB pathway, recent findings indicate that the lack of functional CFTR on the cell surface and not just accumulation of misfolded CFTR in the endoplasmic reticulum (ER) or some other by-products of the CFTR mutation leads to the abnormal function of the NFκB pathway. Weber et al. (2001) evaluated cells with the CFTR G551D mutation that produces a protein that is trafficked normally to the cell membrane but lacks Cl^- channel function. Both G551D- and ΔF508- mutations were associated with the upregulation of NFκB activation and increased production of IL-8 although the NFκB activation in the presence of G551D mutation is only about 2-fold as compared to 7-fold for ΔF508. They also confirmed the upregulation of NFκB in CFTR-antisense cell lines and concluded that cell lines with defective CFTR Cl^- channel activity, regardless of the type of

CFTR defect, have a pro-inflammatory phenotype. Although data also indicate that in addition to chloride transport, ΔF508-induces NFκB activity by other mechanisms. Elucidating the mechanisms by which abnormal Cl^- transport and ΔF508-channel function determine dysregulated NFκB activation is an important area for further investigation. Recently, Marc Chanson, Bruce Stanton, and colleagues demonstrated that deletion of PDZ (postsynaptic density 95, PSD-85; discs large, Dlg; zonula occludens-1, ZO-1) binding domain of CFTR (CFTR-ΔTRL) not only compromises the ability of CFTR to localize to gap junction TNFα protein-complex but also results in activation of downstream NFκB signaling. The data indicate that ΔF508 mutation augments NFκB mediated signaling due to lack of gap junctional communication (GJIC) of CFTR with inflammatory receptors (Figure 2) (Dudez et al., 2008; Grassme et al., 2008; Reiniger et al., 2007). Although none of these studies establishes how CFTR cell surface or lipid-raft expression suppresses inflammation, each independently verifies that inhibition of CFTR conductance or lipid-raft localization mimics the pro-inflammatory effects of CFTR knockdown (antisense) (Perez et al., 2007). Thus, functional CFTR at the cell surface is critical for controlling the NFkB mediated inflammatory signaling and innate immune response. Based on the recent work of Eric Gulbins (Grassme et al., 2008), Marc Chanson (Dudez et al., 2008), GB Pier (Kowalski and Pier, 2004; Reiniger et al., 2007), and our group (Vij et al., 2009), we propose a model in which CFTR localization to lipid-rafts in response to infection modulates raft clustering and signaling to NFκB pathway. In contrast to the case of mutant CFTR, lack of lipid-raft CFTR alleviates the raft clustering that induces the activity of the NFκB pathway.

To summarize, there is a consensus on NFκB as a CF marker and role of CFTR in NFκB mediated innate immune response. Moreover, recent studies suggest that lack of CFTR on the cell surface and/or lipid-rafts results in defective innate immune response by modulating the pathology (NFκB signaling) and physiology (ion transport) of the CF lung. Thus, changes in both CFTR expression and cell surface localization are critical for innate immune response and the development of chronic obstructive and inflammatory lung diseases.

Therapeutic Strategies to Control NFκB Mediated Chronic Lung Disease in Cystic Fibrosis

Controlling the excessive airway inflammation is an important methodology in the treatment of chronic CF lung disease, as it is the prime cause of patient mortality. Recent advances in the study of CF lung disease has indicated that NFκB activation and the resulting IL-8 secretion are one of the major causes of neutrophil mediated deleterious lung pathology. Therefore, selective inhibition of these signaling pathways presents a lucrative target for CF therapy that will add to the present day therapeutic strategies (Table 1). In spite of several therapeutic NFκB inhibitors and their applications in innumerable inflammatory conditions including autoimmune arthritis, asthma, COPD, septic shock, glomerulonephritis, atherosclerosis, and cancer, very few have been translated to the clinic. We discuss here the therapeutic strategies to control the pathogenesis of NFκB mediated chronic CF lung disease and methods to revert the lung disease from chronic stages.

The strategies that have shown some promise include azithromycin, one of the most potent macrolide antibiotics used in CF treatment (Saiman et al., 2003). It was also shown to inhibit NFκB and IL-8 levels in CF airway epithelial cells. In addition, Hollis-Eden Pharmaceuticals, San Diego, recently developed HE3286 (TRIOLEX™), which was selected by Cystic Fibrosis Foundation Therapeutics (CFFT) as a drug candidate to target chronic CF lung disease. HE3286 is a partial inhibitor of NFκB pathway (Hollis-Eden Pharmaceuticals, unpublished observations) and is a promising CF drug as complete blockade of this important inflammatory pathway may achieve immune suppression.

Curcumin, a well known anti-inflammatory compound from turmeric, has wide spread applications in the treatment of other airway inflammatory diseases like COPD and allergic asthma (Sharafkhaneh et al., 2007). Marie E. Egan's work showed the beneficial effect of curcumin in rescuing the mutant ΔF508-CFTR from ER associated degradation resulting in increased functional appearance of the protein on the plasma membrane (Egan et al., 2004), although later studies have demonstrated the inability of curcumin to rescue functional mutant CFTR (Song et al., 2004). Curcumin is also considered an effective drug candidate to control inflammation in CF, by inhibiting NFκB activation (Freudlsperger et al., 2008). Curcumin is also a potent Cox-2 inhibitor (Plummer et al., 1999). We have previously discussed that the use of Cox-2 inhibitors in CF for controlling inflammation may augment the progression of the CF lung disease in a subset of CF patients with mild alleles by inhibiting other downstream pathways like EP-2/EP-4-mediated cAMP levels (Devor and Schultz, 1998; Vij et al., 2008) or by switching off the pro-inflammatory response altogether (Figure 3). They may also reduce the efficacy of other therapeutic strategies used to increase CFTR expression and function in these patients. The commonly used broad-spectrum Cox-2 inhibitor, ibuprofen, was first advocated as a long-term therapy for CF lung disease in 1995, following a favorable report of a 4-year controlled trial. However, its clinical use has been limited primarily due to concerns about adverse effects. Additional clinical studies were proposed to better assess the risk-benefit profile. The results of several studies evaluating clinical efficacy of ibuprofen in CF have been recently reviewed (Konstan, 2008).

We propose that sequential deciphering of NFκB and IL-8 mediated neutrophil chemotaxis in CF may lead to identification of better and more specific therapeutic target(s) to improve the overall CF pathophysiology and lung function (Vij et al., 2008). It is becoming evident from recent studies that CFTR plays a critical role in inflammatory response in addition to its well described ion transport function (Dudez et al., 2008; Mehta, 2008; Reiniger et al., 2007; Vij et al., 2009). Thus, an effective treatment for CF may require the identification of small molecule or therapeutic corrector compounds that rescue the optimal amount of mutant CFTR to the cell surface and cholesterol rich lipid-rafts. As a proof of concept fenretinide and docosahexaenoic acid (DHA) have shown promise in rescuing lipid-raft signaling in CF cells and human studies, respectively (Opreanu et al., 2010; Vilela et al., 2006). Although, it remains an open question if we can restore enough mutant CFTR to the cell surface that can control the chronic CF lung disease. In addition, it is not clear if "so-called" functional ΔF508-CFTR restored on the cell surface is capable of controlling the chronic inflammatory signaling. Although, if this can be achieved, it can lead to the promising therapeutic strategy that can correct ion transport dysfunction while also inhibiting the NFκB mediated chronic inflammation. A comprehensive review by Eitan Kerem (Kerem, 2005) elaborates this strategy, discussing the use of pharmacological compounds in enhancing the expression and function of functional CFTR on the apical membrane. We anticipate that if ΔF508-CFTR can regulate the NFκB signaling pathway similar to recent studies with wild-type CFTR, the efforts to rescue the mutant CFTR to lipid-rafts may help in correcting both hyper-inflammatory response and chloride channel function. We would also like to emphasize here that NFκB is the critical mediator of several homeostatic cellular processes, hence therapeutic strategies to target NFκB need to be highly selective. This makes it all the more important to understand the disease specific mechanisms of NFκB activation and lung disease pathogenesis, in order to design novel molecular therapeutic strategies to selectively target or modulate the NFκB activation in CF or other lung diseases.

Is It Possible to Correct CFTR Function and NFκB Mediated Chronic Lung Disease?

Recent data suggest that the higher inflammation in ΔF508 CF could be a consequence of fewer CFTR molecules at the membrane, as would be predicted also with other rare CF stop mutations such as G542X (McCormick et al., 2002). This hypothesis is also consistent with the recent data on some of the CFTR "correctors." These corrector drugs -- MPB-07, miglustat, and NB-DGJ -- not only increase CFTR expression at the membrane but also reduce the inflammatory response of cells to *P. aeruginosa* infection (Dechecchi et al., 2007; Dechecchi et al., 2008). Although both miglustat and NB-DGJ reduced inflammation, NB-DJG did not restore ΔF508-CFTR channel activity and the other correctors (corr4A and VRT325) of CFTR channel function did not correct inflammation (Talebian et al., 2009), suggesting that the effects of CFTR on inflammation are independent of CFTR channel function. We also recently demonstrated that modulating proteostasis by bortezomib or selective gene correctors has a potential to rescue both mutant CFTR and IκB, an endogenous inhibitor of NFκB, from ubiquitin proteasome mediated degradation (Belcher and Vij, 2010; Vij, 2008; Vij et al., 2006). These studies are also in agreement with other recent reports that showed the increase in NFκB activation and IL-8 secretion in 16HBE14o- cells expressing CFTR antisense construct as compared to the CFTR sense (Weber et al., 2001), and the *in vivo* data demonstrated the inherent defect in NFκB expression (Tirouvanziam et al., 2000; Verhaeghe et al., 2007; Vij et al., 2009).

Recently, we demonstrated that CFTR172 inhibitor increases the baseline activation of NFκB and IL-8 secretion in CF and non-CF cells (Vij et al., 2009), which is in concordance with findings of Perez *et al.*, who showed that inhibition of CFTR with CFTRinh-172 increased secretion of IL-8 in 16HBE14o- cells (Perez et al., 2007). CFTRinh-172 is thought to prevent channel opening of CFTR and thus abrogate the chloride current. Recent data, from the mutation analysis of critical residues, suggests that the inhibitor may act directly on CFTR within the sixth transmembrane helix of the protein, a domain that has a key role in the channel pore formation (Caci et al., 2008), but exactly how the inhibitor acts on CFTR is not fully understood. However, this data indicates that functional channel activity on the cell surface is also required for controlling inflammatory signaling. The mechanism by which CFTR controls inflammation is becoming clear but requires further investigation. Recent data suggest that CFTR interacts with the TNF receptor (R1) in lipid-rafts and modulates IL-8 secretion and gap junction formation (Dudez et al., 2008). There is also evidence that TNF-R1 is a modifier of CF as there is an association between polymorphisms in the TNF-R1 gene and lung disease severity (Stanke et al., 2006). These studies suggest dysfunctional cell surface or raft CFTR signaling as the potential mechanisms for pathogenesis of NFkB mediated chronic lung disease. In conclusion, there is an emerging consensus that the presence of CFTR on the cell surface or lipid-rafts regulates the chronic inflammatory response, making the topic worthy of further investigation. Better understanding of the exact mechanism of CFTR mediated NFκB signaling will help with the design of novel therapeutic strategies to rescue the chronic inflammatory and obstructive lung diseases such as CF (Figure 4).

Perspective

Together, these studies suggest a new paradigm that links CFTR expression in lipid-rafts to alterations in intracellular signal transduction, and regulation of NFκB activation and inflammatory response. The clinical implication of these findings is that treatment of CF patients with anti-inflammatory compounds that rescue CFTR to the cell surface or lipid-rafts can block NFκB mediated chronic inflammation and interdict the pathology induced by

defective CFTR, potentially attenuating the progression of CF or related obstructive lung diseases like COPD and emphysema.

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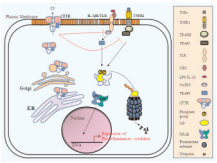

Figure 1.
Role of CFTR in the NFκB mediated innate immune response. The presence of functional CFTR on the plasma membrane and its localization to lipid-rafts are sufficient to control the NFκB mediated hyper-inflammatory immune response. Expression of wild-type CFTR on the membrane inhibits the pro-inflammatory signaling via inflammatory (TNFα, TLR or IL-1β) pathways, which regulate NFκB activation and IL-8 secretion. The precise mechanism by which CFTR regulates the major innate immune response pathways is still unclear. A better understanding of the mechanisms of CFTR-dependent lipid-raft signaling will add to the knowledge of CFTR regulated immune responses and help with the designing of novel therapeutic strategies to control chronic CF lung disease.

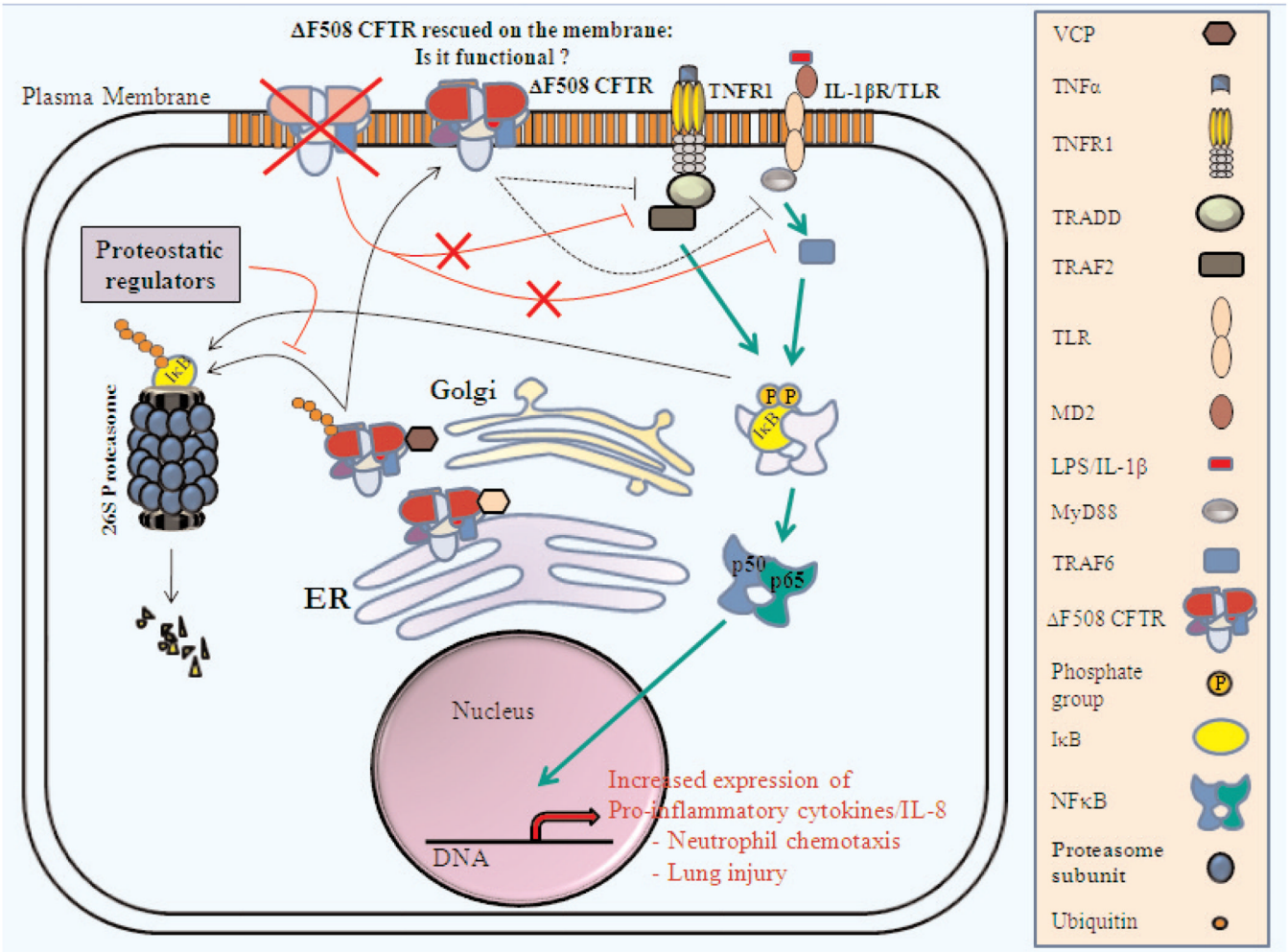

Figure 2.

The hyper-inflammatory immune response in the absence of functional CFTR on the cell surface. Abrogation of the functional CFTR mediates inflammatory response in CF that triggers the chronic proinflammatory signaling. NFκB mediates the hyper-inflammatory response by its over-activation which results in IL-8 and neutrophil mediated chronic CF lung disease. Ubiquitin-proteasome mediated ΔF508-CFTR degradation is a critical mediator of this hyper-inflammatory immune response as it liberates cell surface CFTR mediated NFκB regulation and activity. Moreover, activation of the unfolded protein response (UPR) in the presence of misfolded protein may also trigger the NFκB signaling. Rescue of the mutant CFTR protein on the cell membrane by CFTR "correctors" can restore its chloride efflux defect, although efficacy of rescued mutant CFTR in controlling the NFκB mediated innate immune response needs to be investigated.

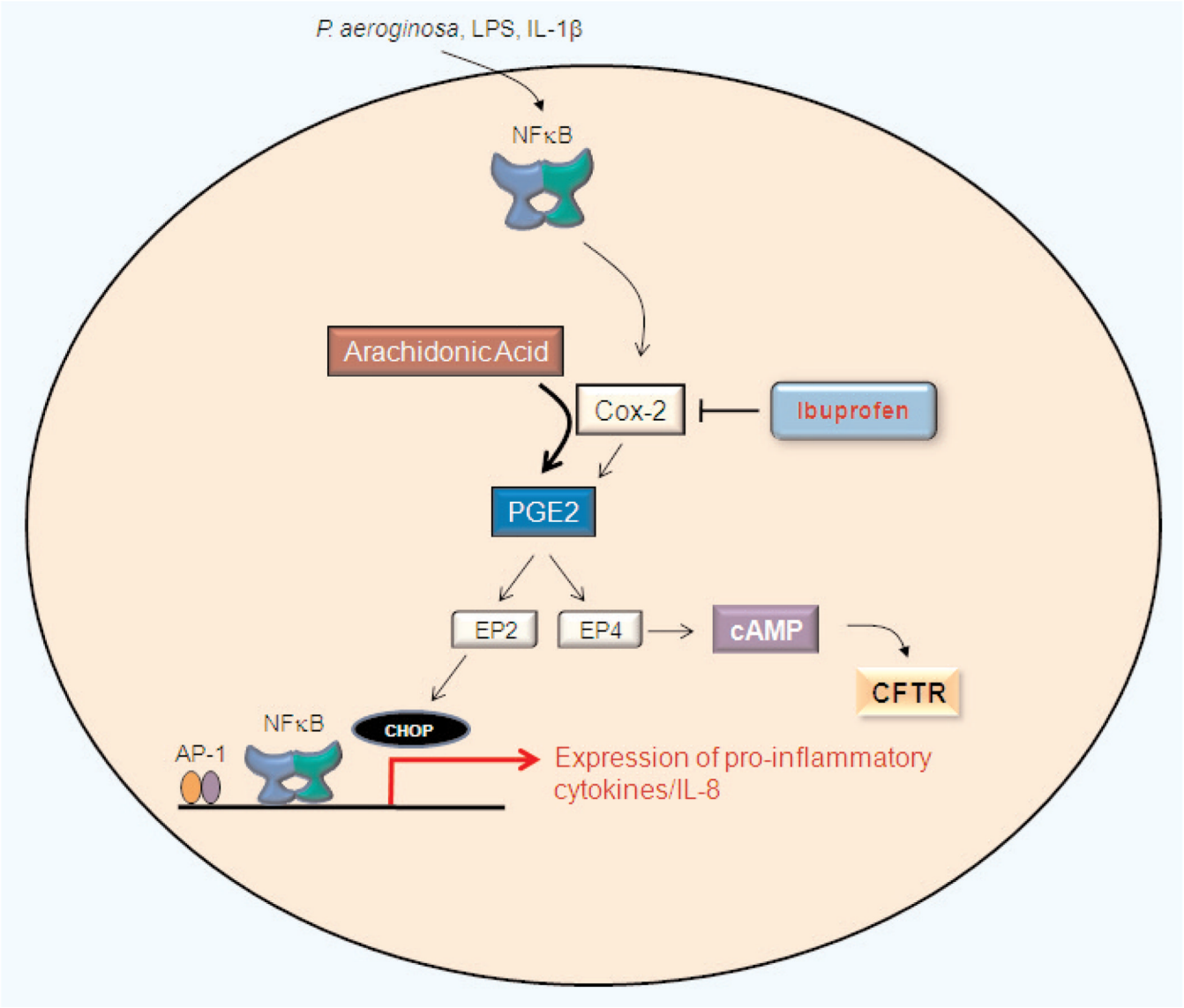

Figure 3.

Cox-2 inhibition suppresses IL-8 levels and cAMP mediated CFTR activation. Pro-inflammatory stimuli (*P. aeruginosa*, IL-1β, or TNFα) increase the levels of PGE-2 via NFκB-mediated Cox-2 induction, which results in induction of IL-8 through the CHOP (C/EBP homologous protein) transcription factor. CHOP is activated via EP-2 (Prostaglandin E2 receptor) in the PGE-2 (Prostaglandin E2)-signaling cascade. The broad-spectrum Cox inhibitor, ibuprofen, suppresses the PGE-2, IL-8, and cAMP levels. Inhibiting Cox-2 may control IL-8 mediated inflammation but it may further deteriorate the CF pathophysiology due to inefficient cAMP-mediated CFTR activation. Use of other Cox-2 inhibitors like curcumin may create a similar scenario that can exacerbate the lung inflammation and obstruction in CF and other chronic obstructive lung diseases like COPD.

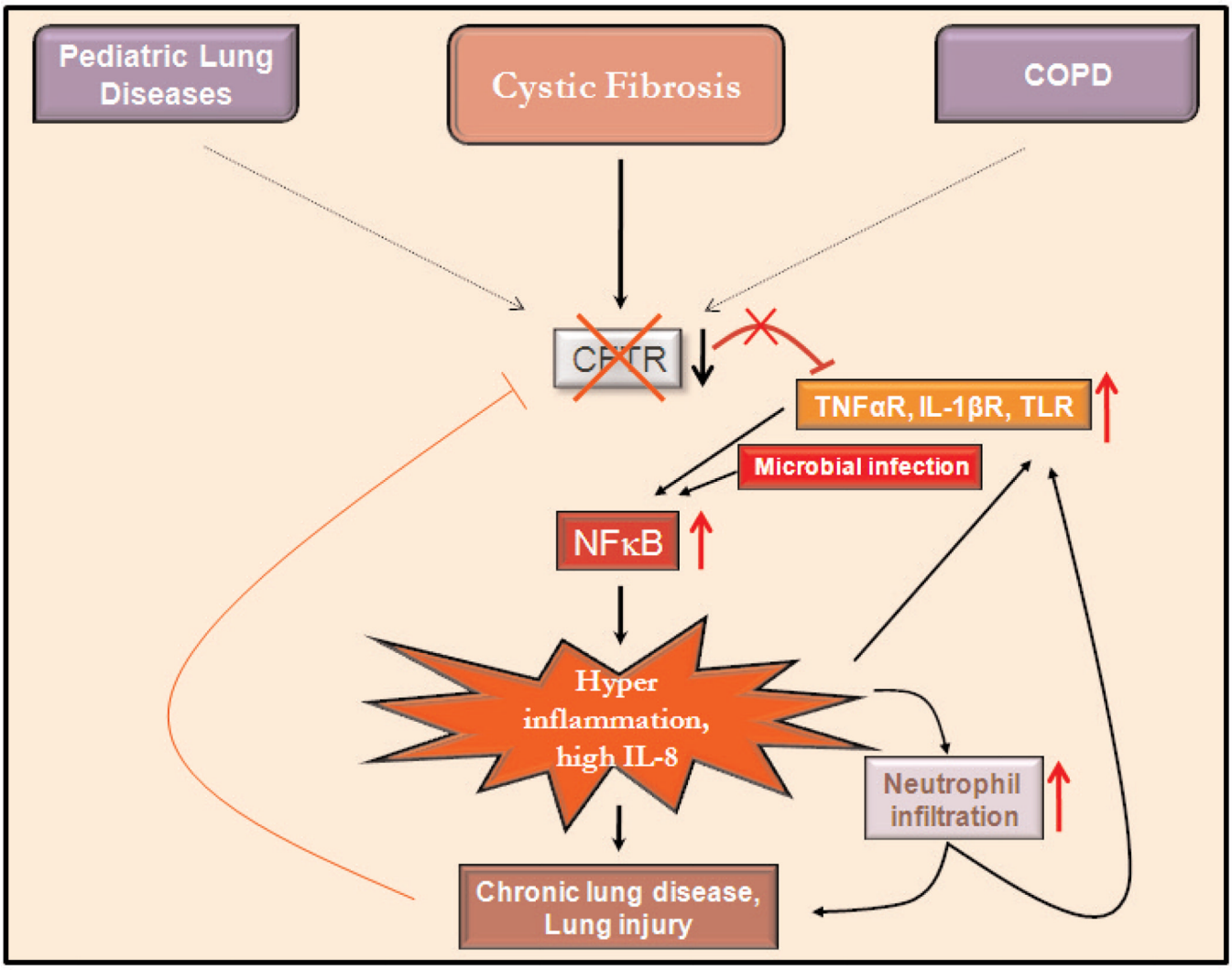

Figure 4.

Role of CFTR in innate immune response in CF and other chronic obstructive lung diseases. Decrease in the functional CFTR protein on the cell membrane and lipid-rafts in CF leads to an enhanced NFκB activation via abrogation of the inhibitory effect on the membrane receptor signaling complexes that triggers the pro-inflammatory cascade. Microbial infection adds up to the NFκB activation, thus further exacerbating the IL-8-neutrophil induced lung pathology. The activated neutrophils result in further induction of pro-inflammatory signaling (TLR, IL-1β, or TNFα) and the lack of CFTR mediated regulatory mechanism creates a cycle of inflammation that leads to pathogenesis of chronic CF lung disease. Severe lung injury again down-regulates the expression of CFTR that contributes to the maintenance of the hyper-inflammatory state. Elucidating the precise cellular mechanisms that contribute to this chronic cycle of inflammation will help develop better drug candidates to halt the progression and persistence of lung damage in CF and other chronic inflammatory lung diseases.

Table 1

Therapeutic Compounds Targeting NFκB Mediated Chronic Lung Disease in Cystic Fibrosis

Drug/Compound Name	Mechanism of Action	Experimental Model	Clinical Status	References
Azithromycin (AZM)	Inhibits NFκB and AP1 activity	*In vivo*	Completed phase III, in clinical use	Saiman et al., 2003
HE3286 (TRI-OLEXTM), Hollis-Eden Pharmaceuticals	Inhibits NFκB activity	*In vivo*	Phase I/II trials in patients with chronic inflammatory conditions	Hollis-Eden Pharmaceuticals, unpublished observations
Curcumin	Inhibits NFκB activity and rescues DF508 CFTR from degradation	*In vitro* *In vivo*	Ongoing safety & efficacy study (data not reported)	Egan et al., 2004; Freudlsperger et al., 2008
Ibuprofen	NSAID, NFκB, and broad spectrum Cox-2 inhibitors	*In vivo*	Completed phase III, in clinical use	Devor and Schultz, 1998; Vij et al., 2008
Fenretinide/Docosahexaenoic acid (DHA)	Regulates lipid-raft and NFκB signaling via ceramide	*In vitro/In vivo*	(DHA) Phase II	Opreanu et al., 2010; Vilela et al., 2006