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Dysregulated Chemokine Signaling in Cystic Fibrosis Lung Disease: A Potential Therapeutic Target

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Abstract

CF lung disease is characterized by a chronic and non-resolving activation of the innate immune system with excessive release of chemokines/cytokines including IL-8 and persistent infiltration of immune cells, mainly neutrophils, into the airways. Chronic infection and impaired immune response eventually lead to pulmonary damage characterized by bronchiectasis, emphysema, and lung fibrosis. As a complete knowledge of the pathways responsible for the exaggerated inflammatory response in CF lung disease is lacking, understanding these pathways could reveal new therapeutic targets, and lead to novel treatments. Therefore, there is a strong rationale for the identification of mechanisms and pathways underlying the exaggerated inflammatory response in CF lung disease. This article reviews the role of inflammation in the pathogenesis of CF lung disease, with a focus on the dysregulated signaling involved in the overexpression of chemokine IL-8 and excessive recruitment of neutrophils in CF airways. The findings suggest that targeting the exaggerated IL-8/IL-8 receptor (mainly CXCR2) signaling pathway in immune cells (especially neutrophils) may represent a potential therapeutic strategy for CF lung disease.

Keywords

Cystic fibrosis; lung inflammation; CFTR; chemokine signaling; IL-8; CXCR2; NF- κ B

INTRODUCTION

Cystic fibrosis (CF), the most common genetic disorder among Caucasians, is an autosomal recessive disease caused by the abnormalities of the protein — cystic fibrosis transmembrane conductance regulator (CFTR) [1]. The most common mutation of gene for CFTR is the deletion of three nucleotides resulting in the absence of phenylalanine at

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CONFLICT OF INTEREST

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position 508, also referred to as F508-CFTR [1]. The primary defect in CF was identified as abnormal ion transport in various epithelia of multiple organs such as the respiratory tract, exocrine pancreas, sweat ducts, reproductive system, intestinal tract, and hepatobiliary system [2]. Although CF is a multisystem disorder, progressive pulmonary disease accounts for most of the morbidity and mortality in CF patients [3]. CFTR is a plasma membrane protein and Camp-regulated chloride channel. Deficient Cl⁻ secretion by defective CFTR channel in combination with excessive Na⁺ absorption across epithelia leads to a reduction in periciliary fluid volume and impairment of the mucociliary clearance, which facilitates the colonization and infection of bacteria in the airway. Chronic pulmonary infection and excessive inflammation eventually lead to pulmonary damage characterized by bronchiectasis, emphysema, and lung fibrosis [4].

Chemokines are the subset of cytokines mediating chemotaxis of cells along a concentration gradient by interaction with their cognate receptors on the membrane of the target cells [5]. To date, chemokines are classified into four sub-classes, *i.e.*, XC-, CXC-, CC- and CX3C- chemokines according to the relative position of the conserved N-terminal cysteine residues. The classical chemokine receptors, a sub-family of G-protein-coupled receptors (GPCRs), are therefore categorized into four subclasses based on their chemokine ligands. Chemokine/chemokine receptor-mediated signalings are relayed by various heterotrimeric G-proteins that further activate distinct effector molecules such as phospholipase, phosphoinositide 3-kinase (PI3K) and tyrosine kinases [6]. The atypical chemokine receptors are identified as G-protein-independent but arrestin-dependent receptors which function to form the chemokine gradients and scavenge chemokines [7]. Interleukin (IL) -8 (also known as CXCL8) is a potent proinflammatory and chemoattractant cytokine that plays a key role in the activation and recruitment of various immune cells (*i.e.*, neutrophils, macrophages, *etc.*) during inflammation [8]. IL-8 is a member of CXC-chemokine family and the GPCRs CXCR1 and CXCR2 are the receptors that mediate IL-8-induced signaling. One of the major functions of IL-8 involved in inflammatory disease is to attract and activate neutrophils [9]. The expression levels, affinity to IL-8, and biological functions differ among the two receptors in different cell types and in different disease conditions [10].

Cystic fibrosis is characterized by severe lung inflammation which is associated with massive overproduction of the proinflammatory cytokines including IL-8. The elevated level of IL-8, along with the consequently excessive neutrophil infiltration mediated by IL-8 in CF lungs, is one of the central mechanisms behind CF lung pathophysiology. Previous studies have reported various malfunctions of CF neutrophils including enhanced granule enzyme activity, increased production of reactive oxygen species (ROS) and IL-8, delayed apoptosis, defective intracellular bacterial killing, *etc.* [11–15]. Moreover, the elevated level of neutrophil elastase and excessive formation of neutrophil extracellular traps (NETs) also contribute to chronic infection of certain opportunistic pathogens such as *P. aeruginosa* and host tissue damage in CF [16–21]. Neutrophil-dominated inflammation in CF drives a vicious cycle of bacterial colonization, airway inflammation, and structural damage, which overwhelms normal resolution and repair mechanisms and pathways. These cause irreversible lung damage, decline of lung function, and premature death of CF patients [22]. IL-8, as the major chemokine recruiting neutrophils during inflammation, is involved in

excessive infiltration of neutrophil in CF airways. Moreover, it also mediates the functions of neutrophils associated with pathogenesis of CF such as inducing NET formation [23].

A thorough understanding of the molecular mechanisms associated with the chronic lung inflammation in CF is essential to the design of novel approaches to reduce the progressive decline of lung function and tissue damage. A variety of chemokines and immune cells are involved in the dys-regulation of immune responses in CF airway pathology. However, overexpression of IL-8 and infiltration of neutrophils are the two major markers representing hyperinflammation in CF airways and associated with the clinical status of CF patients. Hence, this review will focus on the dysregulated signaling involved in the overexpression of chemokine IL-8 and excessive recruitment of neutrophils in CF airway inflammation, which suggests that targeting the exaggerated IL-8/IL-8 receptor (mainly CXCR2) signaling pathway in immune cells (especially neutrophils) may be a potential therapeutic strategy for CF lung disease.

THE REGULATION MECHANISM OF IL-8 EXPRESSION AND SECRETION

IL-8 is produced by macrophages, epithelial cells, airway smooth muscle cells, endothelial cells, *etc.* [24–26]. The gene of IL-8 is located at chromosome 4q12-q21 in human [27]. It is initially produced as a precursor peptide of 99 amino acids which undergoes cleavage to create several active IL-8 isoforms [28]. IL-8 is barely secreted from non-induced cells and its production is rapidly induced by a wide range of stimuli including proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) or IL-1 β [29, 30], bacterial [31–33] or viral [34, 35] components, and cellular stress [36, 37]. Some stimuli, such as IL- β or TNF- α , can up-regulate IL-8 by more than 100-fold [29], whereas others, such as some bacterial products or epidermal growth factor (EGF), just cause a moderate increase in IL-8 secretion [33, 38]. IL-8 production can be induced in many cell types *via* different signaling pathway including the mitogen-activated protein kinases (MaPKs) ERK $\frac{1}{2}$, p38, and JNK, as well as PI3K pathway [39].

Numerous studies have revealed that a sequence within the 5'-flanking region of the IL-8 gene is essential and sufficient for IL-8 induction [40, 41]. The consensus sequences for transcription factors such as CREB, AP-1, NF-IL6, CHOP and NF- κ B were identified in the proximal region of the IL-8 gene promoter [42]. This promoter element contains a nuclear factor NF- κ B element which is required for activation of IL-8 gene transcription in almost all cell types studied. The binding of NF- κ B (P65) to IL-8 promoter and recruitment of RNA polymerase II were detected within 30 minutes after IL-1 stimulation, which indicates the importance of NF- κ B in IL-8 transcriptional activation. The IL-8 promoter also contains the binding sites for activating protein (AP)-1 and CAAT/enhancer-binding protein (C/EBP) which might be not essential for induction but required for maximal gene expression [39–41, 43–46].

Multiple stimulators such as *P. aeruginosa* and proinflammatory cytokines lead to activation of IL-8 transcription *via* a variety of signaling pathways including MAPKs p38, JNK and ERK, as well as the nuclear transcription factors NF- κ B, NF-IL6, and AP-1 [47, 48]. These signaling pathways cooperatively activate IL-8 gene transcription and stabilize IL-8 mRNA

upon stimulation [49–51]. However the redundancy of the phosphoproteins and transcription factors involved in IL-8 expression indicates that no single step can completely inhibit IL-8 gene transcription. Among these pathways, NF- κ B activation has been widely studied and thought to be the most important hub involved in CF lung disease.

CFTR DYSFUNCTION IN AIRWAY EPITHELIAL CELLS MIGHT BE THE PRIMARY FACTOR CAUSING OVEREXPRESSION OF IL-8

Although it's still controversial whether the intrinsic deficiency of CFTR or the bacterial infection is the primary factor leading to the hyper-inflammation in CF airways, substantial evidence has suggested that dysregulated expression and signaling mediated by cytokines and chemokines as well as abnormal infiltration and functions of immune cells are the common features in CF lung disease, especially at the late stage.

The airway epithelial cells (AECs), together with mucins secreted by intraepithelial goblet cells, form a physical barrier. They also express pattern recognition receptors (PRRs) which recognize pathogen associated molecular patterns (PAMPs), resulting in the production of proinflammatory cytokines and chemokines, as well as activation and recruitment of immune cells, such as granulocytes and lymphocytes to the site of infection. Airway surface dehydration and inefficient mucociliary clearance resulting from abnormal CFTR function disable the physical barrier of airway, which predisposes the airway to chronic infection with pathogens, such as *S. aureus* and *H. influenza* at early stage, as well as *P. aeruginosa* and *B. cepacia* complex occurring later on [2]. The factors contributing to the inflammatory progression in CF airways still remain elusive. Some evidence suggests that the hyper-inflammation of the airway is triggered by the infection of bacteria and viruses [52, 53], whereas others argue that the intrinsic deficiency of CFTR functions is the primary factor which causes the CF airway prone to inflammation even in the absence of appreciable colonization of bacteria [54, 55].

AECs are one of the major sources of chemokines including IL-8 in the airway during inflammatory response. Many clinical data shows that airway inflammation occurred at the early age of CF patients in the absence of appreciable colonization of bacteria [55]. This phenomenon substantially supports the argument that the intrinsic deficiency of CFTR functions might be the primary factor which makes the CF airway prone to inflammation [54]. *In vitro* experiments also prove that the basal expression or secretion of IL-8 is upregulated without any stimulation of pathogens or cytokines in AECs with mutated CFTR [55]. It has been argued that the minor infections couldn't be clinically detectable, which might be responsible for the conclusion of up-regulation of proinflammatory cytokines and chemokines without infection [56]. CF patients are often infected with certain opportunistic bacteria, and bronchoalveolar lavage (BAL) samples from different loci of airway show different features [57]. Those factors should be considered when the infection status and inflammatory conditions are evaluated. Moreover, multiple biological factors or chemicals in the air could challenge the airways of the infants with CF. Atmospheric pollutants such as ozone can cause increased IL-8 production in CF airway epithelial cell lines [58]. The common models for studying the proinflammation of CF *in vitro* include the established

airway epithelial cell lines with mutated CFTR, the AECs directly from the CF patients, or the CFTRinh-172-treated AECs with wild-type CFTR [59–61]. Multiple factors such as cell type, genetic manipulation, culturing medium and materials might affect the cell signaling involved in the proinflammatory responses including the secretion of IL-8 [62–64]. Cells from CF patients might have been pre-activated by some pathogens and the effects might still persist, at least partially, even after the samples are cultured in the medium for a period of time. CF disease is a genetic disorder which has been acquired since embryogenesis [2]. Some studies demonstrated that CFTR is critical to the development of lung during embryogenesis [65], and CFTR functions as more than just an ion channel [66]. CFTRinh-172 is a thiazolidinone which selectively and reversibly inhibit CFTR channel in a voltage-independent manner [67]. Some reports suggested that CFTRinh-172 could be an alternative for mimicking the inflammatory process in CF [60]. Inhibition of the Cl^- channel function without interfering the membrane expression of CFTR on the mature cells might induce some pathological changes similar to CF. But the possible off-target, non-specific effects of CFTRinh-172 should also be considered when interpreting the results [68].

PATHOGENS INITIATE OVEREXPRESSION OF IL-8 IN CYSTIC FIBROSIS

CF lung disease is characterized by chronic infection especially by some opportunistic pathogens in airways. Upon infection, AECs detect extracellular or cytosolic/endosomal PAMPs predominantly by the Toll-like receptors (TLRs), a family of type-I transmembrane proteins [69]. TLRs recognize PAMPs through extracellular leucine-rich repeat motifs and mediate signaling through an intracellular Toll/IL-1 receptor homologous region (TIR) domain. TLR activation relays signals through MAPKs/AP-1, IKKs/NF- κ B, and interferon regulatory factors (IRFs) to induce production of inflammatory cytokines and chemokines [70, 71]. The TLRs are expressed on various immune and non-immune cells including B lymphocytes, natural killer (NK) cells, macro-phages, dendritic cells, fibroblast cells, epithelial cells, and endothelial cells [72]. Different types of TLRs can recognize distinct PAMPs, such as TLR2 recognizing lipopeptides or lipoproteins [73], TLR4 recognizing LPS from Gram-negative bacteria [74] and the fusion protein from respiratory syncytial virus [75], TLR5 recognizing a monomer of flagellin [76, 77].

Airway infection induces rapid cytokine and chemokine gene expression in AECs through activation of TLRs [78]. The immune cells are then recruited and activated through these cytokines and chemokines, such as IL-8, CXCL1, and CXCL5 for neutrophils, IL-1 β , CCL2, CCL3, CCL20 and TNF- α for monocyte/macrophage, IFN- α/β and CCL3 for NK cells [79–82]. The expression of TLRs in airway epithelial cells is important in inflammation and immunity in response to inhaled pathogens. However, the uncontrolled or deregulated immune response mediated by TLRs might be the critical trigger for the exaggerated inflammatory responses and lung fibrosis in CF.

P. aeruginosa is the most common pathogen in airways of chronically infected CF patients. Roussel *et al.* have shown that loss of functional CFTR leads to enhanced IL-8 synthesis upon exposure to *P. aeruginosa* diffusible material, which is dependent on TLRs and NADPH oxidase/ROS [59]. The decreased level of extracellular glutathione leads to a greater sensitivity to ROS in CF [83]. The PAMP/TLR signaling might be potentiated by the

excessive ROS in CF, which results in an increased and prolonged production of IL-8. Moreover, ROS is involved in cytokine synthesis by phagocytes and epithelial cells, by inducing the production of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-8, which in turn potentiate ROS production by neutrophils oxidative burst [84].

Activation of an EGF receptor (EGFR) signaling cascade has been shown to be implicated in IL-8 production in AECs [85, 86]. Expression of EGFR and its ligands is increased in the airways of CF patients [87]. TLRs can crosstalk with EGFR to produce certain innate immune responses. It has been reported that multiple TLRs in airway epithelial cells induce IL-8 production, which depends on EGFR activation [88]. *P. aeruginosa* stimulation can induce the expression of the EGFR pro-ligand more strongly in CF AECs. The level of IL-8 is higher and EGFR-mediated signaling might play important role in regulating IL-8 synthesis in CF AECs [89].

ABNORMAL CALCIUM HOMEOSTASIS MIGHT PARTICIPATE IN THE UPREGULATION OF IL-8 EXPRESSION

Bacteria-triggered Ca²⁺ fluxes in airway cells can stimulate transcription of NF- κ B-dependent genes, including IL-8 [48, 90, 91]. Even without pathogen stimulation, the increase of cytosolic Ca²⁺ is sufficient for NF- κ B activation and IL-8 production in AECs [48, 91]. MAPKs and calmodulin-dependent kinases might be involved in Ca²⁺-dependent NF- κ B activation [91–93].

Evidence has shown that intracellular Ca²⁺ mobilization in cells with defected CFTR was enhanced upon stimulation with agonists including UTP, proinflammatory mediators, and pathogens [63, 91, 94, 95]. The elevated Ca²⁺ mobilization might be associated with the increased activity of IP3 receptors resulting from the retention of mutated CFTR protein in endoplasmic reticulum (ER) [96, 97]. A deficient mitochondrial Ca²⁺ uptake related to mitochondrial membrane depolarization might also potentiate the increase of intracellular Ca²⁺ level in AECs with mutated CFTR [98]. Aberrant Ca²⁺ homeostasis and associated NF- κ B activation were studied at the single cell level in a CF bronchial epithelial cell line [99]. Upon IL-1 β stimulation, a greater and more prolonged ER Ca²⁺ release accompanied by the activation of NF- κ B was observed in CFTR-deficient bronchial epithelial cells, as compared to the CFTR-corrected epithelial cells [99]. Antigny *et al.* reported that the membrane presence, but not the channel activity, of CFTR is required to decrease the Ca²⁺ mobilization in corrected CF cells [100]. Thus the abnormal trafficking of mutated CFTR protein might have profound effects on intracellular Ca²⁺ homeostasis.

Transient receptor potential channels (TRPCs)-mediated Ca²⁺ entry is also involved in the regulation of Ca²⁺ homeostasis. Increased Ca²⁺ influx in CF cells is related to TRPC6 which is functionally and reciprocally coupled with CFTR [61, 101, 102]. Store-operated Ca²⁺ entry refers to the sustained Ca²⁺ influx through plasma membrane channels activated by Ca²⁺ release from the ER. Elevated Ca²⁺ signaling in CF cells can be caused by the increased insertion of Ca²⁺ channel Orai1 into the plasma membrane during Ca²⁺ store depletion, which is associated with enhanced IL-8 secretion [103].

DISTURBED BALANCE BETWEEN PROINFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES IS RESPONSIBLE FOR THE SUSTAINED EXPRESSION OF IL-8

The regulated production of proinflammatory and antiinflammatory cytokines is important for the efficient immune response against infection and wound healing. The proinflammatory cytokines such as IFN- γ , IL-1 β , TNF- α and IL-8 are markedly increased in sputum, BAL and peripheral immune cells from CF patients [104–107]. Upon stimulation by pathogens, CFTR-deficient epithelial cells also produce those proinflammatory cytokines at higher level as compared with CFTR-WT cells [59, 108, 109]. The enhanced expression of proinflammatory cytokines and insufficient production of anti-inflammatory cytokines (such as IL-10) lead to the exaggerated inflammatory responses in CF lung disease.

Many PAMPs and damage-associated molecular patterns (DAMPs) can initiate the production and secretion of IL-1 β and TNF- α via NF- κ B pathway. IL-1 β and TNF- α mediated signaling further activates NF- κ B signaling and thus a sustainable activation cycle of NF- κ B signaling pathway can enhance the immune response. In CF cells elevated activity of NF- κ B triggers the formation of this vicious cycle [110–112]. The overexpression of these proinflammatory cytokines in CF might result from the elevated activity of NF- κ B. Positive feedback of these cytokines causes prolonged and enhanced expression of NF- κ B target genes including IL-8. IL-1 β and TNF- α are prototypical proinflammatory cytokines that regulate the chemokine network [113]. A variety of evidence shows that IL-1 β and TNF- α are involved in the upregulation of IL-8 in CF cells. This effect might be associated with the hyper-activated signaling pathways of NF- κ B, AP-1, ERK1/2, p38 and JNK [112, 114, 115].

Positive feedback serves to amplify inflammatory signals. At early stage, the inflammatory signals such as LPS can activate NF- κ B which promotes the production and release of TNF- α and IL-1 β . TNF- α and IL-1 β in turn activate NF- κ B which then enhances the production of other inflammatory mediators including IL-8 and IL-6 [116]. The expression of these later mediators may largely depend on TNF- α and IL-1 β . TNF- α , IL-1 β and endotoxin could also stimulate the production of anti-inflammatory cytokines such as IL-10 [117–119]. IL-10 treatment can suppress NF- κ B activation and IL-8 production in both non-CF and CF bronchial epithelial cells, which is associated with the reduced expression of phosphorylated I κ B and IKK α/β [120]. The low levels of the anti-inflammatory cytokine IL-10 has been observed in CF cells [107, 121].

UPREGULATED NF-KB SIGNALING IS THE HUB INVOLVED IN THE OVEREXPRESSION OF IL-8

NF- κ B is the key transcription factor which regulates the expression of genes involved in innate and adaptive immune response, inflammation, apoptosis, and oncogenesis [122]. It functions as a potent and pleiotropic transcriptional activator in the signal-transducing pathways activated by a wide variety of pathogenic signals and cytokines [122]. The cell type-specific activity of NF- κ B is dependent on its interaction with other transcription

factors on gene regulatory elements. The mammalian NF- κ B family is composed of five structurally related subunits, NF- κ B1 (p50 and its precursor p105), NF- κ B2 (p52 and its precursor p100), c-Rel, RelA (p65), and RelB, which form homodimers and heterodimers with different signaling functions [122, 123]. RelA/NF- κ B1 (p65/p50) heterodimer is often referred to as a 'classic' NF- κ B. As dimeric transcription factors, they recognize a common DNA sequence 5'-GGG(A/G)NN(T/C)(T/C)CC-3' (N is any base) [122, 123]. NF- κ B is retained in the cytoplasm as inactive form by interacting with a family of inhibitory proteins — I κ B. The classic I κ B family mainly includes I κ B α , I κ B β and I κ B ϵ . Other I κ B-like molecules such as Bcl3 and I κ BNS, are localized in nucleus and regulate the transcriptional activity of NF- κ B [124–126]. Nuclear translocation and activation of NF- κ B depends on signal-induced degradation of I κ Bs, which is controlled by a kinase relay module including the I κ B kinase (IKK) α , β , γ signalosome [123].

NF- κ B is required for maximum transcription of many cytokines including TNF- α , IL-1 β , IL-2, IL-6 and IL-12, chemokines such as IL-8, GRO- α , β , γ , MIP-1, MCP-1 and RANTES, which are involved in the acute inflammatory responses [116]. The production of cytokines is predominantly regulated by the transcription rates of cytokine genes instead of secretion in response to the inflammatory stimuli. Therefore transcription factors are critical in regulating inflammation mediated by cytokines and chemokines [116]. The induction of IL-8 gene expression and protein production result from several signaling pathways including NF- κ B -dependent, -independent, and posttranscriptional mechanisms [114, 127]. However, NF- κ B-dependent signaling is the major mechanism responsible for the overexpression of IL-8 in CF [112, 128]. NF- κ B mediated IL-8 secretion and neutrophil influx is a prominent feature of CF [129]. Although it's not very clear how CFTR protein on the plasma membrane controls the activity of NF- κ B, it has been shown that CFTR can negatively regulate the activation of NF- κ B signaling pathway [130–132]. Hunter *et al.* have reported that expression of CFTR protein suppresses basal and TNF- α -induced NF- κ B activity and consequent expression of IL-8 gene [132]. CFTR inhibition with CFTRinh-172 significantly increases NF- κ B activity by approximately 30% in CFTR expressing cells whereas activation of CFTR by elevating cAMP depresses NF- κ B activity by around 25% below baseline [132]. Weber *et al.* also reported that impaired CFTR Cl⁻ channel activity and ER accumulation of mistrafficked CFTR contribute to the endogenous activation of NF- κ B and consequently to the elevated production of IL-8 without infection in the cells with CFTR mutations [133]. The elevated basal level of NF- κ B activation could be further strengthened during infection with pathogens including *P. aeruginosa* [134, 135].

Schmidt *et al.* investigated the mechanism involved in the proinflammatory cytokine-induced activation of NF- κ B in both transient and persistent phases [136]. The biphasic NF- κ B activation is mediated by different MAP3K and I κ B isoforms which are involved in specific complex formation with IKK and NF- κ B. Upon the stimulation by TNF- α and IL-1 α , MEKK3 was essential in the rapid and transient activation of NF- κ B *via* the formation of the I κ B α :NF- κ B/IKK complex, whereas MEKK2 is important in the delayed and persistent activation of NF- κ B by assembling the I κ B β :NF- κ B/IKK complex. These findings provide insight into the regulatory mechanism involved in the cytokine-induced specific and temporal gene expression [136]. Hoffmann *et al.* revealed that I κ B α mediates an oscillatory NF- κ B activation by rapid activation and strong negative feedback, whereas

I κ B β and I κ B ϵ act to dampen the long-term oscillations of the NF- κ B response because of their slow response to IKK activation [137]. Furthermore, unique structure of the I κ BP:NF- κ B complex might contribute to its ability to mediate persistent activation of NF- κ B [138]. These characteristics of I κ B-NF- κ B signaling support the important role of I κ B in the regulation of temporal NF- κ B activation. It has been reported that the basal level of I κ B β is increased in the bronchial epithelia cells (IB3) with mutant CFTR. TNF- α treatment results in increased formation of hypophosphorylated I κ B β and elevated nuclear localization of I κ B β in IB3 cells compared with the other cell lines with wild-type CFTR [112]. Since I κ B β is involved in sustained activation of NF- κ B, and hypophosphorylated I κ BP can function as a chaperone for NF- κ B to be transported into the nucleus [139], excessive expression of I κ BP might be responsible for the over-activation of NF- κ B in CF.

A20 is an inducible cytoplasmic protein which down-regulates NF- κ B signaling *via* ubiquitination and deubiquitination of signaling molecules [140]. The dual functions of A20 facilitate effective inhibition of NF- κ B by inactivating target proteins through deubiquitination, and subsequently targeting those proteins for proteasomal degradation *via* ubiquitination [140, 141]. NF- κ B activation can induce the expression of A20 which in turn switches off the activation of NF- κ B through down-regulating the signaling mediators [142]. Many signaling pathways mediated by IFN- α , IL-1 β , or bacterial/viral products could induce the production of A20, which is essential for the termination of NF- κ B activation and proinflammatory cytokine expression [143, 144]. However, LPS-induced expression of A20 is delayed in CF airway cells [145]. In addition, the formation of functional A20 ubiquitin editing complex is also abnormal. Hence, the aberrant expression and function of A20 might be one of the reasons for the persistent nuclear expression of P65 and overexpression of IL-8 in CF airway cells [145].

TARGETING IL-8/CXCR2 SIGNALING AXIS IN CF LUNG DISEASE

Neutrophil recruitment to sites of infection or injury is a crucial step in the inflammatory response of the innate immune system, protecting the host from invading bacteria. However, uncontrolled neutrophil infiltration often leads to tissue dysfunction and damage [146]. Therefore, blocking neutrophil transmigration across cellular barriers such as mucosal or airway epithelium represents an effective approach to prevent excessive inflammation in many diseases including cystic fibrosis.

CF lung disease is characterized by a chronic and non-resolving activation of the innate immune system with excessive release of chemokines/cytokines including IL-8 and persistent infiltration of immune cells, mainly neutrophils, into the airways. The chemokine receptor CXCR2 is a major receptor regulating recruitment of neutrophils in acute and chronic inflamed tissues [147]. Activation of CXCR2 by its cognate ligands (such as IL-8) induces intracellular signals associated with chemotaxis, recruitment and infiltration of leukocytes (especially neutrophils) from the bloodstream during inflammation [148]. CXCR2 signaling must be tightly regulated to provide both effective immune protection and avoid inflammation-induced pathology. Thus, the mechanisms that fine-tune the CXCR2-mediated inflammatory response are of particular importance. Investigations focused on identifying CXCR2 molecular pathways involved in cellular dysfunctions in CF may help

identify new therapeutic targets that, in combination with current treatments, could improve the life expectancy for CF patients.

It has been well documented that blocking IL-8/CXCR2 biological axis *via* using CXCR2 neutralizing antibodies or selective CXCR2 antagonists, or neutralizing antibodies against cognate CXCR2 ligands (such as anti-IL-8 antibody) potentially inhibited neutrophil migration and infiltration *in vitro* and *in vivo*, and significantly ameliorated inflammation in various inflammatory diseases such as experimental colitis [149–156]. In a recent clinical trial, an oral and well-tolerated CXCR2 antagonist, SB-656933, was administered into adult subjects with cystic fibrosis, who showed trends for improvement in sputum inflammatory biomarkers, although with an increase in systemic inflammatory markers [157]. Therefore, CXCR2 biological axis appears to represent an attractive potential therapeutic target [158]. However, targeting CXCR2 receptor alone may lead to undesired global effects as CXCR2 has also been reported in many other vital cellular functions, such as in preservation of oligodendrocyte function and myelination of neural tissues [159]. Therefore, it is important to identify selective therapeutic targets that might be specific for neutrophil-dominated inflammation as in CF lung disease.

CXCR2 possesses a consensus PDZ (PSD-95/DlgA/ZO-1) motif at their carboxyl termini, and the PDZ motif has been reported to modulate cellular chemotaxis [160]. A variety of PDZ scaffold proteins have been documented to mediate the formation of compartmentalized multi-protein complexes critical for efficient and specific signaling in cells [161–164]. Recently, we have demonstrated that the PDZ motif of CXCR2 plays an important role in regulating neutrophil functions as disrupting this interaction mediated by PDZ motif *via* using an exogenous peptide mimic (mapping CXCR2 PDZ motif) potentially and significantly inhibited CXCR2-mediated calcium mobilization and neutrophil chemotaxis and transepithelial migration, suggesting that CXCR2 signaling complex could be a potential therapeutic target for neutrophil dominant inflammatory diseases, such as cystic fibrosis [147]. Results from this study establish the basis using the CXCR2 PDZ-targeting peptide inhibitor as lead sequence/compound to develop potent pharmacological compounds with enhanced affinity, selectivity, bioavailability, and metabolic stability that could effectively and specifically tackle CF lung inflammation in future clinical trials.

MicroRNAs (miRs) are endogenous noncoding RNAs which regulate gene expression post-transcriptionally by targeting mRNAs for degradation and/or translational inhibition [165]. Studies have reported that miRs are associated with elevated expression of IL-8 in CF lung disease. Bhattacharyya *et al.* studied the differential expression of miRs in F508-CFTR and WT CFTR lung epithelial cell lines by screening a miR library. Especially, the expression of miR-155 was found more than 5-fold elevated in CF lung epithelial cells in culture, compared with control cells. MiR-155 was also increased in lung epithelial cells and circulating neutrophils collected from CF patients, as compared with non-CF samples. The up-regulation of miR-155 promoted PI3K/Akt signaling and production of IL-8 by inhibiting SHIP1 expression [166]. Another study by Fabbri *et al.* revealed miR-93 was highly expressed in CF bronchial epithelial cells in basal conditions, and decreased upon infection of *P. aeruginosa*, which was responsible for a stabilization of the IL-8 mRNA and the increased expression of IL-8 [167]. Multiple cytokines and chemokines are involved in the

pathophysiologic process of CF lung disease. Targeting one cytokine or chemokine might not achieve significant and long-term clinical effects. Considering that a single miR can target several mRNAs [168], miRs might be an optimal candidate for treatment of hyper-inflammatory conditions of CF lung disease if the association between specific miRs and the pathophysiology of cystic fibrosis lung disease has been well established.

CONCLUDING REMARKS

The discovery of CFTR gene 25 years ago has led to the much clearer understanding of the molecular and cellular pathogenesis of CF. However, the processes that initiate and perpetuate CF lung disease still remain incompletely understood. The mechanisms and pathways responsible for lung inflammation in CF are still under debate. The hallmarks of CF lung disease are respiratory infections by opportunistic pathogens and the deregulated inflammatory responses. Multiple defective inflammatory responses have been linked to the deficiency of CFTR, including dysregulated innate and acquired immunity, abnormal lipid metabolism, defective molecular signaling, *etc.* [169]. Diverse therapeutic strategies have been developed to disrupt pathophysiologic cascade in CF lung disease, such as CFTR potentiators and correctors [170], dornase alpha [171], and antibiotics [172], which may indirectly promote the resolution of over-inflammation in CF airway. Drugs directly targeting the inflammatory mediators, such as leukotriene B₄, have been shown to slow the decline in lung function and improve survival [169, 173]. Clinical trials using anti-inflammatory drugs in CF patients have been conducted for more than 30 years. Although the outcome shows promise but the progress is still relatively small. Ibuprofen has been the only anti-inflammatory drug currently recommended for the long-term treatment of CF airway inflammation [174]. The newly FDA-approved drug, Orkambi (lumacaftor 200 mg/ivacaftor 125 mg) [175, 176], for CF patients of 12 years and older who carry the most common CFTR mutation (F508), is very costly, though the clinical studies demonstrate that one of the components, ivacaftor, indeed rescued the impairment of neutrophils from patients with the G551D mutation [177]. Therefore, there is still a relatively long way to go before safe, effective, and affordable therapies emerge.

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