Mucus obstruction and inflammation in early cystic fibrosis lung disease: Emerging role of the IL-1 signaling pathway

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Abstract
Mucus plugging constitutes a nutrient-rich nidus for a bacterial infection that has long been recognized as a potent stimulus for neutrophilic airway inflammation driving progressive lung damage in people with cystic fibrosis (CF). However, mucus plugging and neutrophilic inflammation are already present in many infants and young children with CF even in the absence of detectable bacterial infection. A series of observational studies in young children with CF, as well as investigations in animal models with CF-like lung disease support the concept that mucus plugging per se can trigger inflammation before the onset of airways infection. Here we review emerging evidence suggesting that activation of the interleukin-1 (IL-1) signaling pathway by hypoxic epithelial cell necrosis, leading to the release of IL-1α in mucus-obstructed airways, may be an important mechanistic link between mucus plugging and sterile airway inflammation in early CF lung disease. Furthermore, we discuss recent data from preclinical studies demonstrating that treatment with the IL-1 receptor (IL-1R) antagonist anakinra has anti-inflammatory as well as mucus modulating effects in mice with CF-like lung disease and primary cultures of human CF airway epithelia. Collectively, these studies support an important role of the IL-1 signaling pathway in sterile neutrophilic inflammation and mucus hypersecretion and suggest inhibition of this pathway as a promising anti-inflammatory strategy in patients with CF and potentially other muco-obstructive lung diseases.

KEYWORDS
mucus obstruction, hypoxic necrosis, sterile inflammation, IL-1 signaling, anakinra

1 | INTRODUCTION

Chronic progressive lung disease continues to determine most of the morbidity and mortality of patients with cystic fibrosis (CF) that is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and remains one of the most common life-limiting hereditary diseases in the Caucasian populations. CF lung disease is a prototypical muco-obstructive lung disease characterized by mucus plugging, chronic neutrophilic inflammation, and persistent polymicrobial infection of the airways from early life onward resulting in progressive structural lung damage with diffuse bronchiectasis and destruction of the lung parenchyma. Since recruited into the airways, activated neutrophils release increased levels of proteolytic enzymes such as neutrophil elastase (NE) and cathepsin G (Cat G), neutrophil extracellular traps (NETs), reactive oxygen species such as hydrogen peroxide, and proinflammatory cytokines such as IL-8. These neutrophil effector molecules play important roles in antibacterial host defense, but are also key drivers of inflammation and airway damage in CF. Especially NE has been identified as a key risk factor of the onset and progression of bronchiectasis and lung function decline in patients with CF from infancy to adulthood. Moreover, in concert with other inflammatory...
mediators, neutrophil-derived proteases exert profound detrimental effects on airway epithelia such as worsening of the CF basic ion transport defect (via degradation of CFTR and proteolytic activation of the epithelial Na\(^+\) channel ENaC) and induction of mucus hypersecretion, thus further aggravating mucociliary dysfunction and mucus plugging in patients with CF.\(^{14-18}\) The CF basic ion transport defect leads to dehydrated airway surfaces, hyperconcentrated and highly viscous airway mucus and impaired mucociliary clearance that has long been recognized as a key abnormality in CF lung disease rendering CF airways vulnerable to bacterial infection.\(^{7,19-23}\) Accordingly, the chronic neutrophil-dominated inflammation was for a long time thought to be secondary to infection with CF pathogens such as \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus}. Emerging evidence suggests that, especially, in infants and young children with CF, mucus plugging may trigger sterile inflammation that may play an important role in the pathogenesis of early lung disease before the onset of chronic bacterial infection.\(^{5,24-27}\) However, the mechanism linking mucus plugging with inflammation in early CF lung disease remain poorly understood. Here we review evidence from observational studies in infants and young children with CF and studies in animal models with CF-like lung disease that support the concept that mucus plugging is associated with airway inflammation, even in the absence of bacterial infection. Second, we discuss recent data supporting a link between mucus plugging and sterile neutrophilic inflammation via the release of interleukin-1\(\alpha\) (IL-1\(\alpha\)) from epithelial cells that die under the hypoxic conditions of mucus-obstructed CF airways. Finally, we outline how these findings offer novel opportunities for anti-inflammatory therapies in early CF lung disease.

2 | AIRWAY MUCUS PLUGGING AND NEUTROPHILIC INFLAMMATION OCCUR IN THE ABSENCE OF BACTERIAL INFECTION IN EARLY CF LUNG DISEASE

Mucus plugging of the small airways was reported as one of the earliest and most striking features of lung disease in autopsy studies of infants with CF in the 1940s, and it was postulated that these mucus plugs may be a key predisposing factor for bacterial infection and inflammation.\(^{28}\) With the widespread implementation of CF newborn screening\(^{29,30}\) and longitudinal observational studies of CF infants diagnosed in the first weeks of life that were spearheaded by the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF), it became possible to study the onset and progression of early CF lung disease including the relationship between early mucus obstruction, inflammation, and infection.\(^{21,32}\) Using multiple breath washout (MBW) and cross-sectional chest imaging by computed tomography (CT) or magnetic resonance imaging (MRI) as sensitive outcome measures of lung structure and function, and bronchoalveolar lavage (BAL) as outcome measure of inflammation and infection, it was found that many infants with CF show early signs of lung disease before the onset of infection with conventional CF pathogens. MBW studies showed that early abnormalities in lung function are already evident in a subgroup of infants with CF and that the prevalence and severity of airflow obstruction increase in preschool years.\(^{33-37}\) These findings were corroborated by a series of imaging studies utilizing chest CT and MRI showing a high prevalence of mucus plugging associated with air trapping and abnormal lung perfusion due to hypoxic pulmonary vasoconstriction, and signs of inflammation such as bronchial wall thickening and early bronchiectasis in infants and preschool children with CF.\(^{5,36,38-42}\) When MBW or chest imaging was combined with BAL studies, it became apparent that in this age group mucus plugging and inflammation were often present in the absence of detectable bacterial infection based on culture-dependent diagnostics.\(^{5,43,44}\) The concept of sterile inflammation in early CF lung disease has been supported by a recent study using culture-independent techniques to determine the lower airways microbiome in infants and preschool children with CF.\(^{45}\) This study showed that most infants with CF had negligible bacterial densities, but already neutrophilia detectable in their BAL during the first year of life. Between the age of 1 to 2 years, most patients acquired an oral-like microbiota, which was followed by a pathogen-dominated microbiota from the age of 3 years onward, and this dysbiosis of the lower airways was associated with increased inflammatory markers in BAL.\(^{45}\) Another recent study found that the total mucin concentration and the appearance of mucus flakes were increased in BAL of young children with CF compared with non-CF controls irrespective of bacterial infection, and that airway mucus content correlated with markers of inflammation, hypoxia, and oxidative stress in BAL.\(^{24}\)

A link between airway mucus plugging and sterile inflammation has also been observed in independent animal models showing that CF-like mucus obstruction per se can trigger the onset and progression of inflammation and lung damage. The CF ferret exhibits CF-like lung disease with rapid onset postnatal airway mucus plugging, bacterial infection, and neutrophilic inflammation.\(^{46}\) When CF ferrets were treated life-long with broad-spectrum antibiotics, airway infection was effectively prevented, but mucus plugging was still present and associated with airway neutrophilia, bronchial wall thickening, and bronchiectasis.\(^{26}\) Similar findings were obtained in mice with airway-specific overexpression of the \(\beta\)-subunit of the epithelial Na\(^+\) channel (\(\beta\)ENaC-Tg) that exhibit CF-like airway surface dehydration and impaired mucociliary clearance.\(^{47}\) The \(\beta\)ENaC-Tg mouse develops a spontaneous lung disease that shares key features with CF including early-onset mucus plugging, neutrophilic inflammation, structural lung damage, airway dysbiosis, and increased susceptibility to CF pathogens when raised under conventional specific pathogen-free (SPF) conditions.\(^{25,48,49}\) Interestingly, when \(\beta\)ENaC-Tg mice were raised under germ-free conditions, they showed levels of mucus plugging, airway inflammation and structural lung damage that were comparable to conventional \(\beta\)ENaC-Tg mice.\(^{25}\) Collectively, these observational studies in young children and animal models with CF-like lung disease support the concept that mucus plugging is a potential trigger of sterile inflammation, and that these early abnormalities can precede bacterial infection and structural lung damage in young children with CF.
Mucus plugging is invariably associated with regional hypoxia in the airways of patients with CF. Direct measurement of oxygen partial pressure in the airway lumen revealed hypoxic and even anoxic zones within mucus plugs.\textsuperscript{50} In addition, CF airway epithelia was reported to show increased oxygen consumption, likely reflecting higher energy expenditure related to increased ENaC-mediated Na\textsuperscript{+} absorption.\textsuperscript{51-55} The regional hypoxia together with increased oxygen demand may induce hypoxic injury and necrosis of airway epithelial cells as indicated by microscopy studies of airway sections from patients with CF and COPD.\textsuperscript{56,57} These studies revealed translucent degenerative airway epithelial cells lining the small airways that showed characteristic morphological features of hypoxic cell death\textsuperscript{58} and correlated in numbers with the severity of mucus plugging. In many common diseases of other organs that are associated with regional hypoxia including myocardial infarction and stroke, hypoxic cell death is a strong trigger for sterile inflammation.\textsuperscript{59} Upon sterile cell injury, the immune system senses altered host molecules released by injured and dying cells (damage-associated molecular patterns; DAMPs) by pattern recognition receptors and mounts an inflammatory response.\textsuperscript{60,61} IL-1, one of the most extensively studied due to their strong pro-inflammatory effects.\textsuperscript{62} IL-1\textalpha{} is expressed in the cytoplasm of epithelial and mesenchymal, as well as hematopoietic cells.\textsuperscript{56,62} Upon necrotic cell death or oxidative stress, IL-1\textalpha{} is released in a constitutively active form and acts as a DAMP that alerts the surrounding tissue to cell damage.\textsuperscript{63,64} IL-1\textbeta{} is mainly expressed by monocytes, macrophages, as well as in neutrophils in response to activation by microbial stimuli (pathogen-associated molecular patterns; PAMPs) that signal through Toll-like receptors (TLRs).\textsuperscript{65,66} In contrast to IL-1\textalpha{}, IL-1\textbeta{} is generated as an inactive precursor that requires intracellular cleavage by inflammasome-activated caspase-1 to attain biological activity as a cytokine and is secreted by vesicle-mediated secretion and/or direct transport across the plasma membrane involving pyroptosis.\textsuperscript{60} Extracellular cleavages of the IL-1\textbeta{} precursor is also possible by neutrophil-derived proteases, which can thereby generate active IL-1\textbeta{} in a local inflammatory microenvironment.\textsuperscript{67} Furthermore, both IL-1\textalpha{} and IL-1\textbeta{} also induce IL-1\textbeta{} production, which can further amplify the immune response in an autocrine/paracrine fashion and is presumed to be a key factor in sustained inflammation.\textsuperscript{68} Binding of IL-1 cytokines to IL-1R1 expressed on the surface of immune cells and nonhematopoietic tissue resident cells leads to recruitment of the adapter molecule myeloid differentiation primary response gene 88 (MyD88). This elicits strong proinflammatory signaling through downstream activation of NF-kappaB and downstream upregulation of pro-inflammatory mediators such as IL-8.\textsuperscript{69,70} Of note, other members of the TIR domain superfamily, such as TLRs also share this signal transduction pathway, including recruitment of MyD88 and activation of transcription factor NF-kappaB.\textsuperscript{71} In sterile inflammation, IL-1R1 has an important role in neutrophil recruitment to sites of tissue injury.\textsuperscript{59} Mice lacking IL-1R1 have markedly reduced neutrophilic inflammation in response to dead cells, and reduced collateral damage by inflammation. By the use of neutralizing monoclonal antibodies to IL-1\textalpha{} or IL-1\textbeta{}, it was shown that sterile neutrophilic inflammation in response to injury is dependent on the release of IL-1\textalpha{} from necrotic cells, but not on IL-1\textbeta{}. On the contrary, knocking out IL-1R1 or inhibition with endogenous interleukin-1 receptor antagonist (IL-1Ra) did not prevent neutrophilic inflammation in response to microbial stimuli suggesting that pathogen recognition is TLR-dependent but IL-1R1-independent.\textsuperscript{59} When viewed in combination, these findings suggested the activation of IL-1 signaling by hypoxic cell death as a candidate pathway for sterile neutrophilic inflammation in early CF lung disease (Figure 1: upper panel).

Animal models of CF lung disease provide unique opportunities to explore the role of novel candidate pathways in the pathogenesis. Longitudinal studies of \textbeta{}ENaC-Tg mice with CF-like lung disease demonstrated that early mucus plugging in neonatal pups is associated with systemic hypoxia, as well as local cellular hypoxia of the airway epithelium accompanied by the appearance of translucent and vacuolated epithelial cells undergoing necrotic degeneration and cell death.\textsuperscript{69} Similar to findings in airway sections from CF patients, the abundance of necrotic cells correlated with the severity of mucus plugging\textsuperscript{72} and was decreased upon prevention of mucus plug formation by preemptive treatment with the Na\textsuperscript{+} channel blocker amiloride.\textsuperscript{73} One key observation was that this hypoxic epithelial necrosis preceded airway neutrophilia in neonatal \textbeta{}ENaC-Tg mice indicating necrotic cell death as a major trigger of early inflammation in this model of CF-like lung disease.\textsuperscript{59} These necrotic bronchial epithelial cells were found to be a prominent source of IL-1\textalpha{} that was consistently elevated in BAL of neonatal \textbeta{}ENaC-Tg mice.\textsuperscript{56} To test the contribution of the IL-1 signaling pathway to the pathogenesis of airway inflammation in vivo, \textbeta{}ENaC-Tg mice were crossed with IL-1R1-deficient mice. Studies in the progeny of this cross showed that genetic deletion of IL-1R1 abrogated airway neutrophilia almost completely and reduced structural lung damage in \textbeta{}ENaC-Tg mice.\textsuperscript{56} Of note, lack of IL-1R1 also reduced mucus obstruction and the associated pulmonary mortality in \textbeta{}ENaC-Tg pups.\textsuperscript{56} Furthermore, pharmacological inhibition of IL-1R1 signaling with anakinra, the recombinant form of the naturally occurring antagonist IL-1Ra, reduced airway inflammation, mucus content and lung damage in adult \textbeta{}ENaC-Tg mice with established lung disease.\textsuperscript{56} The observation that disabling of IL-1 signaling reduced mucus plugging in addition to inflammation in vivo, is consistent with studies showing that IL-1\textalpha{} as well as IL-1\textbeta{} induced expression of the secreted airway mucins MUC5AC and MUC5B, and thus mucus hypersecretion in primary human bronchial epithelial cells, and that this effect was prevented by...
pretreatment of cultures with the IL-1R antagonist anakinra. In addition, it was shown that IL-1β activates CFTR-mediated Cl−/fluid secretion in airway cultures in addition to the induction of mucin secretion. We, therefore, speculate that the inability of IL-1β to induce CFTR-dependent Cl−/fluid secretion in CF airways may contribute to mucus hyperconcentration and plugging in response to insults (e.g., aspiration and viral infection) during early CF pathogenesis. Taken together, these data from in vivo and in vitro model systems support an important role of the IL-1 signaling pathway in the pathogenesis of sterile neutrophilic inflammation and mucus hypersecretion, and suggest that pharmacological inhibition of this pathway may be a promising therapeutic strategy to target both abnormalities in early CF lung disease (Figure 1: upper panel).

4 | TRANSLATIONAL STUDIES SUPPORT A ROLE OF IL-1 SIGNALING IN EARLY CF LUNG DISEASE

The clinical relevance of disease mechanisms identified in model systems needs to be validated in patients. Overall, the IL-1 signaling pathway has so far received little attention in the context of CF. However, several lines of evidence from emerging translational studies support the involvement of this pathway in the pathogenesis of CF lung disease. First, a recent study investigated the relationship between IL-1 signaling, neutrophilic inflammation and structural lung changes in young children with CF. In a cohort of 102 young children with CF, IL-1α, and IL-1β were detected in BAL even in the absence of bacterial infection. Both IL-1 cytokines were increased
in the presence of infection and correlated with airway neutrophilia and other markers of neutrophilic inflammation such as IL-8 and free NE activity. Interestingly, when inflammation markers were correlated with the extent of structural lung disease detected by chest CT, IL-1α showed the strongest association with structural lung disease in the absence of bacterial infection.79 Second, a recent association study using an informative intragenic microsatellite marker identified IL-1R as a genetic modifier in CF.90 In F508del homozygous CF patients from the European Twin and Sibling Study who were born between 1957 and 1990, this study found an enrichment of a specific allele in the IL1R1 locus in patients who reached at least school age, suggesting that IL-1 signaling was linked to survival in children with CF born during that period.80 Third, IL-1α and IL-1β were found to be elevated in the sputum of older CF patients with chronic airway infections14,81,82 and independent studies identified IL1B encoding IL-1β as a CF modifier in North American and European CF cohorts.83,84 These human studies are consistent with an important role of the IL-1 signaling pathway in the pathogenesis of early CF lung disease, where this pathway may be predominantly activated by IL-1α released from hypoxic cells undergoing necrosis in mucus-obliterated airways before the onset of airway infection. Further, these data suggest that IL-1β that is induced in hematopoietic cells in response to bacterial infection may contribute to IL-1 mediated pathogenesis during later stages of CF lung disease (Figure 1: upper panel).

5 IL-1 SIGNALING AS A NOVEL THERAPEUTIC TARGET IN CF LUNG DISEASE

Collectively, these results obtained from functional and genetic studies in model systems and patients with CF indicate that inhibition of IL-1 signaling may be a promising therapeutic strategy in patients with CF and potentially other lung diseases characterized by mucus plugging and chronic neutrophilic inflammation. First, inhibition of IL-1 signaling is predicted to be a potent anti-inflammatory strategy to reduce sterile neutrophilic inflammation driven by the release of IL-1α in mucus-obliterated airways in the absence of infection, but may also reduce overwhelming inflammation mediated by IL-1β in chronically infected CF airways.56,85 Second, therapeutic targeting of the IL-1 signaling pathway may also reduce mucus expression and mucus hypersecretion that contribute to increased mucus concentration and viscoelasticity and thus aggravate mucociliary dysfunction and mucus plugging in CF airways (Figure 1: lower panel). Several therapeutic strategies have been developed and to date, three biologics have been approved for a spectrum of chronic inflammatory diseases that may be repurposed for CF: the IL-1Ra (anakinra), a soluble decoy receptor (rilonacept) and a neutralizing monoclonal anti-IL-1β antibody (canakinumab).86 Clinical trials with a neutralizing anti-IL-1α antibody, bermekimab are currently underway for the treatment of type 2 diabetes, psoriasis and colorectal cancer.87-89 Anakinra is the prototype of IL-1 blocking biologics that block the activity of both IL-1α and IL-1β and is approved for a wide range of indications including rheumatoid arthritis, gout, pericarditis, and rare autoimmune inflammatory syndromes in adults, as well as in children.90 Anakinra is a generally well-tolerated drug administered daily by subcutaneous injection. It has a short half-life and an excellent safety record, and preclinical studies in βENaC-Tg mice suggest anakinra as a promising inhibitor of IL-1α signaling in CF.56 Bermekimab and canakinumab, as neutralizing antibodies against IL-1α and IL-1β respectively, may represent alternative treatment options in CF offering the potential advantages of more selective targeting of the two IL-1 cytokines and a less frequent and thus more convenient subcutaneous administration due to their prolonged half-life.86 Whereas early inflammation may be dominated by IL-1α released from necrotic cells, IL-1β may become the dominant IL-1 cytokine in the inflammatory microenvironment of chronic CF airways disease, especially after the onset of bacterial infection.86 However, the timing of IL-1α and IL-1β release and their relative contribution to neutrophilic inflammation and mucus secretion have not been elucidated, and the benefit of selective blockade of IL-1α versus IL-1β to inhibit self-sustaining airway inflammation and mucus hypersecretion in early CF lung disease remains to be determined.

As with any anti-inflammatory strategy, a potential hurdle of therapeutic targeting of IL-1 signaling is the risk of compromising host defense, which may worsen the outcome of acute and/or chronic pulmonary infections. Studies in mice indicate that the inflammatory response to microbial stimuli may not be affected by inhibition of the IL-1 signaling pathway, because pathogen recognition via TLR signaling remains intact.59 However, preclinical testing of anakinra in animal models with CF-like lung disease infected with Pseudomonas aeruginosa or other CF pathogens is pending. In the clinical arena, long-term experience with anakinra in patients with rheumatoid arthritis demonstrated a good safety profile with no increase in the occurrence of sporadic bacterial infections, nor reactivation of opportunistic infections.91 Furthermore, anakinra was successfully administered to patients with chronically active infections.92,93 However, close monitoring of adverse events including increased susceptibility for acquisition and/or exacerbation of chronic airway infections will be critical in clinical trials testing inhibition of IL-1 signaling as an anti-inflammatory and mucus modulating strategy in CF and other mucob-oblstructive lung diseases (Figure 1: lower panel).

6 SUMMARY AND OUTLOOK

A growing body of evidence including observational studies in infants and young children with CF, as well as investigations in by multiple animal models of CF lung disease, support the concept that airway mucus plugging per se can drive neutrophilic airway inflammation, even before the acquisition of significant bacterial infection.52,24-26,43,45 Recent studies suggest that activation of the IL-1 signaling pathway by the release of IL-1α from airway epithelial cells that undergo hypoxic necrosis in mucus-obliterated airways may be an important mechanistic link between mucus plugging and sterile inflammation in early CF lung disease.56 In CF airways, mucus plugs generate local hypoxia and thus promote hypoxic epithelial necrosis, which is a well-known stimulus of sterile inflammation in other diseases.50,56,60 Dying cells release IL-1α, which is constitutively active and activates the IL-1R1/MyD88 signaling pathway resulting in the
expression of neutrophil chemoattractants such as IL-8 that recruit neutrophils into the airways (Figure 1: upper panel).54 The relevance of this pathway to the pathogenesis was first supported by studies in \( \beta \)ENaC-Tg mice demonstrating that genetic deletion or pharmacological inhibition of IL-1R1 by treatment with the IL-1Ra anakinra reduced airway neutrophilia, mucus obstruction and structural lung damage in this model of CF lung disease.54 In addition, recent studies in CF primary airway epithelial cultures identified inhibition of IL-1 signaling with anakinra as an effective strategy to block increased mucin expression/ mucus hypersecretion under inflammatory conditions in vitro.74 In the meantime, the pathophysiological relevance of this pathway has been further corroborated by genetic and observational studies in CF patients that support a role of IL1R1 as a modifier of CF lung disease80 and demonstrated a strong association between IL-1\( \alpha \) in BAL and structural lung damage in young children with CF in the absence of bacterial infection.79 IL-1 blocking therapeutics are already approved for a spectrum of chronic inflammatory diseases including rheumatoid arthritis and other autoinflammatory conditions and the preclinical studies in CF model systems suggest that the IL-1Ra anakinra, or other biologics blocking the IL-1 signaling pathway, may be a promising candidates for drug repurposing in CF.56,74 Inhibition of IL-1 signaling in early CF lung disease, ideally before the onset of airway infections, may have the potential to break an otherwise self-perpetuating cycle of mucus plugging, inflammation and mucus hypersecretion that sets the stage for chronic bacterial infection and progressive structural lung damage. Furthermore, inhibition of mucus hypersecretion and nonresolving neutrophilic inflammation by therapeutic targeting of the IL-1 signaling pathway may also offer benefits to patients with established CF lung disease (Figure 1: lower panel). Given the limited effects of current anti-inflammatory and mucus-modulating drugs and the high unmet need for patients with CF,54 the time may be ripe to test the safety and efficacy of IL-1 blockade as a novel therapeutic approach to control airway inflammation and mucus hypersecretion in CF and potentially other muco-obstructive lung diseases in clinical trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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