Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies

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Hypoxia and sterile inflammation is characteristic in cystic fibrosis airways leading to lung damage in early life http://ow.ly/NnHf304acAZ

Cite this article as: Montgomery ST, Mall MA, Kicic A, *et al*. Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *Eur Respir J* 2017; 49: 1600903 [https://doi.org/10.1183/13993003.00903-2016].

ABSTRACT Cystic fibrosis is one of the most common autosomal recessive genetic diseases in Caucasian populations. Diagnosis *via* newborn screening and targeted nutritional and antibiotic therapy have improved outcomes, however respiratory failure remains the key cause of morbidity and mortality. Progressive respiratory disease in cystic fibrosis is characterised by chronic neutrophilic airway inflammation associated with structural airway damage leading to bronchiectasis and decreased lung function. Mucus obstruction is a characteristic early abnormality in the cystic fibrosis airway, associated with neutrophilic inflammation often in the absence of detectable infection. Recent studies have suggested a link between hypoxic cell death and sterile neutrophilic inflammation in cystic fibrosis and other diseases *via* the IL-1 signalling pathway. In this review, we consider recent evidence regarding the cellular responses to respiratory hypoxia as a potential driver of sterile neutrophilic inflammation in the lung, current knowledge on hypoxia as a pathogenic mechanism in cystic fibrosis and the potential for current and future therapies to alleviate hypoxia-driven sterile inflammation.

Received: May 05 2016 | Accepted after revision: Aug 31 2016

Support statement: US Cystic Fibrosis Foundation, Cystic Fibrosis Australia and the German Federal Ministry of Education and Research (82DZL004A1) provided funding.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

Introduction

Cystic fibrosis is one of the most common autosomal recessive genetic disease in Caucasian populations of European descent [1, 2], with an incidence of 1 in 3630 live births in Australia [3]. Diagnosis *via* newborn screening and targeted nutritional and antibiotic therapy has improved outcomes so that the median long-term survival is now into middle adulthood [4–6]. However, respiratory failure remains the key cause of morbidity and mortality and current preventative therapies targeting airways disease have limited efficacy [4, 5, 7]. Cystic fibrosis results from mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene; mutations in *CFTR* result in the absence or reduction of functional CFTR anion channels in the apical membrane of airway epithelial cells, affecting secretion of chloride (Cl^-), bicarbonate (HCO_3^-) and other anions [8]. Reduced epithelial Cl^- transport [9] combined with excessive epithelial sodium (Na^+) transport *via* the epithelial Na^+ channel (ENaC) [10–14] results in dehydration of the airway surface liquid and impaired mucociliary clearance [15]. Depletion of airway surface liquid increases the concentration and viscosity of the mucus layer, which is exacerbated by hypersecretion of mucins in chronic airway inflammation [16] and leads to the formation of thickened mucus plaques on airway surfaces and plugging of the airway lumen [17]. Increased epithelial oxygen consumption associated with increased ENaC-mediated Na^+ absorption [18] combined with luminal hypoxia due to mucus plugging creates steep oxygen gradients within adherent mucus, unique to the cystic fibrosis airway [17]. The hypoxic niches within thickened mucus provide a nidus for motile bacteria able to penetrate the mucus encouraging anaerobic bacterial growth and colonisation [17].

Progressive respiratory disease in cystic fibrosis is characterised by chronic neutrophilic airway inflammation, typified by increased membrane-bound and free neutrophil elastase activity [19, 20]. Neutrophilic airway inflammation is associated with structural airway remodelling, bronchiectasis and decreased lung function [20–23]. Mucus obstruction is a characteristic early abnormality in the cystic fibrosis airway [23], associated with neutrophilic inflammation [24] often in the absence of detectable infection [20, 22], leading to the question of what drives early sterile inflammation in cystic fibrosis. Recent studies have suggested a link between hypoxic cell death and sterile neutrophilic inflammation in cystic fibrosis and other diseases *via* the IL-1 signalling pathway [25–29]. In this review, we consider recent evidence regarding the cellular responses to respiratory hypoxia as a driver of sterile neutrophilic inflammation in the lung, current knowledge on hypoxia as a pathogenic mechanism in cystic fibrosis and the potential for current and future therapies to alleviate hypoxia-driven sterile inflammation.

Physiological and transcriptional responses to respiratory hypoxia

Respiratory hypoxia is uniquely characteristic of the cystic fibrosis airway; although, young children with cystic fibrosis are typically normoxic. Two respiratory issues evident in children with cystic fibrosis are regional hypoxia in the airway lumen due to obstructive mucus plugging [20, 22, 23] and increased epithelial oxygen consumption most likely due to elevated Na^+/K^+ ATPase activity involved in increased ENaC-mediated Na^+ absorption [10, 11, 15, 18]. Both abnormalities may result in airway epithelial cells vulnerable to cellular hypoxia and ensuing necrosis [27]. Hypoxaemia is also present in patients with chronic or advanced lung disease [30]. Increased oxygen consumption creates steep oxygen gradients within thickened mucus in airway surface liquid and hypoxic niches primed for anaerobic growth of *Pseudomonas aeruginosa*, driving antibiotic resistance in biofilms observed in cystic fibrosis [17, 31]. However, significant inflammation in the absence of respiratory infection is present at diagnosis in a majority of infants with cystic fibrosis, and significant gas trapping is observed [20, 32].

Physiological responses to respiratory hypoxia

Alveolar oxygen pressure during normoxia is unusually high [33–35], and thus respiratory hypoxia occurs at a higher oxygen tension compared with other organs. Even moderate levels of alveolar hypoxia experienced at high altitudes are sufficient to trigger an inflammatory phenotype. Elevated presence of macrophages, neutrophils and inflammatory cytokines have been observed in bronchoalveolar lavage fluid (BALF) isolated from humans exposed to hypobaric hypoxia [36]. With significant gas trapping [20, 32] and episodes of nocturnal oxygen desaturation [37] observable in infants with cystic fibrosis, the physiological response to hypoxia may play a role in early lung damage in cystic fibrosis. The physiological response of the pulmonary vascular system to hypoxia is hypoxic pulmonary vasoconstriction, resulting from hypoxia-induced K^+ current inhibition and membrane depolarisation in pulmonary arterial smooth muscle cells [38, 39]. Significant abnormal pulmonary perfusion has been observed in infants and young children with cystic fibrosis suggesting perfusion deficits are associated with airway disease, even when clinically stable [23]. Chronic pulmonary vasoconstriction can lead to pulmonary hypertension, and vascular remodelling caused by chronic hypoxia and hypertension may contribute to structural remodelling observed in early cystic fibrosis lung damage [32, 40].

Transcriptional responses to respiratory hypoxia

The transcriptional response to hypoxia has also been extensively studied *in vivo* and *in vitro*, elucidating important factors involved (figure 1). Hypoxia-inducible factor (HIF)-1 plays a vital role in the response to hypoxia, inducing the transcription of proteins to increase oxygen availability. The HIF-1 complex exists as a heterodimer, consisting of subunits HIF-1α and HIF-1β [41]. HIF-1β is constitutively expressed, whereas HIF-1α expression is facultative and regulated in an oxygen-dependent manner. During normoxia, HIF-1α is hydroxylated by prolyl hydroxylase domain (PHD) proteins using oxygen as a substrate, leading to interactions with the von Hippel–Lindau (VHL) E3 ligase complex, subsequent ubiquitination, and proteosomal degradation of HIF-1α [42–44]. As the degradation of HIF-1α requires oxygen for PHD activity and ubiquitination *via* VHL, hypoxic conditions result in the stabilisation and translocation of HIF-1α [43]. In cystic fibrosis, lack of CFTR function affects HIF-1α stabilisation, with *in vitro* experiments finding lower expression of HIF-1α in cystic fibrosis airway epithelial cells during hypoxia when compared to healthy controls [45]. This is possibly due to impaired control of intracellular reactive oxygen species (ROS) [46] resulting from loss of CFTR transport of the antioxidant glutathione [47, 48], affecting the adaptive response to hypoxia in cystic fibrosis [49].

NF-κB transcription during hypoxia

Even though HIF-1α is the predominant transcription factor activated during hypoxia, activation of NF-κB is another critical and largely HIF-1α-independent transcriptional response [50]. During hypoxia, activation of NF-κB is rapid, persistent and IκB kinase (IKK) dependent, instigated by the phosphorylation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor- alpha (IκBα) [50, 51]. Inhibition of PHDs during hypoxia increases IKK-β catalytic activity [52], resulting in greater IκBα phosphorylation and NF-κB activation [51, 53]. The initial activation of NF-κB is too rapid

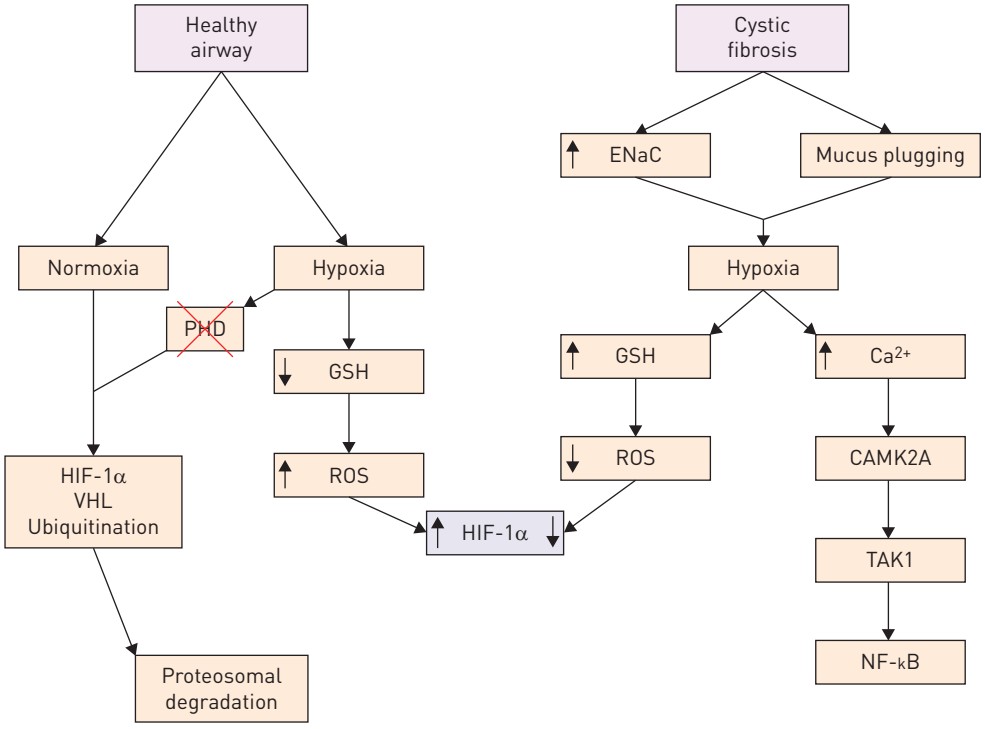

FIGURE 1 Transcriptional response to hypoxia in normal and cystic fibrosis airways. Hypoxia-inducible factor (HIF)-1α plays a central role in the transcriptional response to hypoxia. In normal airways, under normoxic conditions HIF-1α is hydroxylated by prolyl hydroxylase domains (PHDs) and through subsequent interactions with the von Hippel–Lindau (VHL) E3 complex, marked for ubiquitination and proteosomal degradation. Under hypoxic conditions, PHD activity is inhibited and HIF-1α is stabilised. Hypoxia also results in the efflux of intracellular glutathione (GSH) *via* cystic fibrosis transmembrane conductance regulator (CFTR), causing intracellular reactive oxygen species (ROS) to accumulate, contributing to increased HIF-1α stabilisation. In cystic fibrosis airways, impaired ventilation due to airway mucus obstruction and increased epithelial sodium channel (ENaC) activity result in cellular hypoxia of airway epithelial cells. Hypoxia causes Ca^{2+} influx and calcium/calmodulin-dependent protein kinase type II (CAMK2) activation, transforming-growth-factor-β-activated protein kinase 1 (TAK1) activation and subsequent nuclear factor κB (NF-κB) activation. In the absence of functioning CFTR, there is increased intracellular GSH and lower ROS generation, resulting in reduced stabilisation of HIF-1α.

for a transcriptional response to hypoxia; instead Ca^{2+} dependent activation of Ca^{2+}/calmodulin-dependent protein kinase II (CAMK2) occurs prior to the inhibition of PHDs [54]. This demonstrates hypoxia-induced IKK-IκBα phosphorylation and subsequent NF-κB activation is largely HIF-1α independent [50], with basal activation of NF-κB required for HIF-1α accumulation under hypoxia [55]. However, activation of NF-κB during hypoxia is reduced in cells lacking functional CFTR, suggesting an impaired response in cystic fibrosis [56]. These data highlight the multifaceted transcriptional and physiological response to respiratory hypoxia, and the interconnectivity between classic hypoxia-response genes and the NF-κB inflammatory pathway. Furthermore, these mechanisms may be relevant in cystic fibrosis lung disease, with reduced NF-κB activation [56] and lower HIF-1α stabilisation in cells lacking functional CFTR, contributing to an impaired response to hypoxia in cystic fibrosis.

Hypoxia and inflammation in cystic fibrosis

Chronic neutrophilic airway inflammation is a key factor in driving mortality and morbidity present in cystic fibrosis. Neutrophil elastase, the primary product of activated neutrophils, is important in host defence against bacteria such as *Pseudomonas aeruginosa* [57], but elevated neutrophil elastase can also result in cleaving of chemokine receptors on immune cells, compromising their capacity to kill bacteria [58]. Elevated neutrophil elastase also exacerbates ion transport imbalance in cystic fibrosis, activating near-silent ENaC transport and degrading CFTR channels and disabling ion transport [59, 60]. Detection of free neutrophil elastase in BALF is the most significant predictor for presence of bronchiectasis in early cystic fibrosis [22] and neutrophil elastase was linked to structural lung damage in mice with cystic fibrosis-like lung disease [19]. Airway infection is a predominant and persistent cause of neutrophilic inflammation in cystic fibrosis [61]. However, inflammation can be seen in asymptomatic children with cystic fibrosis without lower airway colonisation [32], and the pathogenesis of non-infectious inflammation in these cases is poorly understood. Recent studies in βENaC-overexpressing mice with cystic fibrosis-like lung disease [11, 62] led to novel insights how mucus obstruction may be linked to neutrophilic inflammation in early cystic fibrosis lung disease. First, a study utilising this model of cystic fibrosis lung disease derived in germ-free conditions observed airway mucus obstruction and persistent inflammation in the absence of bacteria, with significantly higher neutrophil presence in BALF compared with germ-free wildtype mice [63]. These results suggest that mucus plugging *per se* can trigger chronic airway inflammation even in the absence of bacterial infection. Early neutrophilic inflammation is important in the pathogenesis of cystic fibrosis, with neutrophils and airway mucus plugging present in infants at diagnosis [20, 32]. Second, studies in βENaC-overexpressing mice with cystic fibrosis-like lung disease demonstrate that airway mucus plugging is associated with cellular hypoxia resulting in necrosis of airway epithelial cells [24]. Additionally, a recent study demonstrated that necrosis of airway epithelial cells is a characteristic finding in cystic fibrosis patients and that necrotic airway cells are correlated with the severity of mucus plugging in small airway lung sections [27]. These results suggest that elucidating the link between hypoxia-driven epithelial necrosis and early neutrophilic inflammation may be vital in attenuating cystic fibrosis-associated morbidity and mortality.

Sterile inflammation

Unlike inflammation resulting from infection, driven by the toll-like receptor network, sterile inflammation during hypoxia is mediated *via* the activation of interleukin-1 receptor (IL-1R) (figure 2) [25, 27]. Activation of IL-1R occurs when hypoxic cells undergoing necrosis release IL-1α, which acts as a danger-associated molecular pattern molecule [25, 27]. The inflammatory role of IL-1 has been thoroughly investigated in a number of inflammatory [64] and hypoxic diseases such as ischaemia, where it influences inflammation in the cerebrum [29], as well as renal [65] and cardiac systems [66, 67]. Chen *et al.* [25] determined that IL-1α, not IL-1β was responsible for the inflammatory response to cell death using *in vivo* knockout murine models. A recent study by Fritzsching *et al.* [27] also demonstrated that epithelial necrosis in the airways of βENaC-overexpressing mice is associated with an increase in neutrophils in BALF, as well as elevated IL-1α levels. Interestingly, genetic deletion and pharmacological inhibition of IL-1R resulted in significantly reduced airway neutrophilia airway mucus obstruction and structural lung damage, but had no effect on airway epithelial necrosis or IL-1α in BALF [27].

Necrotic airway cells trigger a sterile inflammatory response by the passive release of IL-1α, which is trafficked to the nucleus and contained during apoptosis [68]. However during necrosis, IL-1α is released from cells in biologically active uncleaved and cleaved forms [25, 69]. Furthermore, extracellular IL-1α released from primary airway epithelial cells exposed to oxidative stress is able to trigger an inflammatory phenotype in lung fibroblasts [70], reiterating the inflammatory capacity of IL-1α during necrosis. IL-1α exists natively as a precursor protein that is cleaved *via* calpain [71], but cleavage can be inhibited by binding of IL-1α to IL-1R2 [72]. Intriguingly, IL-1R2 expression is reported to be absent in both immortalised airway epithelial cell lines and primary airway epithelial cells, which suggests airway

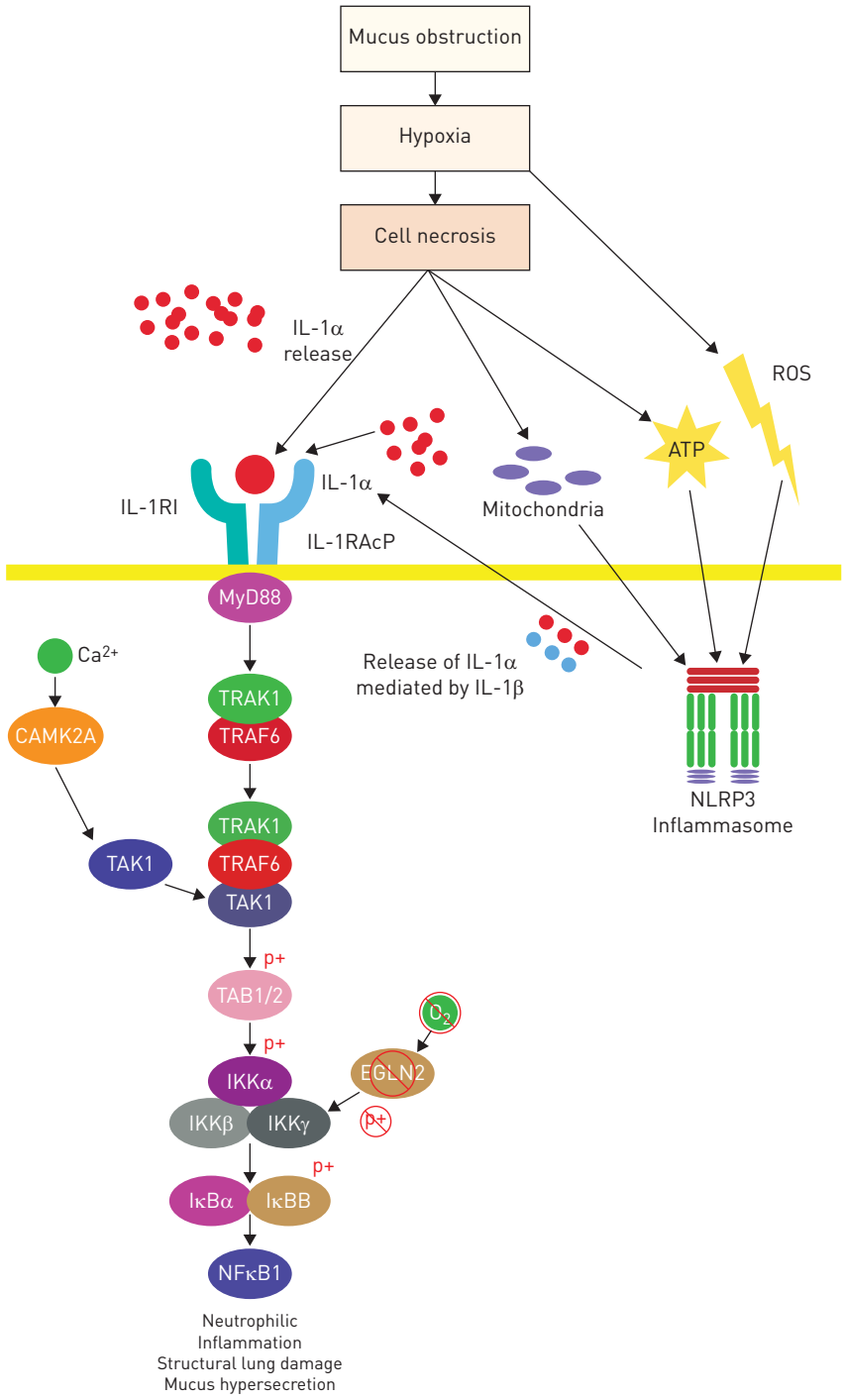

FIGURE 2 Transcriptional inflammatory response to hypoxia and cellular necrosis. Cellular hypoxia results in the release of IL-1α through two mechanisms – hypoxic necrosis of epithelial cells passively releasing IL-1α, or through Nod-like receptor protein 3 (NLRP3) inflammasome activation *via* reactive oxygen species (ROS) generation and cell products released from cells undergoing necrosis. Binding of IL-1α to IL-1R recruits MyD88 to the IL-1R:IL-1RAcP complex, and results in activation of transforming-growth-factor-β-activated protein kinase 1 (TAK1) by interacting with IL-1 receptor-activated protein kinase (IRAK1):tumour necrosis factor receptor-associated factor 6 (TRAF6). TAB1 phosphorylates IκB kinase (IKK) γ, which binds to IKKα and IKKβ activating the IKK complex. The IKK complex phosphorylates nuclear factor κB (NF-κB) inhibitors IκBα and IκBβ, subsequently activating NF-κB1. Initial activation of NF-κB during hypoxia is mediated by intracellular Ca^{2+} activating calcium/calmodulin-dependent protein kinase type IIA (CAMK2A), which activates TAK1 and the subsequent pathway prior to IL-1α release. Hypoxia also inhibits egl-9 family hypoxia-inducible factor 2 (EGLN2) degrading IKKβ, increasing catalytic activity and greater NF-κB activation.

epithelial cells lack the ability to regulate IL-1α activity due to an inability to express IL-1R2 [73], which may contribute to the hyper-inflammatory response observed in cystic fibrosis.

IL-1α role and regulation

Aside from the release from necrotic cells, there is a paucity of data about the regulation and physiological role of IL-1α. Previously, it was believed that IL-1α was only secreted passively from cells undergoing non-apoptotic cell death [25]; however, recent studies have demonstrated alternative control of IL-1α. Willart *et al.* [74] have shown that IL-1α is secreted from airway epithelial cells following exposure to house dust mite, potentially affecting the inflammatory response in cystic fibrosis patients with reduced allergen clearance due to impaired mucociliary clearance. Recent studies have determined that the Nod-like receptor protein 3 (NLRP3) inflammasome regulates the active release of IL-1α and IL-1β [75–77]. Stimulation *via* toll-like receptor signalling is sufficient to upregulate pro-IL-1β and to induce cell surface expression of IL-1α, but is insufficient for processing and extracellular release [75, 77]. Activation of the NLRP3 inflammasome results in IL-1β processing and release and extracellular secretion of IL-1α rather than cell surface expression [75–77]. Fettelschoss *et al.* [75] confirmed differential IL-1α expression *via* biotinylation of surface IL-1α and NLPR3 activation, and discovered IL-1α secreted after inflammasome activation is not cleaved from the cell surface, but rather secreted from intracellular compartments. The pathways for IL-1α surface expression and IL-1α secretion are distinctly different, and dependent upon the activation of caspase-1 and the presence of IL-1β for secretion to occur [75]. As IL-1α does not contain a caspase-1 cleavage site, dependency upon inflammasome activation and IL-1β can be explained by binding of IL-1α and IL-1β for transport *via* IL-1β during transfer from the cytoplasm to extracellular space *via* caspase-1 [75]. Active secretion of IL-1α during inflammasome activation is further compounded by necrotic airway cells during hypoxia, as ATP and mitochondria released from necrotic cells activate the NLRP3 inflammasome [28]. Hypoxia plays a role in inflammasome activation; mitochondrial ROS generated during hypoxia [78] have been demonstrated to activate the NLRP3 inflammasome, leading to pulmonary inflammation [79, 80]. Inflammasome activation also results in significantly increased neutrophil influx into the airway, supporting a hypothesis for IL-1α regulation in sterile inflammation driven by hypoxia [28, 81]. The NLRP3 inflammasome is primed for activation by flagellin from bacteria such as *Pseudomonas aeruginosa* [82], explaining increased levels activated caspase-1 evident in cystic fibrosis mice [83], and potentially exacerbating NLRP3 activation in cystic fibrosis.

Neutrophilic inflammation following bacterial infection is mediated by toll-like receptor binding activating MyD88 and subsequently NF-κB [84]. However, in sterile inflammation the activation of MyD88 and NF-κB instead results from binding of IL-1α to IL-1R [25] (figure 2). Fritzsching *et al.* [27] determined that IL-1α released from necrotic airway epithelial cells and subsequent activation of IL-1RMyD88 signalling is an important pathway in the onset and perpetuation of neutrophilic inflammation in mice with cystic fibrosis-like lung disease. NF-κB is the key factor for neutrophil recruitment during inflammation [85], chiefly through release of the neutrophil chemoattractant IL-8 [86]. Hypoxia significantly increases IL-8 produced by alveolar macrophages [87], facilitating increased neutrophil influx to hypoxic areas already inundated by increased neutrophilia from inflammasome activation [81, 87]. In addition to increasing neutrophil influx, hypoxia also increases trans-epithelial migration of neutrophils [88] and subsequently the adherence of neutrophils to airway epithelial cells [89, 90], potentially exacerbating neutrophilic inflammation in the airway. Macrophages and neutrophils lacking HIF-1α expression have reduced infiltration and activation *in vivo*, with HIF-1α a basal regulator of energy metabolism in these cells [91]. Neutrophil survival is also influenced by HIF-1α [92, 93] and hypoxic neutrophils release significantly more neutrophil elastase [94], which was demonstrated to cause significant destruction of intestinal epithelium after acute alveolar hypoxia [95]. Finally, hypoxia impairs the killing of bacteria due to reduced oxidative burst capacity and inhibited glycolysis-induced ATP production [91, 95]. During hypoxia, necrotic cells drive inflammation by passive and active IL-1α release and subsequent IL-1R activation, resulting in neutrophil recruitment to the airway. These mechanisms may help explain the exaggerated neutrophilic inflammation present in early cystic fibrosis, and provide novel targets for anti-inflammatory therapies.

Potential for therapy

IL-1 receptor

IL-1R provides a novel target for attenuation of sterile inflammation in cystic fibrosis and potentially other chronic inflammatory lung diseases (figure 3). In rheumatoid arthritis, a disease characterised by IL-1α driven inflammation, inhibition of IL-1R with IL-1R antagonist (IL-1Ra) is efficacious and commonplace [96]. However, there are limited data on the use of IL-1Ra to limit pulmonary inflammation in a disease setting. It has been demonstrated that IL-1Ra may reduce oxidative lung injury and neutrophil influx in rats administered IL-1Ra post-insult with IL-1 [97]. In a murine model of acute lung injury due to ventilation, IL-1Ra reduced airway neutrophil influx and expression of neutrophil chemoattractant and maintained

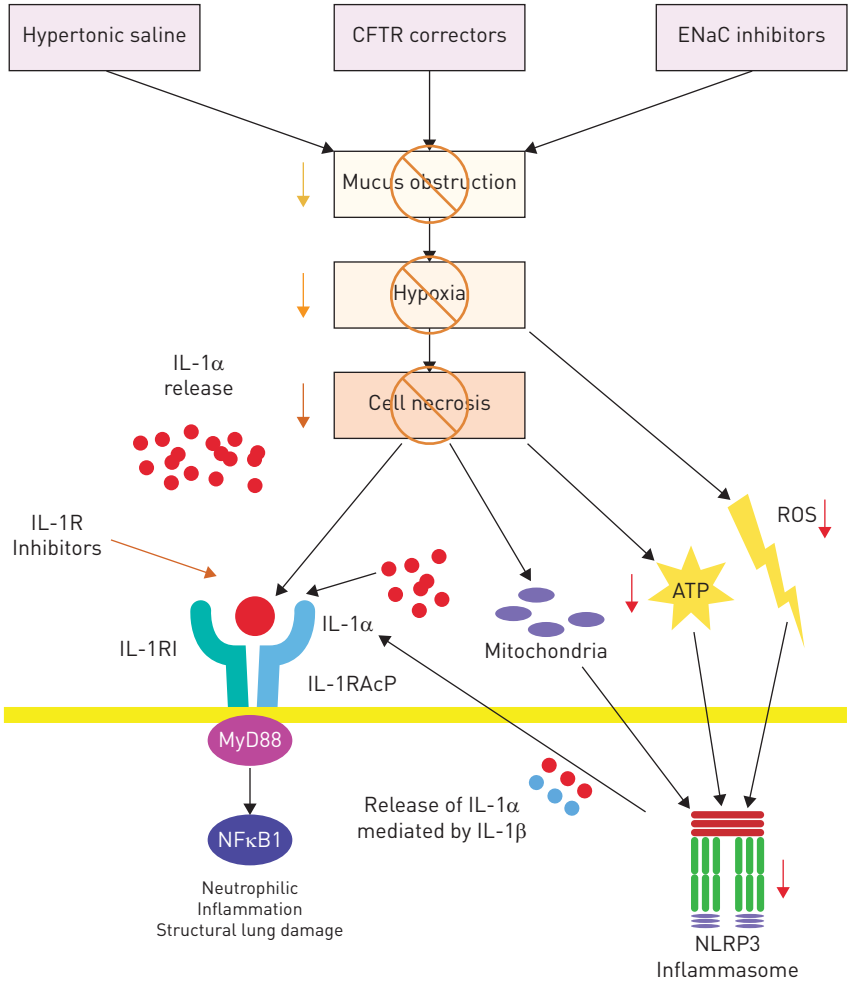

FIGURE 3 Potential therapies to alleviate hypoxia-driven inflammation. Potential therapies to alleviate hypoxia-driven inflammation under investigation target either mucous obstruction causing the hypoxia, or inhibition of IL-1R to disrupt the IL-1-nuclear factor κB (NF-κB) inflammatory pathway. IL-1R inhibitors such as IL-1Ra and IL-1R antibodies bind to IL-1R, inhibiting stimulation of IL-1α driven inflammation. Inhalation of hypertonic saline, cystic fibrosis transmembrane conductance regulator (CFTR) modulators or epithelial sodium channel (ENaC) inhibitors improve airway surface hydration and mucociliary clearance in cystic fibrosis airways, alleviating mucus obstruction and resulting hypoxia. ROS: reactive oxygen species; NLRP3: Nod-like receptor protein 3.

epithelial permeability [98]. Treatment of mice with IL-1Ra also prevented development of pulmonary fibrosis after inhalation of silica, predominantly affecting lung interstitial cells [99]. Neutrophils isolated from cystic fibrosis patients release significantly less IL-1Ra compared with neutrophils isolated from healthy individuals [100], which may suggest an imbalanced anti-inflammatory capacity in the cystic fibrosis airway. In a cystic fibrosis-like murine setting, pharmacological inhibition of IL-1R using IL-1Ra resulted in almost complete abrogation of airway neutrophilia and significantly reduced levels of keratinocyte chemoattractant, an IL-8 orthologue in mice [27]. Inhibition of airway neutrophilia was associated with a significant reduction in structural lung damage as determined from distal airspace enlargement characteristic of the murine model [24, 27]. However, the moderate levels of reduction in airway mucus obstruction following IL-1Ra treatment were not sufficient to improve luminal O_2 delivery to levels that results in reduced epithelial necrosis [27]. Even though inhibition of the IL-1R-MyD88 pathway resulted in reduced influx of neutrophils, recruitment and activation of macrophages remained intact in both the cystic fibrosis-like murine model and in mice with necrosis-induced inflammation [25, 27]. Therefore, blocking IL-1R signalling *via* IL-1Ra may limit the damaging effects of neutrophils without compromising antibacterial host defence [25, 27].

Mucus obstruction

Other potential therapeutic approaches include improved oxygenation *via* prevention or reduction of airway mucus obstruction (figure 3). One common therapy currently recommended for improving mucus clearance in cystic fibrosis is the inhalation of aerosolised hypertonic saline. Although the exact mechanism of action

of hypertonic saline is unknown, it is believed to draw water from epithelial cells and the serosal compartment *via* establishment of an osmotic gradient. Hypertonic saline is effective at transiently improving mucociliary clearance, demonstrated in both cystic fibrosis and non-cystic fibrosis disease [101, 102] and was shown to reduce mucus plugging and mortality effectively in mice with cystic fibrosis-like lung disease [103, 104]. Pharmacological intervention with the classical ENaC blocker amiloride to inhibit Na^+ absorption from cystic fibrosis airway surfaces has also been investigated as a therapy to reduce mucus obstruction, but resulting in no clinically significant improvements in cystic fibrosis patients with established lung disease [105, 106]. This is likely due to low potency and short half-life of amiloride on airway surfaces [107]. However, a recent study utilising a cystic fibrosis-like murine model demonstrated that preventative treatment with amiloride starting in neonatal mice resulted in significantly reduced airway mucus obstruction, epithelial necrosis and airway inflammation, supporting that airway epithelial necrosis and associated inflammation can be reduced by therapeutic targeting of mucus plugging [104, 108]. Long-acting ENaC inhibitors with higher potency have been developed [109–111], but whether mucus obstruction and inflammation in established lung disease are abrogated by new therapeutics remains to be seen.

CFTR modulation

CFTR modulators, aimed at rescuing mutant CFTR function, such as ivacaftor and lumacaftor also have potential to alleviate airway mucus obstruction. Ivacaftor is a potentiator that improves Cl^- transport of a series of mutant CFTR proteins that are expressed at the apical plasma membrane including gating mutations such as G551D, mutations causing reduced Cl^- channel conductance such as R117H, and the common mutation F508del following correction of its trafficking defect [112–115]. Lumacaftor partially corrects the protein folding defects present in F508del-CFTR, improving the trafficking of F508del to the plasma membrane [116]. Both ivacaftor and lumacaftor work to restore CFTR function of responsive mutations [112, 116], resulting in improving mucociliary clearance and clinical outcomes [117, 118]. Clinical trials involving combination therapies of ivacaftor/lumacaftor in patients with cystic fibrosis homozygous for F508del showed significant but modest improvements in lung function in cystic fibrosis patients older than 12 years of age, but it is currently too early to judge clinical significance of observed forced expiratory volume improvement [119]. Although safety and pharmacokinetics of ivacaftor in children under 6 years of age has been assessed [120], no controlled clinical trials have been performed, so use of both ivacaftor and lumacaftor is limited to after structural lung damage and airway mucus obstruction is already present. Clinical trials of ion transport modulators in young children with no detectable bacterial infection designed to determine if mucus obstruction and pulmonary inflammation is improved are warranted to provide insight regarding the roles of such interventions in preventing cystic fibrosis lung disease.

HIF-1α

Hypoxia-driven inflammation is characteristic of ischaemic and inflammatory diseases such as acute lung injury and colitis. Increased understanding of HIF-1α regulation and downstream pathways is leading to new opportunities for pharmacological intervention and attenuation of hypoxia-driven inflammation. HIF-1α is protective during hypoxia, important in maintaining epithelial barrier function [121]. This has been confirmed by a number of studies using a murine model of colitis [122, 123] and acts through increased expression of HIF-1-regulated barrier-protective genes, including, intestinal trefoil factor and CD73 [121]. The respiratory epithelium is important in respiratory hypoxia, with HIF-1α stabilisation in alveolar epithelium demonstrated to be protective during acute lung injury [124]. This is achieved through increased glycolytic capacity, improved mitochondrial respiration and concomitant attenuation of lung inflammation [124]. Hypoxia-induced HIF-1α stabilisation also affects wound healing; HIF-1α and target gene vascular endothelial growth factor (VEGF) are detectable during wound re-epithelialisation [125]. Furthermore, VEGF is also upregulated during hypoxia [126] and has significant implications in epithelial wound repair under these conditions [127]. These studies suggest that pharmacological manipulation of HIF-1α *via* PHD inhibition may be a therapeutic pathway to induce airway epithelial repair and potentially attenuate inflammation. Hydroxylase inhibitors such as iron chelators [128] are one therapeutic option that has shown protective effects in colitis [122, 123]; however, they are largely non-specific for PHD inhibition. A more suitable approach may be through a recently identified novel class of PHD inhibitor [129], which acts by preferential binding directly to the PHD active sites. One of these novel inhibitors, TM6008, was observed to reduce cell death after hypoxic exposure [130]. Therefore, TM6008 may have potential to reduce the IL-1α driven inflammation that is associated with necrotic cell death in hypoxic disease [27].

Conclusion and future perspectives

Mucus obstruction leading to localised respiratory hypoxia is characteristic in early cystic fibrosis, likely to contribute to early cystic fibrosis airway pathology. Transcriptional and physiological responses to hypoxia are altered in cystic fibrosis, with reduced HIF-1α and NF-κB activation in cells lacking functional CFTR potentially exacerbating inflammation during hypoxia. Neutrophilic inflammation and neutrophil

recruitment driven by IL-1α released from necrotic cells resulting from hypoxia is exacerbated by NLRP3 inflammasome activated secretion of IL-1α. Once neutrophils are recruited to the airway, hypoxia significantly increases their survival, trans-epithelial migration and elastase release. Potential therapies to alleviate inflammation driven by IL-1R activation include: the use of IL-1R antagonists to inhibit activation by IL-1α; alleviation of mucus obstruction and resulting hypoxia *via* use of hypertonic saline to promote mucociliary transport; inhibition of ENaC-mediated Na^+ absorption with ENaC blockers and restoring CFTR channel function with CFTR modulators to improve airway surface hydration and mucus clearance and thus prevent mucus obstruction. Therapies to prevent or alleviate inflammation driven by hypoxia are in early stages of trials, or have previously shown no significant clinical effect. However, these studies used relatively crude clinical endpoints and none have yet been investigated as interventions to prevent the onset of structural lung disease [105, 106, 110, 119, 130]. Currently, the majority of targets for therapy are involved in the initial activation of the inflammatory pathway, but there is little research into activators further downstream in the pathway (figure 3). Future research could potentially target key genes in the NF-κB activation process, although such targets are less specific for hypoxia-driven inflammation and may have system wide effects including further impairment of host defence mechanisms in cystic fibrosis airways. Further study into the mechanisms of hypoxia-driven inflammation is required to identify feasibly actionable drug targets. These may include the replication of animal model studies using human-derived samples. Such studies would directly characterise human responses to hypoxia in the airway and elucidate specific mechanisms of cell death and IL-1α-driven inflammation. Answering these questions will result in deeper knowledge of the inflammatory environment within the respiratory system of patients with cystic fibrosis, with potential to identify targets for treatment to limit sterile inflammation in early childhood.

Acknowledgements

The authors thank Luke Garratt (Telethon Kids Institute, Perth, Australia) for help with manuscript preparation.

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