



Advances in airway inflammation: Clinical implications for cystic fibrosis

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The progression and severity of cystic fibrosis (CF) disease results from a failure of pulmonary innate immune functions, reduced mucociliary clearance, and failure of immune mechanisms to successfully opsonize and kill bacteria in airways. In the early stages of CF disease, the reason for the high prevalence of inflammatory cells in airways is currently unclear. This airway inflammation is characterized by a sustained accumulation of active neutrophils, high proteolytic activity and high levels of pro-inflammatory cytokines such as interleukin (IL)-8 found in the airway secretions.

“In CF airways, the excessive inflammatory response is multifactorial”

In the airways of CF patients, the inflammatory response to a defined bacterial load is greater and more excessive than in normal airways. Recently, there are a number of endogenous factors that have been identified as important modifiers in the susceptibility of CF airway cells to *Pseudomonas aeruginosa* infection. Elevated level of furin in CF airway cells cause hypersusceptibility to *Pseudomonas aeruginosa* exotoxin A-induced cytotoxicity. Elevated level of ceramide in CF airway cells has been also claimed to mediate bacterial infection, inflammation and cell death in the respiratory tract of a *cftr*-deficient mouse model. Elevated levels of total iron and iron-binding protein ferritin have been also reported in CFTR-deficient airway epithelium, facilitating therefore the *Pseudomonas aeruginosa* biofilm growth. Despite these intensive investigations, the relative importance of all these factors to the clinical manifestations of CF airway disease remains uncertain.

The sequence of events at the onset of airway inflammation has been the subject of debate following the finding of neutrophil dominated inflammation in the absence of bacterial or viral pathogens in bronchial lavages obtained from CF infants. High IL-8 content and neutrophil-predominant airway inflammation in CF begins early during the neonatal period, increases throughout childhood and adolescence, and is associated with persistent airway infection.

It is important to remain that CF disease results from mutations in the CF transmembrane conductance regulator (CFTR) gene affecting all exocrine organs through the body. In airways, new data support the hypothesis that lack of CFTR protein activity in airway epithelial cells is responsible for the onset of the inflammatory cascade in the CF lung. The proof that CF lung inflammation can occur prior to any infection came from naïve human CF lung grafts developed in severe combined immunodeficiency mice. In this *ex vivo* model, we demonstrated that sterile human CF lungs secreted high levels of IL-8, prior to any infection and host (mouse) neutrophils progressively accumulated in CF lungs leading to tissue destruction. Consequently, these observations have led to a proposal that increased neutrophil dominated inflammation, or alternatively the failure to down regulate inflammation in airways, is intrinsic to CF and operates independently of an infectious stimulus.

“CF is not only an epithelial disease, but also a neutrophil disease.”

In the past decade, important advances have been made in the understanding of the mechanisms underlying massive neutrophilic inflammation in CF airway. It is currently believed that inflammatory signalling in airway epithelium plays a critical role in orchestrating the response of the lungs to a broad variety of insults in the context of CF. Cell culture systems using primary human bronchial epithelial cells and respiratory cell lines have revealed that CF cells exhibit enhanced proinflammatory signalling compared to non-CF cells. This intrinsic inflammation was mainly characterized by an increased level of inflammatory mediators (e.g., IL-8 and IL-6) compared to non-CF bronchial epithelial cells, either in the absence or in the presence of bacterial stimulation. This enhanced production of proinflammatory cytokines released at the airway epithelium surface might also facilitate bacterial proliferation and contribute to an enhanced level of sialyl-Lewis x and 6-sulfo-sialyl-Lewis x epitopes in human airway mucins of CF patients.



The NF- κ B cell signaling in airways appears to be a focal point for control of lung inflammation through regulated production of mediators that participate in recruitment and activation of neutrophils, modulation of apoptosis, and control of epithelial barrier integrity. Consequently, dysregulated NF- κ B activation in the epithelium may provide a common pathway for driving the excessive inflammatory response and massive neutrophilic infiltration in CF airways. Neutrophils are attracted to CF lungs very early on and in high numbers due to pre-existing unknown stress conditions (e.g., upregulated cytokine secretion, pH, osmolarity). Interestingly, Tirouvanziam and colleagues recently established that CF airways contain a significant fraction of nonapoptotic viable neutrophils. They demonstrated that profound functional and signalling changes readily occur within viable neutrophils when recruited to CF airways. Compared with their blood counterparts, airway neutrophils have undergone conventional activation, as shown by decreased intracellular glutathione (GSH), increased lipid raft assembly and surface mobilization of CD11b+ and CD66b+ granules. Thus, these authors have identified a subset of neutrophils within CF airways with a viable but dysfunctional phenotype.

“Considering the persistence of neutrophils in airways not only as a transient fraction designed for rapid death, but also as potential contributors to the disease with a lifespan of several days, should considerably change the focus of research on neutrophils homing in airways of CF patients. “

Thus, we consider that CF is not only an epithelial disease but also a neutrophil disease. A comprehensive approach that will include a better understanding of the function of CFTR protein as a regulator of the airway inflammation and the knowledge of the fraction of viable non-apoptotic neutrophils homing in airways of patients with cystic fibrosis disease will provide useful information for a better treatment of lung inflammation of patients with CF.

“New treatment approaches fighting the deleterious effects of inflammation on the airway epithelium. “

Macrolide antibiotics administered in sub-antimicrobial doses improve pulmonary function and decrease exacerbation frequency for patients with CF. Macrolides have been introduced as CF treatment in recent years and are thought to act both on pro-inflammatory cytokines and neutrophil activation. These clinical trials were recently confirmed by a multicentre, randomised, double blind, placebo controlled trial conducted in young CF patients. Long-term use of low dose azithromycin was associated with a beneficial effect on lung disease expression, even before infection with *Pseudomonas aeruginosa*. Consistent with this, macrolide antibiotics possibly reduce mucin production as well as neutrophil migration in airways. GSH is an antioxidant that is found to be reduced in blood neutrophils and in ASL of CF patients. *N*-acetylcysteine (NAC), a well known antioxidant GSH prodrug has been recently tested on a small cohort of CF patients. Upon treatment, neutrophil burden in CF airways was decreased but pulmonary function measures were not improved, due probably to short-term treatment. While gene therapy does not appear to be a therapeutic option in the near future, CFTR pharmacotherapy is currently being developed as an alternative to reduce F508del-CFTR degradation and to improve chloride function in CF lung. Two drugs that increase chloride secretion via an alternative chloride channel, Moli1901 and denufosol, have been shown to be safe and efficacious in clinical trials. Osmotic therapy to increase mucus hydration using inhaled dry-powder mannitol and hypersaline solution may be an alternative approach and was demonstrated to be safe and well tolerated in children and adults with mild-to-moderated CF.

“In 2008, it is likely that we will see the first results from trials of small druglike molecules with activities of corrector and potentiator of CFTR function to rehydrate airway surfaces of CF patients.”

To conclude, it is currently believed that inflammatory signalling in airway epithelium plays a critical role in orchestrating the response of the lungs to a broad variety of insults in the context of CF. Consequently, airway inflammation has been increasingly considered to be a therapeutic target for CF disease. Anti-inflammatory treatment is moving towards earlier intervention in CF pathophysiology which is an exciting development to decrease the rate of decline in lung function and thus offer longer lives for patients with CF.

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