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Review

Lung inflammation in cystic fibrosis: Pathogenesis and novel therapies ☆

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ABSTRACT

Despite remarkable progress following the identification of the causing gene, the final outcome of cystic fibrosis (CF) remains determined mainly by the progressive reduction of lung function. Inflammation of the airways is one of the key elements of the pathogenesis of the disease: it is responsible for the destruction of lung architecture, resulting in progressive loss of respiratory function. Bronchial infection induces an intense inflammatory reaction characterized by a massive invasion of neutrophils, the properties of which seems altered in CF. Moreover, the inflammatory process is also marked by a profuse release of soluble pro-inflammatory mediators, such as interleukin (IL)-6, IL-1β and IL-8 cytokines. In contrast, release of the anti-inflammatory mediator IL-10 is reduced, thus reflecting a pro-/anti-inflammatory imbalance. The inflammation/infection pair seems hard to dissociate, and the origin of the baneful consequences of the persisting excessive inflammatory responses remains to be cleared up: does inflammation follow or rather precede infection?
Recent data suggest that uncontrolled inflammation is constitutive in CF. Countering it at early stages of the disease in order to prevent irretrievable damages in lungs remains a major priority in treating patients with CF. In this review, we discuss the usefulness and limitations of mouse models of CF to study the pathogenesis of human lung inflammatory disease, and the development of new potential strategies to reduce the inflammatory burden in the airways.

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Introduction

Morbidity and mortality observed in CF are principally related to lung alterations characterized by a vicious circle of obstruction, infection and chronic inflammation of the airways. The sequence of events leading to progressive destruction of the lung architecture and loss of respiratory function is not fully understood. It has been suggested that the initiating event is dehydration of the fluid layer lining the surface of the tracheobronchial tree [1]. Depletion of airway surface layer (ASL) volume develops, in large part, as a consequence of abnormal transepithelial ion transport related to loss of function of CF Transmembrane Conductance Regulator (CFTR) protein [1], and it also seems regulated by local levels of nucleotide and nucleoside, ATP and adenosine [2]. Preclinical studies on various experimental models of animal and human origins all agree on a key role of the CFTR in the homeostasis of fluids on the surface of epithelia. The protein mainly acts as a low conductance cAMP-dependent chloride channel, but it also regulates several other membrane transport proteins, most notably the epithelial sodium channel ENaC [3,4]. Transepithelial ion fluxes, mainly of chloride and sodium, are coupled to water movements that maintain adequate hydration of the epithelium surface. CFTR dysfunction impairs the water balance of

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ASL, reducing the volume of its aqueous film and thickening its mucus film produced by submucosal glands with a minor contribution of goblet cells, endangering the mucociliary clearance [5–7]. The thick dehydrated sputum obstructs the airways and prevents elimination of dust, bacteria and other impurities from the lungs, thus making them more vulnerable to repeated bacterial infections and chronic inflammation.

In addition to typical transepithelial ion transport abnormalities, other epithelial cell dysfunctions, including decreased sialylation of cell membranes [8–10], defective bacterial internalization [11], imbalanced omega3/omega6 polyunsaturated fatty acid (PUFA) metabolism [12–22] and cell membrane accumulation of ceramides [23–26] have allowed to make the link between mutated CFTR protein and chronic inflammation in CF airways.

Decreased sialylation of CF cell membranes has been reported in CF epithelial cells; it is associated with increased concentrations of the gangliotetraosylceramide asialoganglioside M1 (asialo-GM1), which serves as a cell receptor for the two main pathogens for CF patients, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [8,9]. Binding of *P. aeruginosa* and *S. aureus*, but not of *Escherichia coli*, the latter not associated to significant pulmonary disease in CF, was reportedly increased in polarized CF bronchial and pancreatic epithelia [9]. However, studies on the role of asialo-GM1 as an epithelial cell receptor for *P. aeruginosa* have provided contradictory results. Indeed, no enhanced binding of several laboratory strains and fresh clinical isolates of *P. aeruginosa* to asialo-GM1-treated cells was observed [10]. It has been reported that in normal lungs, CFTR protein itself serves as a cellular receptor for binding, endocytosing, and clearing *P. aeruginosa* [11]. Internalization of *P. aeruginosa* was about a hundred times larger in epithelial cells expressing functional CFTR compared to cells lacking it or expressing the mutant F508del CFTR protein. The first extracellular domain of CFTR was suggested to be the specific ligand for the bacterium. If binding to functional CFTR is the initial step of the process clearing *P. aeruginosa* from the lungs, a direct link is established between mutations in CFTR and a higher vulnerability to trigger and perpetuate infections [11].

Abnormal essential fatty acid (EFA) metabolism could be related to excessive inflammatory responses in CF [12–14]. Pancreatic insufficient patients display a more disturbed lipid metabolism [15], but it would be too simple to ascribe EFA deficiency in CF to reduce intake or to alter gastrointestinal handling (digestion, absorption, transport) as the sole pathophysiological mechanism. Reduced EFA circulating values have been found as early as in the first weeks of life in infants with CF [16]; they were also present in well-nourished young CF patients who do not receive a low-fat diet and do not present with fat malabsorption [17,18]. The underlying mechanisms of altered EFA metabolism in CF seem indeed to be multifactorial [19]: increased lipid turnover in cell membranes, increased oxidation of fatty acids for energy needs, increased production of eicosanoids linked to an exacerbated inflammatory status and decreased desaturase activity. A characteristic imbalance between arachidonic acid (a representative derivative of omega6 PUFA) and docosahexaenoic acid (DHA, a representative derivative of omega3 PUFA) have been identified in patients with CF [13,15–17,20] and in animal models of the disease [21,22].

Ceramides, a class of sphingolipids, were reported to accumulate in an age-dependent manner in CF respiratory cells [23]. Accumulation of ceramides has been related to pulmonary inflammation, apoptosis and death of respiratory epithelial cells, deposits of DNA in bronchi and high susceptibility to severe *P. aeruginosa* infections [24–26].

Regardless of the cause, chronic inflammation progressively leads to suppurative pulmonary disease characteristic of CF. As inflammation and infection are extremely difficult to dissociate, this opens the debate on the origin of the inflammation: does it follow or rather precede the infection? Recent research has shed some new light on the issue of the origin of uncontrolled inflammatory overreactions in CF. The fact that muco-modulating agents, although effective in improving rheology and transportability of the hyperviscous mucus, fail to modulate inflammation in CF [27,28] could outline a direct link between inflammation and the genetic defect in the disease. Another argument in favor of this hypothesis is the detection of pulmonary inflammation in asymptomatic patients very early in life [29–33], even in newborns [29]. Clinical observations suggest that, in CF, inflammation precedes infection [29–33]; however, the cellular and soluble mediators as well as the intracellular signaling pathways involved in the intense inflammatory responses remain very poorly understood.

In this review, we describe the characteristics of airway inflammation and its relationship to progressive lung disease in CF patients. We discuss the usefulness and limitations of mouse models of CF to study the pathogenesis of human lung inflammatory disease, and the development of new potential strategies to reduce the inflammatory burden in the airways.

Characteristics of CF human inflammation

Chronic bacterial infection is assuredly the leading cause of progressive suppurative inflammation in CF lungs. However, several lines of evidence have suggested that altered pro-inflammatory responses in CF epithelia, described in the past as single barrier cells, may manifest independently of any detectable infection. The introduction of CF newborn screening has led to a better understanding of the early natural history of CF lung disease, as it has allowed assessing the evolution of pulmonary signs and clinical course of CF lungs shortly after birth [29–33]. In newborns with CF, lungs appear structurally normal and bacterial cultures of respiratory secretions often fail to yield specific pathogens. However, bronchoalveolar lavage (BAL), undertaken for culture and measurements of pro- and anti-inflammatory cytokines in infants with CF aged from 1.5 to 71 months, has shown neutrophilic-dominant lower airway inflammation with elevated concentrations of interleukin (IL)-8, the principal neutrophil chemoactractant in CF lungs, even in the absence of any detected pathogen [30].

CF-associated airway inflammation is characterized by a profuse influx of neutrophils into the lungs; however other types of leukocytes, including eosinophils [34], lymphocytes [35] and monocytes [36] could also play a role. The fact that several neutrophil cellular functions seem to be deregulated in CF [37,38] could solve the enduring paradox of an overwhelming infiltrate of inflammatory cells that fails to resolve infections. Neutrophil elastase (NE), a serine protease released from primary neutrophil granules, has been found to be elevated in CF airways very early in life [39]. NE has been claimed to be a very informative biomarker of disease progression, with higher sputum levels being associated with more rapid lung function decline and bronchiectasis [39]. The enzyme perpetuates the vicious cycle of inflammation: its broad substrate specificity is related to disruption of structural tissue components, such as elastin and fibronectin, and to activation of the proform of matrix metalloprotease (MMP)-9, contained in tertiary neutrophil granules [40]. MMP-9, a marker involved in matrix extracellular proteolysis, may also contribute to the increased transmigration capacity of neutrophils, observed in isolated CF cells [41]. Besides, higher concentrations of pro-inflammatory mediators such as IL-6, IL-8 and IL-1β have been found in the BAL of children with CF [29]. Similarly, increased levels of IL-17, also involved in lung neutrophil infiltrate, have been found to be increased in CF [35]. Conversely, reduced concentrations of IL-10 [42] and of lipoxins [43], which possess anti-inflammatory properties, were measured, highlighting an imbalance in inflammatory signals in CF. Moreover, airway neutrophils isolated from CF patients showed a blunted phagocytic capacity that could contribute to altered host defense functions and poor bacterial clearance [44].

On cell death, CF neutrophils release DNA, which increases mucous viscosity, and abundant oxidases, which contribute to the occurrence of oxidative stress. The abnormal flux of reactive oxygen species in CF lungs exacerbates pulmonary deterioration and favors progression of bronchiectasis [45].

Lungs of CF patients are often colonized or infected in infancy and early childhood with organisms such as *S. aureus* and *Haemophilus influenza*. In