Immunopathophysiologic Mechanisms of Cystic Fibrosis Lung Disease

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Abstract

Cystic fibrosis is a life-threatening autosomal recessive disorder with a highly variable clinical presentation. The pathophysiology is related to the mutant transmembrane conductance regulator (CFTR), a chloride channel that is encoded by the CF single gene located on chromosome 7. The variability of the clinical presentations, even among patients carrying the same mutation, is extensive enough to justify the hypothesis that other pathophysiologic mechanisms participate in the evolution of the disease phenotype. Presented here are recent lines of research on the contributing factors to respiratory tract morbidity, as well as the innate defense mechanisms in the CF lungs, the cytokines and chemokines that influence the inflammatory processes, the antioxidative system, and the composition of the airways surface fluid. These studies concluded that the clinical presentation is determined by pathology of the CFTR as well as by other mechanisms, some of which are related to the CFTR functions and others to the products of modifier genes as well as the influence of the environment.

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Cystic fibrosis is the most common lethal inherited disorder with autosomal recessive inheritance among Caucasians [1]. It is caused by a mutation in the gene located on the long arm of chromosome 7 that encodes the CF transmembrane conductance regulator, which is a chloride ion channel, located on the apical membranes of epithelial cells and regulated by cyclic AMP [2]. The underlying defect in CF is caused by lack of functional expression of CFTR, which leads to the high viscosity of airway secretions and, consequently, to decreased mucociliary clearance [3]. Examination of altered ion and water transport alone, however, failed to elucidate the path from gene to pathogenesis in the CF lung. Recent studies provide evidence that the role of CFTR may extend beyond chloride permeability, and that its dysfunction influences the expression of other gene products, including proteins essential to the inflammatory processes, other membrane ion transport, and cell signaling. The proteins that are encoded by the modifier genes in CF affect the different phenotypes among patients with the same mutation [4].

There is a complex pathophysiologic cascade leading to lung damage in CF, and some of the mechanisms underlying the lung disease are summarized in this review [Table 1].

The airway surface liquid

The contribution of the airway surface liquid to the pathophysiology of CF airways is explained by three hypotheses involving abnormal ASL composition, volume and physical properties. The ASL is composed of two layers: a periciliary fluid phase through which the cilia beat freely, and an overlying mucus gel phase [5]. The low volume hypothesis postulates that the loss of CFTR

Table 1. Pathophysiologic mechanisms in cystic fibrosis lung disease

Mechanisms	Reference
CFTR-related pathophysiology	
Defective chloride transport	[2]
Reduced bicarbonate secretion	[10]
Defective sodium channel regulation with hyperabsorption of Na+	[6,7]
Defective glutathione transportation	[34]
Modifier gene-related pathophysiology	
Increased and prolonged NFκB activation	[13,14]
Dysregulation of cytokines	[12]
IL-10 deficiency	[15,16,20]
Excessive production of TGF-β1	[28]
MBL deficiency	[31]
HLA-class II polymorphism	[32]
TNF-α overproduction	[22]
Other pathophysiologic mechanisms	
Neutrophil dysfunction	[24,25]
Decreased lipoxin concentration	[26]
Reduced level of SLPI	[36]
Impaired sialylation of glycolipid receptors	[37]
Attenuated responses of TLR4 to environmental *P. aeruginosa*	[38]
BPI-ANCA antibodies	[39]
Increased adhesion molecules	[40]

CF = cystic fibrosis
CFTR = CF transmembrane conductance regulator
ASL = airway surface liquid

function causes increased epithelial sodium channels activity, resulting in hyperabsorption of Na^+, with consequent increased fluid resorption from the lumen leading to dehydrated ASL that interferes with ciliary function [6,7]. The high salt hypothesis postulates that the high concentration of NaCL in the ASL of CF patients compared to healthy people causes inhibition of the activity of endogenous antimicrobials, such as defensins and cathelicidins, which provide an important innate defense mechanism of the airways under normal circumstances [8,9].

The low pH hypothesis is based on a defective CFTR-dependent bicarbonate transport, which consequently acidifies the ASL. The mucociliary clearance mechanism is inhibited by the abnormally acidic ASL [10].

Dysregulation of cytokine secretion

The inflammatory response in CF airways is excessive relative to the burden of infection [11]. High levels of the pro-inflammatory cytokines interleukin-1, tumor necrosis factor-alpha, IL-6 and IL-8 were found in the sputum, bronchoalveolar lavage fluid and serum of CF patients, even during stable clinical conditions [12]. Nuclear factor-kappa B is a transcription factor that is mobilized to the cell nucleus in response to bacterial stimulation, and there it regulates the genes involved in chemokine and cytokine expression. NFκB exists together with its inhibitor IκB in the cytoplasm as a complex. A lack of cytosolic IκB, and high levels of constitutively activated NFκB, associated with an up-regulation of IL-8 was demonstrated in human ΔF508 CF bronchial tissues as well as in cultured human CF bronchial glands [13].

IL-8 is a major neutrophil chemoattractant peptide that ensures that activated neutrophils continue to accumulate in the airways [12]. Excessive activation of the transcription factor NFκB leads to increased production of IL-8 in response to respiratory pathogens [14]. Bronchoalveolar lavage fluid from CF patients contains reduced amounts of IL-10 compared to healthy controls [15]. Stimulated T cells from CF patients produced less IL-10 *in vitro* than did stimulated T cells from healthy volunteers [16]. IL-10 has potent immunoregulatory and anti-inflammatory activities, which include inhibiting production of IL-8, TNF-α and IL-1β [17] and increasing production of IκB, the inhibitor of NFκB activation [18]. IL-10 also increases apoptosis of polymorphonuclear neutrophils [19]. The decreased level of IL-10 in the CF airways may be a preexisting immunoregulatory abnormality that allows inflammation to persist even after acute infectious stimuli have been removed. Recurrent episodes of abnormally persistent inflammatory responses may then lead to a vicious cycle of chronic inflammation that could cause lung damage [20].

Although bacterial colonization was traditionally considered the main cause of chronic airway inflammation, regulation of airway inflammation is the result of the interactions of multiple pro- and anti-inflammatory signals.

TNF-α

TNF-α is a pro-inflammatory cytokine produced in abundance by resident macrophages in the lower respiratory tract of CF patients. It was shown that macrophages from CF patients display significantly higher TNF-α mRNA expression and TNF-α production when compared to those from normal subjects [21]. This cytokine contributes to the pathophysiology of CF by inducing cachexia and weight loss, and there is an inverse relationship between TNF-α concentration in the CF patient's sputum and levels of forced expiratory volume in one second [22].

Neutrophils

The neutrophils are a cornerstone of the innate defense mechanism, and their function was found to be abnormal in CF. The stimulated neutrophils from CF patients release a higher level of elastase, oxidants and IL-8 [23] than those from healthy subjects in *ex vivo* studies. They also showed an exaggerated recruitment response to IL-8 [24]. Neutrophil apoptosis was found to be accelerated by various mechanisms, and the DNA released from the destroyed cells was shown to contribute to the increased viscosity of the sputum [25].

Lipoxins

Lipoxins are arachidonic acid metabolite lipid mediators, functionally distinguishable from most other eicosanoid mediators because of their anti-inflammatory actions. They modulate neutrophilic inflammation by inhibition of neutrophil chemotaxis, adherence and transmigration, and suppress their activation. Lipoxin concentrations were significantly suppressed in the airway fluids of CF patients compared with those of patients with other inflammatory lung conditions. These finding suggest that there is a pathophysiologically important defect in lipoxin-mediated anti-inflammatory activity in the CF lung [26].

Modifier genes

The widely diverse phenotypic expression of CF is likely influenced by the class of CFTR mutation, by environmental factors, and by modifier genes. A modifying locus is an inherited genetic trait that is distinct from the disease locus that leads to a quantitative or qualitative difference in the disease phenotype [27].

Transforming growth factor

TGF-β1 is produced by many cells throughout the body. It is produced by bronchial epithelial cells in human lung tissue and promotes proliferation of fibroblasts and deposition of collagen with subsequent fibrosis. Production of TGF-β1 varies between individuals and partly depends on gene polymorphism. High TGF-β1 producers develop significantly more lung fibrosis in response to inflammatory triggers. Polymorphism of TGF-β1 gene (codon 10), which is associated with its high production in CF patients, was found to be correlated with more severe lung disease and more rapid deterioration in pulmonary func-

IL = interleukin
NFκB = nuclear factor-kappa B
TNF-α = tumor necrosis factor-alpha
TGF-β1 = transforming growth factor-beta 1

tions compared to CF patients with the same CFTR mutations but who are low producers of TGF-β1 [28].

Mannose-binding lectin

Mannose-binding lectin, which promotes opsonization and phagocytosis, is an important component of the innate immune defense against bacterial and viral infections during infancy, before the adaptive immune system develops specific immune responses [29]. MBL is a product of a single gene whose variant alleles that produce low serum MBL concentrations are associated with an increased risk of infection [30]. The presence of one or more copies of a dysfunctional MBL gene is associated with diminished lung function in CF patients who are chronically infected by P*seudomonas aeruginosa*. Low levels of MBL were found to be correlated with more severe lung disease and shorter life expectancy [31].

HLA class II polymorphism

There is a strong association between some major histocompatibility complex class II alleles and allergic disease. Allergic manifestations are frequently found in CF patients, and some studies suggested that atopic patients suffered from more severe disease than non-atopic patients. HLA-DR7 was found to be associated with an increase in total immunoglobulin E and in colonization with *P. aeruginosa*, and the genetic variation in resistance and susceptibility to *P. aeruginosa* in CF patients could implicate HLA class II loci [32].

Antioxidant and antiprotease system

It is now well established that oxidative stress is of central importance in CF pathogenesis and contributes early to the decline of lung function. The imbalance between the antioxidant defense provided by reduced glutathione and the oxidative stress resulting from the inflammation and infection process contributes to the clinical severity in CF [33].

Glutathione

Glutathione is an important antioxidant that is found in high concentrations in normal epithelial lining fluid of the lower airways. CF airways are exposed to significantly increased levels of oxidative stress resulting from the characteristic chronic pulmonary inflammation and infection. Glutathione, whose transportation is influenced by CFTR, was found to be decreased in CF bronchial epithelial lining fluid. The glutathione deficiency in the respiratory epithelial surface favors oxidative stress lung damage and an excessive inflammatory response [34].

α1-antitrypsin

α1-AT is one of the few antiproteases capable of inactivating neutrophil elastase. The extremely high level of neutrophil elastase in the airways of CF patients indicates that there is an imbalance between α1-AT and elastase, even though normal to elevated levels of α1-AT have been reported. It was shown that α1-AT genotype is not a major contributor to the variability of pulmonary disease severity in CF [35].

Secretory leukocyte protease inhibitor

SLPI is a major local protease inhibitor in the upper airways, and it also participates in the anti-inflammatory and antimicrobial responses of the airways. The SLPI level was found to be reduced in CF patients, resulting in increased inflammation and increased infection by S*taphylococcus aureus* [36].

Altered receptor-pathogen interactions

Adherence to asialo-glycolipid receptors

The respiratory pathogen that most contribute to the morbidity of CF patients is P. *aeruginosa*, which binds to asialo-glycolipid receptors. Such asialylated receptors are not normally available on the airway surfaces to any great degree, but they are significantly increased in areas of cell damage. Cells with CFTR mutations have increased amounts of asialylated glycoconjugates due to an impaired process of glycolipid receptors sialylation, and so there is increased adherence of respiratory pathogens [37].

Although CF is a monogenic disease, there is a wide variability in clinical phenotype expression among patients with the same mutation due to mutant CFTR-related functions, modifier genes, excessive inflammatory response, dysregulation of cytokines secretion, and impaired innate immunity as well as interaction of these factors and the environment

Toll-like receptors

The major gram-negative bacteria surface component lipopolysaccharide and its component lipid A are recognized by human Toll-like receptor-4. These bacteria can modulate the structure of their LPS on invasion of host tissue to resist killing by the innate immune system. The acylation state of LPS may affect the LPS-mediated immune responses. Isolates from the airways of CF-affected individuals synthesize hexa-acylated LPS in contrast to penta-acylated LPS, which are isolated from the environment and are poorly recognized by human TLR4. Attenuated responses of human TLR4 to environmental P. *aeruginosa*, which has an LPS penta-acylated structure, and the vigorous response to hexa-acylated LPS may have significant implications for airway disease in CF [38].

MBL = mannose-binding lectin
α1-AT = α1-antitrypsin
SLPI = secretory leukocyte protease inhibitor
LPS = lipopolysaccharide
TLR = Toll-like receptor

Bactericidal/permeability-increasing protein anti-neutrophil cytoplasmic antibodies

BPI is a membrane-associated protein found in the azurophilic granules of neutrophils and an important host defense by possessing bactericidal activity as well as endotoxin-neutralizing activity. CF patients colonized with P. *aeruginosa* have autoantibodies of IgA and IgG isotype directed against BPI. These BPI-ANCA antibodies interfere with bacterial phagocytosis and are associated with more severe pulmonary disease [39].

Adhesion molecules

Increased levels of soluble adhesion molecules in serum and other body fluids have been demonstrated in several inflammatory diseases. Significantly increased levels of sICAM-1 and sE-selectin but not sVCAM-1 were found in clinically stable CF patients. Serum levels of these adhesion molecules increased even more at the time of exacerbations compared with levels at the time of stable clinical conditions. The up-regulation of these molecules may play an important role in the pathogenesis of CF airways inflammation, and could be an indication of its severity [40].

Conclusion

Although CF is a monogenic disease, it appears that there is a wide variability in clinical phenotype expression among patients with the same mutation. Several mechanisms have been proposed to link the CF genotype to clinical disease, some of which include the CFTR-related functions, modifier genes, excessive inflammatory response, dysregulation of cytokine secretion, impaired innate immunity and others. The vast amount of research into the pathophysiologic mechanisms in CF has contributed much to better understanding of the disease, paving the way to advances in therapeutic options.

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BPI = bactericidal/permeability-increasing protein
Ig = immunoglobulin
ANCA = anti-neutrophil cytoplasmic antiodies
ICAM = intracellular adhesion molecule
VCAM = vascular cell adhesion molecule

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