Role of Infection in Cystic Fibrosis Lung Disease

Subjects: Respiratory System

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Cystic fibrosis (CF) is an autosomal genetic multisystemic disease. The basic defect lies in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein caused by mutations in the respective gene. Infection play an important role in the pathophysiology of cystic fibrosis, and it is one of the significant causes of morbidity and mortality in CF.

cystic fibrosis CFTR inflammation infection

1. Introduction

Cystic fibrosis (CF) is an autosomal genetic multisystemic disease [1]. The basic defect lies in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein caused by mutations in the respective gene [1]. The defective anion channel located in the epithelial cell membrane results in defective ion transport, which in turn leads to airway surface liquid depletion and thick mucus airway secretions that impair mucociliary clearance [2]. Production of thick mucus in the airways leading to airway obstruction is a major cause of symptoms and lung disease progression in CF. A vicious cycle of airway obstruction, infection, and inflammation that plays a pivotal role in the pathogenesis and progression of CF lung disease has been long described [3]. CF lung disease remains the major cause of death despite recent major advances in therapeutics of the disorder [4]. Early-life intermittent lung infection with pathogens such as *Pseudomonas aeruginosa* gradually becomes chronic and leads to more inflammation and lung damage. Treatments disrupting this vicious cycle improve outcomes and ameliorate lung damage, thus prolonging the life of people with CF and improving lung function and quality of life. The recent development of CFTR modulators seems to alter the milieu in the CF airways reducing inflammation and possibly infection, and it is expected to alter the long-term outcomes of the disease dramatically [5]. Research is ongoing for the development of new antimicrobial and anti-inflammatory agents in an effort to improve management, especially in combination with the new CFTR modulators and correctors. As bacterial resistance to antibiotics has increased, new approaches are being investigated to combat lung infection.

2. Role of Infection in the Development and Progression of Cystic Fibrosis Lung Disease

2.1. Microbial Interactions

The presence of thick mucus and the vicious cycle of infection and inflammation has an impact on the lung microbiome in patients with CF [6]. The interaction between different bacteria in the lungs of patients with CF may be associated with the severity of inflammation in the lungs [6]. There are conflicting data on the role of different bacterial species, especially the role of facultative anaerobes of the usually so-called “oropharyngeal flora” in the lung of individuals with CF and if the interactions lead to more or less infection and inflammation [7]. Reduced lung microbial diversity and microbiota dominance by certain bacteria were associated with reduced lung function in a study by Cuthbertson et al. [8]. Lung disease was classified as normal/mild, moderate, or severe based on the level of percent predicted $FEV_{1,}$ and bacterial operational taxonomic units (OTUs) were divided into core and satellite taxa. Within each lung disease category, four OTUs that are known to cause CF lung disease (*P. aeruginosa, S. aureus, S. maltophilia,* and *B. cepacia*) were found to have core status, and two (*H. influeanzae* and *A. xylosoxidans*) satellite status. A linear relationship was demonstrated between microbial diversity and dominance with FEV_1. The dominance of known pathogenic OTUs, especially *P. aeruginosa,* was shown to correlate with decreasing lung function [8].

Another recent study looked at the possible protective role of commensal strains (aerobic and anaerobic) toward less inflammation [6]. In this study using airway epithelial cells and the ex-vivo murine precision-cut lung slices murine lung model, it was shown that ex-vivo simultaneous infection of commensals, especially *Streptococcus mitis* with *P. aeruginosa,* was associated with reduced inflammatory response [6]. However, the role of *Streptococci* in lung infection and inflammation is not clear, with some studies showing protective and other studies showing a synergistic effect with *P. aeruginosa* co-infection [9][10][11].

Recent studies have also shown that the interaction between microbes in cystic fibrosis is quite complex [12][13][14]. Communities of anaerobes seem to interact, especially with *P. aeruginosa* [15]. The proportion of so-called “fermenters,” which are anaerobes that are thought to be “benign” versus the so-called “pathogens,” is constantly changing and depends on several factors. It is thought that fermenters usually grow in lung areas with low oxygen tension and use sugars to grow, while pathogens grow in lung areas with high oxygen tension and use amino acids for growth [16].

A study by Ghuneim et al. tested in vitro a mathematical model of interactions between the different CF microbial communities [17]. This mathematical model was biofilm-based and was developed by the same researchers in order to predict the changes in microbial populations based on oxygen and ph gradients [16]. In this study, different antibiotics were used, and their effect on the concentration of different microbial populations was investigated. The most common bacteria were found to be *Pseudomonas, Streptococcus, Veillonella, Hemophilus, Fusobacterium, Prevotella, Staphylococcus, Achromobacter,* and *Neisseria* and the bacterial genera that were primarily responsible for the community differentiation were *Pseudomonas, Streptococcus* and *Staphylococcus.* Different antibiotics had different impacts, but all antibiotics made an impact on the CF microbiome, thus highlighting that the dynamics of microbial interactions in the CF lung are constantly changing and also depend on antibiotic use [17].

The presence of anaerobes is not always benign, and their number in the CF lung is not constant but fluctuates over time [7]. Anaerobes are often found in the lungs of patients with CF, and their presence in higher numbers has

often been associated with better lung function. However, some virulence factors produced by anaerobes could augment known CF pathogens' virulence by enhancing antimicrobial resistance and acting synergistically in airway colonization and infection [7]. Anaerobes of the oropharyngeal flora also appear to be a diverse group of bacteria, with, for example, *Porphyromonas* being associated with better FEV_1 and *Streptococcus anginosus* being associated with lower FEV_1, while for *Prevotella,* there are conflicting data [18][19]. Of note, *Streptococcus pyogenes* could be associated with pulmonary exacerbations [20]. A recent review by Blanchard and Waters highlights the important role of anaerobes in CF lung infection in addition to known pathogens, namely *P. aeruginosa, S. maltophilia, B. cepacia, A. xylosoxidans*, non-tuberculous mycobacteria and *A. fumigatus* [21]. More specifically, the detection of anaerobes is associated with a worse clinical response to antimicrobials and a greater decline in lung function. Less anaerobe diversity was related to more severe lung disease. It seems that the heterogeneous oxygen gradient of the CF lung in combination with the thick mucus impending on mucociliary clearance predisposes patients to anaerobic infection [21].

Significant interactions exist not only between pathogens and anaerobes, which are often considered to be benign oropharyngeal flora but also between pathogens. A cystic fibrosis foundation (CFF) patient registry analysis from 2003–2011 had shown that the presence of methicillin-sensitive *S. aureus* seemed to inhibit infection with *P. aeruginosa,* and in turn, the presence of *P. aeruginosa* seemed to prevent colonization with *B. cepacia* complex, *A. xylosoxidans,* and *S. maltophilia*, while colonization with *B. cepacia* complex was associated with a lower chance of subsequent colonization by any other bacterium or *Aspergillus* species. The microorganisms most likely to persist and lead to chronic infection were *P. aeruginosa*, *B. cepacia* and methicillin-resistant *S. aureus* (MRSA). All three were associated with a reduced chance of MSSA being isolated in the following years [22]. The strongest association in this study was the negative association of MSSA with *P. aeruginosa* for the following year, implying that these two bacteria have antagonistic effects in the CF lung. In this study, it is postulated that *P. aeruginosa* and *B. cepacia* inhibit other bacteria by dominating the microbiome and decreasing the microbiome diversity of the CF lung [22].

A more recent longitudinal cohort study that followed patients from 2004 to 2017 with a mean time of follow-up of 10.5 years was published and showed that *P. aeruginosa* and *S. aureus* co-infection is not uncommon in patients with CF, and it can persist for a long time. Co-infection with *P. aeruginosa* and *S. aureus* was noted with both MSSA and MRSA [23].

2.2. Factors Promoting Microbial Persistence in the CF Lung

The capability of microbes to persist in the CF lung has been related to both host and bacterial factors. Biofilm formation, quorum sensing, secretion systems, antimicrobial resistance, hypermutation, microevolution, and adaptive modifications are all factors that affect the ability of microorganisms to persist in the CF lung and cause chronic infection [24]. Even though *P. aeruginosa* remains the prototype for adaptation in the CF lung, other bacteria such as *A. xyloxidans* and *S. maltophilia* have recently been shown to be important for the progression of lung disease [24].

Initial early infections with *P. aeruginosa* are with strains that are more virulent and which cause acute infections. If the eradication treatment fails and the infection becomes chronic, the *P. aeruginosa* phenotype changes over time and becomes persistent [5]. The reasons behind eradication failure are not totally clear and seem to be both bacterial- and host-related [5].

Studies that followed patients long-term and sequenced *P. aeruginosa* strains have shown that mutations accumulate as a clone dominates and persists due to adaptation that happens progressively in the CF lung. There are some strains that are fit to persist, and occasionally a patient might be infected by a different strain that will displace the existing *P. aeruginosa* population in the patient's lung. This strain usually originates from another chronically infected patient [25]. Bacterial mutations that are important for chronic infection affect genes that are involved in biofilm formation, mucoid phenotype, antibiotic resistance, motility, quorum sensing, and reduction of virulence factor production [5][25]. In addition, the production of type IV secretion toxins ExoS and ExoT by *P. aeruginosa* seems to impair phagocytosis by both neutrophils and alveolar macrophages [5].

2.3. Host Factors That Predispose Chronic Infection

Anatomically CF upper and lower airways, and the CF lung had been considered to have structural differences starting in early life compared to the respiratory system in individuals without CF. This finding has been demonstrated in both animal models and in imaging studies, including young children [26][27]. Recent investigations in infants who were diagnosed by neonatal screening and underwent chest CT scan, bronchoscopy and infant lung function testing have demonstrated that lung disease is milder at one year of age than previously reported in patients at the age of 3 months [28][29]. Abnormal CFTR function results in acidic airway surface liquid (ASL). The acidity of the airways is a major factor that leads to many defects in host lung defense and promotes microbial colonization [26]. Acidity in the CF lung, combined with congenital differences and thick mucus that cannot be detached from the submucosal glands and the airways, sets the scene for chronic infection, inflammation, and lung damage [30]. CFTR dysfunction also results in higher NaCl concentrations in the ASL that is associated with inhibition of the innate lung defense [31].

The CF lung environment is not only acidic but also hypoxic and even anaerobic in some areas due to mucus plugging and energy consumption by lung epithelial cells and neutrophils [8][25]. The steep oxygen gradient, in combination with the lack of nutrients like iron and zinc, are selective pressures toward strains that cause chronic *P. aeruginosa* infection [25].

Nutrient availability is also different in the CF airway. CF sputum has considerably more iron than sputum in healthy individuals [31]. Iron is consumed by *P. aeruginosa* and promotes growth as well as the formation of virulence factors and biofilm [31]. Even though glucose levels are relatively lower in CF sputum, this is not true in patients with CFRD hyperglycemia, increasing the risk for bacterial acquisition and growth [31].

The lung host defense systems in patients with CF are not correctly regulated, and the susceptibility to infection is more pronounced as a result of the dysfunction of mostly neutrophils and macrophages [32].

Normally airway cells phagocytose pathogens and then desquamate. Desquamation protects the lung from injury. The capability of the CF airway cells for phagocytosis is reduced, leading to the speculation that the deficient CFTR protein is the channel through which phagocytosis occurs [26]. In support of this speculation, in epithelial cell cultures, it has been noted that plasma membrane blebs are formed after phagocytosis of *P. aeruginosa* [26]. Blebs seem to be related to *P. aeruginosa* lipopolysaccharide (LPS) and are associated with increased epithelial cell apoptosis. Experiments in CF mice show that exposure to *P. aeruginosa* LPS is associated with an abnormal immune response and lung structural changes [26].

Hypersecretion of IL-1β as part of immune system dysfunction affects both *P. aeruginosa* and *B. cenocepacia* survival in the CF lung [26]. Another dysregulation of the immune system is the expression of toll-like- receptors that recognize LPS, flagellin, peptidoglycan, and lipoproteins of the bacterial cell wall predisposing to infection by *P. aeruginosa*.

The impact of F508del on favoring infection has not been clarified, and the mechanism is controversial. Some evidence suggests that it is due to unfolded protein response in combination with the infection and inflammation status in CF. The unfolded protein response is thought to be triggered by the accumulation of the dysfunctional CFTR protein in the cytoplasm after the endoplasmic reticulum retains the misfolded CFTR protein. [33].

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