Bacterial Host Interactions in Cystic Fibrosis

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Bacterial host interactions in cystic fibrosis
Máire Callaghan and Siobhán McClean

Chronic infection is a hallmark of cystic fibrosis (CF) and the main contributor to morbidity. Microbial infection in CF is complex, due to the number of different species that colonise the CF lung. Their colonisation is facilitated by a host response that is impaired or compromised by highly viscous mucous, zones of hypoxia and the lack of the cystic fibrosis transmembrane regulator (CFTR). Successful dominant CF pathogens combine an effective arsenal to establish infection and counter-attack the host response, together with an ability to adapt readily to an unfavourable environment. Hypermutability is common among CF pathogens facilitating adaptation and as the host response persists, progressive destruction of the normal architecture of lung tissue ensues with catastrophic consequences for the host.

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Introduction
Cystic fibrosis is caused by mutations in the CFTR gene leading to a disrupted chloride channel. It is well established that the greatest contributor to patient morbidity and mortality is chronic lung disease, caused by a constant cycle of infection and inflammation throughout the patient’s life (Figure 1). The CFTR mutation leads to defective regulation of chloride and sodium, resulting in increased water absorption, depletion of airway surface liquid (ASL) and dehydrated mucous [1]. Consequently, the purulent sputum and mucus plugs together with an ineffective inflammatory response, all contribute to the chronic infections that are central to CF lung disease.

From early childhood, CF patients experience recurrent pulmonary infections from a range of pathogens. In spite of intensive antibiotic therapy, certain organisms persist, leading to pulmonary exacerbations, hospitalizations and patient death (Box 1). These include Pseudomonas aeruginosa, Burkholderia cenocepacia complex (Bcc) and Achromobacter xylooxidans [2], with Bcc being the most problematic. It was recently demonstrated that chronic colonisation by Bcc resulted in a greater lung function decline than by the other two pathogens [3]. CF patients are also susceptible to colonisation by other pathogens, including Staphylococcus aureus (both Methicillin-resistant and sensitive), genus Pandoraea, Stenotrophomonas maltophilia and non-tuberculous Mycobacteria (NTM) [4–6] although the role of these latter four pathogens in CF lung disease is unclear. For example, a recent paper investigating 21 patients colonised with St. maltophilia without any other Gram negative organism, showed that there was a comparable decline in lung function in the two years after colonisation, to the three years before its identification [6]. Furthermore, the identification of high levels of anaerobic organisms in CF sputum [7] has added to the complex microbial population in the CF lung. These CF-associated anaerobes were not susceptible to antibiotics with known efficacy against anaerobes and the clinical significance of anaerobes in CF is not yet fully understood [8]. Limited information exists on the host–microbial interactions of many of these organisms and this review will focus only on the current understanding of the more clearly defined CF pathogens (Box 1).

Role of CFTR in CF lung colonisation
A direct role of the CFTR mutation in CF pathogenesis has been attributed to normal CFTR acting as a pathogen receptor involved in the internalisation and subsequent clearance of P. aeruginosa [9], but this mechanism is pathogen-specific. Mutated CFTR has also been attributed to be the cause of reduced internalisation of one Bcc species, Burkholderia dolosa, but not the more virulent Burkholderia cenocepacia in respiratory epithelial cells [10]. Furthermore, in contrast to P. aeruginosa, S. aureus was more invasive of CF cells compared to non-CF cells [11], indicating that CFTR is not the only route of bacterial uptake and invasion into the epithelium.

Alterations in the phenotype of CF airway epithelial cells also provide receptors for pathogens to adhere to. For example, CF airways show alterations in membrane glycoproteins and glycolipids which are directly linked to the CFTR defect (reviewed by [12]). The ratio of asialylated to sialylated glycolipids is higher in CF cells compared to non-CF cells, providing additional receptors for P. aeruginosa and Bcc. This is significant as invasion of lung epithelial cells by Bcc depends on asialylated glycolipids [13]. In addition, alterations in specific fucosyl residues of
The cycle of infection and inflammation combined with bacterial adaptation allows bacteria to chronically colonise the CF host.

Box 1 Examples of bacteria isolated from CF lung and their contribution to CF lung disease

Definite pathogen in CF
- P. aeruginosa
- Burkholderia cepacia complex (particularly B. cenocepacia)
- Methicillin resistant Staphylococcus aureus

Likely pathogen in CF
- Achromobacter xylosoxidans
- Non-tuberculous Mycobacteria

Unclear role in CF lung disease
- Genus Pandoraea
- Methicillin sensitive Staphylococcus aureus
- Inquilinus limosus
- Raistonia species
- Haemophilus influenzae
- Anaerobic bacteria (e.g. Prevotella sp., Bacteroides sp., Porphyromonas sp.)

Unlikely pathogen in CF
- Stenotrophomonas maltophilia

membrane glycopeptides of CF cells provide receptors for a fucose-specific P. aeruginosa lectin that is involved in P. aeruginosa pathogenicity [14]. Furthermore, increased glycosaminoglycan levels have been observed in CF airways as recently reviewed by Reeves et al. [15] and may contribute to CF lung disease. For example, increased heparan sulphate prolongs the efficacy of cytokines, such as interleukin (IL)-8, and also acts as a receptor for P. aeruginosa. Finally, it has recently been shown that CFTR deficiency in mice led to a reduction in acid sphingomyelinase activity, allowing ceramide accumulation in lung tissue [16**]. The elevated ceramide resulted in increased cell death and subsequent deposition of DNA in the respiratory tracts, providing sites for P. aeruginosa attachment [16]. This accumulation of ceramide was also observed in CF patients and the treatment of a CF patient with the acid sphingomyelinase inhibitor, amitriptyline resulted in a greater than 10% improvement in lung function [17].

The host immune response in the CF lung
The airway epithelium recognizes and responds to pathogens through the interaction between host pathogen recognition receptors and pathogen-associated membrane proteins. Toll like receptors (TLRs) such as TLR-4 on epithelial surfaces interact with lipopolysaccharide resulting in the activation of cytokine and anti-microbial peptide genes. TLR-2 signaling by P. aeruginosa has been shown to initiate cleavage of intracellular junctional
proteins to accommodate neutrophil transmigration [18]. The neutrophils while important in pathogen clearance, undergo necrosis resulting in DNA release and increased mucus viscosity, compounding the problem of bacterial attachment. Another aspect of host defense that is compromised in CF is macrophage clearance of pathogens. Recent evidence suggests that dysfunctional CFTR in macrophages impairs bacteriocidal activity against P. aeruginosa [19], but the specific role of CFTR in macrophage killing remains undefined and controversial. Many humoral components of immunity are also ineffective in CF. Host defense peptides (HDPs) are impaired due to the dehydrated mucus of the ASL [1] and secretion of proteases in the CF lung further compromises immune function. In particular, neutrophil elastase predominates and in addition to destroying a range of anti-microbial proteins, degrades mucin [20] and increases IL-8 secretion from epithelial cells [21]. This is significant because Bcc strains have enhanced growth in the presence of IL-8 [22]. Dysregulation of matrix metalloproteases (MMPs) in CF is another contributor to CF lung disease and to bacterial colonisation. Adult CF patients show elevated levels of serum MMP-1, MMP-8 and MMP-9 [23] and elevated MMP-9 in lower airway secretions [24]. Recently, we have shown that while P. aeruginosa predominantly activates MMP-2 in CF lung epithelial cells, B. cenocepacia infection activated MMP-9 only, the latter resulting in a delay in wound healing [25].

The host response in CF may be further affected by gender. Female CF patients have poorer survival rates than males. This has been attributed to estradiol reducing the ASL volume of CF epithelia in vitro [26]. More recently, treatment of P. aeruginosa-infected male CFTR-knockout mice with 17β-estradiol resulted in an increased secretion of pro-inflammatory-chemokines and chemoattractant-chemokines compared to controls [27], indicating that estrogens also have a direct effect on host response. Many of the elevated cytokines were Th17 mediators, which is significant in the context of the IL-23/IL-17 dependence of neutrophil recruitment during P. aeruginosa infection [28]. A 99% reduction in lactoferrin mRNA and a higher lung bacterial burden, was also observed in estrogen treated mice, relative to the control group. Many aspects of the host response in CF are therefore either impaired or dysregulated by chronic infection and may be further modulated by hormones, with potential significant consequences for the host.

Adaptation of bacterial pathogens to the CF lung

Adaptations of CF pathogens facilitate colonisation in the challenging host environment or the avoidance of host immune detection and antibiotic attack. The clinical impacts of many of these adaptations have been comprehensively reviewed by Hauser et al. [29**]. Hypermutable bacteria have an increased mutation rate of up to 1000-fold. It has been well established that hypermutable populations have been identified among CF pathogens, including P. aeruginosa, H. influenzae and S. aureus, contributing to their adaptability. This hypermutability of CF pathogens is illustrated by a study demonstrating that 68 genetic alterations accumulated in P. aeruginosa isolates from a chronically colonised CF patient over 8 years. Many of these genetic alterations were also identified in isolates from 29 other patients and were virulence genes associated with the initiation of infection which were selected against over time [30]. In a follow-up study, it was apparent that anti-microbial resistance genes were not over-represented among these hypermutated genes suggesting a more general adaptation to the CF lung [31] rather than to treatment regimens.

Phenotypic changes in P. aeruginosa during chronic infection have been well documented. P. aeruginosa isolated from patients with acute respiratory infection are generally non-encapsulated, expressing a variety of invasive virulence factors including flagella, type IV pili, multiple secreted toxins and degradative enzymes whereas P. aeruginosa isolates from chronically infected patients typically lack invasive virulence factors and convert to a mucoid phenotype during the establishment of chronic infection. This phenotype is associated with biofilm formation and resistance to phagocytosis. Phenotypic changes in Bcc isolates during the course of infection have also been described. In contrast to P. aeruginosa, Bcc changes from a mucoid to a non-mucoid phenotype during chronic colonisation and patients which were infected exclusively with non-mucoid Bcc had a more rapid decline in lung function than those infected with mucoid bacteria [32**]. Bcc mucoid isolates also lost expression of virulence factors and acquired a mutation in a quorum sensing (QS) gene during chronic infection [33]. Furthermore, proteomic analysis of clonal B. cenocepacia variants obtained during long-term colonisation showed an increase in peptidoglycan synthesis enzymes, iron uptake and chaperone proteins in later isolates, further demonstrating the adaptation of these organisms to the lung environment [34].

S. aureus can establish infection in the CF lung by adaptation using some of the strategies already discussed for P. aeruginosa. It also forms robust biofilms and ica expression is upregulated under hypoxic conditions, further promoting biofilm formation in oxygen deprived regions of the CF lung [35]. Small colony variants (SCV) of S. aureus frequently cultured from the CF lung are associated with higher rates of intracellular invasion [11]. There is also some evidence of hypermutable strains of S. aureus in CF and similar to other successful CF pathogens, this organism also downregulates certain virulence genes during chronic infection [36].
All three of these CF pathogens modulate virulence mechanisms during chronic infection, however the phenotypic changes that occur in these different organisms over time are very varied and the significance of many of these changes to the establishment of infection and to CF lung disease is not fully understood.

**Specific strategies to evade host response**

Bacterial invasion of the epithelium may provide an effective escape from the host response or antibiotic therapy for some pathogens or maybe a host clearance mechanism for others. Whatever the consequences of bacterial uptake by epithelial cells, phagocytosis by the immune cells direct the pathogen towards death by either oxidative or non-oxidative means. In addition to biofilm formation providing resistance to phagocytosis, additional strategies are also used to overcome this host response mechanism. *P. aeruginosa* can evade phagocytosis by becoming non-motile [37]. Similarly, *Staphylococcus aureus* forms polysaccharide encased cells under oxygen limited conditions which confer resistance to neutrophil killing [35]. Bcc strains are inherently resistant to non-oxidative killing [38] and some Bcc strains can also survive within macrophages by inhibiting phagosome–lysosome fusion [39].

Many CF pathogens disrupt epithelial integrity by opening the intracellular tight junctions, providing a possible mechanism for infiltration of pathogens within lung tissue and enabling access to receptors on the basolateral side of the epithelium, potentially provoking further inflammation. *P. aeruginosa* disrupts tight junctions relatively slowly as measured by a gradual drop in transepithelial electrical resistance and a concomitant decrease in the expression of the tight junction protein, ZO-1 within 24 h of infection [40]. By contrast, Bcc induces a more rapid disruption in epithelial integrity with a reduction in ZO-1 within 4 h, suggesting that different mechanisms are involved [41]. Certain *Pandoraea palonica* isolates also impair epithelial integrity within 4 h [4]. This difference in the rate of epithelial barrier disruption may be significant, since *P. aeruginosa* is not associated with septicemia in CF patients, while Bcc-associated septicemia has been widely reported.

**Quorum sensing facilitates adaptation**

Virulence of many pathogens is regulated by QS signaling systems that are dependent on bacterial cell density. Three QS systems operate in *P. aeruginosa*, las, rhl and pqs which function in an interconnecting network, facilitating co-operation and cheating within *P. aeruginosa* populations [42]. The virulence gene regulation of *P. aeruginosa* is well studied and many virulence determinants including motility and biofilm formation are QS dependent as reviewed recently [43,44]. QS signaling molecules also appear to have direct effects on host cells via calcium signaling. It was recently shown that the *P. aeruginosa* QS autoinducer molecule 3O-C12-HSL dramatically disrupted lung epithelial integrity by calcium-mediated alterations in tight junction protein interactions [45]. QS also regulates many *S. aureus* virulence genes as recently reviewed by Goerke and Wolz (2010) [36]. In particular, the agr QS system has a negative effect on *S. aureus* biofilm formation. During chronic infection, QS mutations occur to varying extents in CF pathogens. Although the diversity of QS deficient variants of *P. aeruginosa* in the CF lung is high [46], and agr *S. aureus* mutants are regularly isolated from CF lungs, QS mutations were infrequently observed among clinical *B. cenocepacia* isolates and those with QS mutations were growth impaired relative to wild type [47]. These findings suggest significant species differences in these regulatory networks and again highlight that CF pathogens implement different strategies to establish chronic infections.

**Variable oxygen concentrations and low iron**

Successful CF pathogens have adapted to survive in a range of oxygen concentrations found in the CF lung. *P. aeruginosa* grows maximally in a microaerobic environment and the presence of cyanide in CF sputum suggests that regions of the CF lung facilitate the optimal growth of *P. aeruginosa* under microaerophilic conditions [48]. *P. aeruginosa* also adapts well to anaerobic niches in the lung and *P. aeruginosa* CF isolates have increased transcription of genes involved in both denitrification and fermentation [49]. Bcc are also cyanogenic under biofilm and colonial growth conditions [50]. Therefore, both of these pathogenic species are capable of adapting to varied oxygen availability which is fundamental to their survival and proliferation in the CF lung.

In addition to oxygen, a key element for the survival of all pathogens is iron. Pathogens overcome the iron depleted environment in the lung, primarily by the production of iron chelating siderophores, transported across the membrane via specific receptors. Pyoverdine and pyochelin are both produced by *P. aeruginosa* and levels of intracellular iron in this species affect surface motility and biofilm maturation. Production of siderophores by Bcc are reviewed by Thomas (2007) [51]. Ornibactin is the most significant siderophore produced by Bcc organisms and has been implicated in their survival in a mouse model [52]. In addition to the production of siderophores, some CF pathogens can also use exogenous siderophores from other species to acquire iron. *P. aeruginosa*’s iron-regulated genes, respond to ornibactin from *Burkholderia* [53] and over thirty different siderophore receptors have been identified in *P. aeruginosa* strains as reviewed in [54] facilitating the uptake of exogenous siderophores. To counteract the iron scavenging mechanisms of invading pathogens, the host secretes the siderophore binding protein, siderocalin and phagocytes also acquire iron from some bacterial siderophores. However, recent evidence of
increased expression of siderophore receptors by clonal *B. cenocepacia* variants obtained during long-term colonisation [34] suggests an adaptive strategy by this pathogen to overcome any host response to limit iron availability.

**Adaptation to high concentrations of antibiotics**

Most CF pathogens are notoriously difficult to eliminate with antibiotic therapy. Many CF isolates are more resistant to antibiotics in the biofilm mode of growth [55,56]. Slow bacterial growth and reduced metabolic activity, in addition to the physical barrier of the biofilm matrix all contribute to this phenomenon. In addition to biofilm formation, CF pathogens also undergo mutations of antibiotic target sites and proactively eliminate antibiotics by enzymatic cleavage as in the case of β-lactam antibiotics or by efflux mechanisms. Four multidrug efflux systems are reported to play a role in the antibiotic resistance of *P. aeruginosa* CF isolates with the MexXY-OprM system playing the predominant role in aminoglycoside resistance [57]. A recent study of the genetic pathways involved in Bcc antibiotic resistance revealed that they were also multifactorial and include beta lactamases, novel efflux pumps, a phenylacetic acid degradative pathway and phosphohydrolases [58]. Both *P. aeruginosa* and Bcc therefore have an array of strategies which allow them to tolerate high doses of antibiotics delivered to the lung. The challenge in developing novel antimicrobial therapies is to identify new targets which can by-pass or overcome the array of bacterial resistance mechanisms.

**Future directions**

The key to understanding the complex interactions between host and pathogen is having relevant models for their study. However, the development of representative CF animal models has been challenging. Mouse models have been limited as CF-mice generally exhibit a normal lung physiology without mucous plug obstruction. More recently developed CF-pig and CF-ferret models demonstrated mucous gland secretions that resemble those of human CF [59,60]. The lungs of newborn CF-piglets showed evidence of airway obstruction and reduced eradication of instilled bacteria [61]. CF-ferrets showed a higher abundance of bacteria in two-day old animals, but this difference was not maintained in animals that died after one week [59]. Analysis of these models continues and it may be several years before they become effective tools for host-pathogen interaction studies. Until then, many microbial pathogenesis studies in CF will continue to be performed with immortalised lung epithelial cells, ideally in the more physiologically relevant polarised format. A more relevant approach, albeit of scarce supply, is using primary cultures from explanted lungs of CF patients at transplant. The difficulties with this model were recently highlighted by Broidie et al., where many cultures were overgrown by bacteria which colonised the patients [62]. Those successful bacteria-free cultures formed a polarised epithelium, expressing phenotypes of well differentiated CF primary cells, yet this differentiated phenotype did not extend beyond two passages [62], limiting their use for detailed investigation of host–microbial interactions.

Finally, further research is required into the host–microbial interactions of the less studied CF pathogens, for example, *A. xylodiscus, Pandoraea* and NTM, and more importantly, inter-bacterial interactions in the polymicrobial community that persists in the CF lung.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


   This paper presents a clear hypothesis regarding a link between the CFTR defect, mucus production and susceptibility of CF lung to colonisation by pathogens.


   This review describes the current epidemiology of respiratory tract infection in CF and the changes which have occurred in recent years. Important items of common and emerging pathogens are discussed and the factors that restrict the interpretation of data for monitoring microbial epidemiology in CF are also highlighted.


9. Schroeder TH, Lee MM, Yacono PW, Cannon CL, Gerecke AA, Golan DE, Pier GB: CFTR is a pattern recognition molecule that
Host-microbe interactions: bacteria


This paper provides a mechanism for the role of ceramide accumulation in CF chronic lung disease, and demonstrates that inhibition of acid sphingomyelinase with amitriptyline resulted in reduced P. aeruginosa colonization of mice.


This is an excellent comprehensive review on the adaptations that CF pathogens undergo while establishing a chronic infection and clearly links these to the relevant clinical data, highlighting the clinical impact of each adaptation.


This article and the subsequent one [33] challenged the previously held theory that mucoid Bcc isolates, like mucoid P. aeruginosa were associated with increased virulence. It clearly demonstrates that CF patients colonised exclusively with non-mucoid Bcc showed a more rapid decline in lung function. They also demonstrated that the phenotypic switch from mucoid to non-mucoid could be induced by antibiotics.


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