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Pseudomonas aeruginosa Biofilm Formation in the CF Lung and Its Implications for Therapy

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1. Introduction

Numerous microorganisms colonize or are associated with the airways of individuals with Cystic Fibrosis (CF). Impairment of the mucociliary clearance in CF lungs leads to a greater number of microbes present for the simple fact that they are not physically removed (Gibson, Burns et al. 2003; Boucher 2004). Microbes thrive in the large mucus plugs in CF airways, probably due to optimal growth temperatures and the abundance of nutrients. Additionally, CF patients display defective antimicrobial peptide activity in their lungs, which can further enhance microbial colonization (Gibson, Burns et al. 2003; Boucher 2004). As a result of these abnormalities, CF lungs are extraordinarily susceptible to infection with a number of bacteria, fungi, and viruses, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Haemophilus influenzae*, *Aspergillus fumigatus*, *Candida albicans*, Respiratory Syncytial virus, and Influenza Virus (Govan and Deretic 1996; Lyczak, Cannon et al. 2002; Saiman and Siegel 2004; Lipuma 2010). The relative abundance and rate of isolation of these various microorganisms varies over time. For instance, early in life, *S. aureus* is the most often isolated microbe, but by adolescence to young adulthood, *P. aeruginosa* becomes the predominate microorganism isolated from the airways (Gibson, Burns et al. 2003; Pressler, Bohmova et al. 2011).

P. aeruginosa is a Gram-negative bacterium that causes opportunistic infections. With a large number of genes involved in metabolism of many different substrates, as well as numerous regulatory genes, this bacterium has the genetic flexibility to colonize a wide range of different habitats (Stover, Pham et al. 2000; Yoon and Hassett 2004; Gomez and Prince 2007). Though typically considered an obligate aerobe, *P. aeruginosa* had been shown to undergo anaerobic respiration, in particular by denitrification processes utilizing nitrate, nitrite, or nitric oxide as terminal electron acceptors (Davies, Lloyd et al. 1989; Yoon and Hassett 2004). Furthermore, *P. aeruginosa* produces an arsenal of virulence factors, including pili, flagella, exopolysaccharides, proteases, elastase, lipases, iron chelators (pyoverdine and pyochelin), and a number of different toxins, including pyocyanin, hydrogen cyanide, exotoxin A, and the Type III Secretion System (T3SS) toxins ExoS, ExoT, ExoU, and ExoY (Lyczak, Cannon et al. 2000; Ran, Hassett et al. 2003; Shaver and Hauser 2004; Sadikot, Blackwell et al. 2005; Yahr and Wolfgang 2006; Gomez and Prince 2007). Utilizing these pathogenic tools, *P. aeruginosa* can infect a wide range of hosts, including animals (Rahme, Stevens et al. 1995), plants (Rahme, Stevens et al. 1995), insects (Miyata, Casey et al. 2003), nematodes (Gallagher

and Manoil 2001), and fungi (Hogan and Kolter 2002). In humans, in addition to CF lung infections, *P. aeruginosa* can cause acute pneumonia (especially in the context of ventilator-associated pneumonia), burn wound infections, ulcerative keratitis, otitis media, otitis externa, bacteremia, urinary tract infections, and meningitis (Lyczak, Cannon et al. 2000; Sadikot, Blackwell et al. 2005; Moore and Flaws 2011).

The unique pathogenic characteristics of *P. aeruginosa* also promote efficient infection in the CF lung. Studies suggest that 20%-25% of CF infants have had a positive *P. aeruginosa* culture in the United States, and infection rates steadily increase with increasing age, such that 80% of adults 25 years old and older are chronically infected with *P. aeruginosa* (Gibson, Burns et al. 2003; Stuart, Lin et al. 2010; Woodward, Brown et al. 2010). Initially, individuals with CF experience intermittent infection, wherein transient colonization is followed by *P. aeruginosa*-free periods (Hoiby, Frederiksen et al. 2005; Stuart, Lin et al. 2010). Clinically, intermittent infection has been defined as either 1) "at least 1 isolate of [*P. aeruginosa*] with normal [*P. aeruginosa*] antibody levels," or 2) "[*P. aeruginosa*] cultures were positive in 50% or less of the 12 months" (Stuart, Lin et al. 2010). Other definitions are possible. These initial, intermittent strains are thought to originate from the environment, but there have also been cases of epidemic strains, demonstrating the potential for direct or indirect person-to-person spread (Salunkhe, Smart et al. 2005; Lipuma 2010; Mowat, Paterson et al. 2011; Saiman 2011). Following the intermittent colonization period, *P. aeruginosa* eventually establishes chronic infection (Gibson, Burns et al. 2003; Pressler, Bohmova et al. 2011). One of the hallmark characteristics of chronic *P. aeruginosa* infection in the CF lung is a switch in the colony morphology of *P. aeruginosa* isolates from a non-mucoid to a mucoid phenotype (Gibson, Burns et al. 2003; Yoon and Hassett 2004). It has been estimated that this conversion to mucoidy takes approximately 1.8 years to occur (Stuart, Lin et al. 2010). Because of mucoidy and other changes (as described below), the bacteria in the chronic state survive the intense immune reaction that occurs in the CF lung as well as the high-dose antibiotic treatment given to CF patients to kill infecting microbes (Lyczak, Cannon et al. 2002; Gomez and Prince 2007). Because of their location within the mucus airway plugs, these mucoid bacteria are further protected from immune clearance (Worlitzsch, Tarran et al. 2002; Bjarnsholt, Jensen et al. 2009). Thus, once the chronic infection is established, *P. aeruginosa* persists for the life of the individual. This chronic colonization is the cause of much of the morbidity and mortality associated with CF (Gibson, Burns et al. 2003).

Chronic *P. aeruginosa* infection also contributes significantly to the economic burden associated with treatment and care for individuals with CF. Recent studies have calculated an average of $48,098 (US) in overall medical costs per CF patient per year in the United States, with similar estimates for some European countries (Ouyang, Grosse et al. 2009). Thus, with approximately 30,000 CF individuals in the United States (Gibson, Burns et al. 2003), CF accounts for over $1.4 billion (US) in medical expenditures in the United States alone. This calculation is a gross underestimate because it omits increased costs for transplantation, malnutrition, CF-associated diabetes, and other complications (Ouyang, Grosse et al. 2009). Additional analysis has estimated that the costs of treatment with the anti-Pseudomonal antibiotic tobramycin can reach $22,481 per person per year in the United States, which is nearly half of the aforementioned per person total expenditures ($48,098) (Woodward, Brown et al. 2010). It is evident, then, that development of more effective anti-Pseudomonal therapies might lead to decreased *P. aeruginosa* infection rates and decreased economic burden. Development of new drugs will come through a better understanding of the mechanisms used by *P. aeruginosa* to establish chronic infection.

2. Transition to the chronic infection phenotype

2.1 Biofilm formation

It is generally well accepted that the chronic nature of *P. aeruginosa* in the CF lung results from the association of the bacteria into organized structures called biofilms (Gibson, Burns et al. 2003; Gomez and Prince 2007). Biofilms are communities of microorganisms bound to a surface, or to each other. During biofilm formation, bacteria undergo phenotypic, and often genotypic, changes that lead to self-aggregation and transition to a lifestyle distinct from their free-swimming (planktonic) counterparts (Costerton, Lewandowski et al. 1995). Numerous infectious states involve a biofilm component, including *P. aeruginosa* infection in the CF lung, infectious kidney stones, bacterial endocarditis, otitis media, chronic prostatitis, urinary tract infections, periodontitis, and medical device-related infections (Costerton 2001; Donlan and Costerton 2002; Parsek and Singh 2003). Often, these biofilm infections are chronic and/or recurrent.

While the characteristics of biofilms vary depending upon microbial species and growth conditions, there are several general properties that can be used to describe and define biofilms (Figure 1). Focusing specifically on *P. aeruginosa*, biofilm formation is initiated as planktonic bacteria bind to a surface via their polar flagella (O'Toole and Kolter 1998; Sauer, Camper et al. 2002), although pili have also been shown to mediate attachment to cells and other surfaces (Woods, Straus et al. 1980; Chiang and Burrows 2003). At this point, the bacterium can spin in place as the flagellum continues to rotate (Sauer, Camper et al. 2002; Hinsa, Espinosa-Urgel et al. 2003; Caiazza and O'Toole 2004). However, this initial attachment is reversible because polarly-bound bacteria can detach and swim away from the site of initial attachment (Sauer, Camper et al. 2002; Hinsa, Espinosa-Urgel et al. 2003; Caiazza and O'Toole 2004; Monds, Newell et al. 2007). This initial reversible attachment stage has been referred to as a "sampling" of the surface before full commitment to biofilm formation has been made (Caiazza and O'Toole 2004). Full commitment to the biofilm mode of growth is signaled as the initially-bound *P. aeruginosa* rods lay down on the surface along their long axis (Sauer, Camper et al. 2002; Caiazza and O'Toole 2004). The bacteria become irreversibly bound at this point and remain on the surface (Stoodley, Sauer et al. 2002)(Figure 1: Inset). Next, through type IV pilus-mediated twitching motility, the individual bacteria begin to associate into structures called microcolonies (O'Toole, Kaplan et al. 2000; Sauer, Camper et al. 2002). Through the efforts of specific signaling molecules called quorum sensing signals (described below), and the resultant change in gene expression, these microcolonies mature into large structures, which can reach a thickness of 100 µM, depending upon the growth conditions (Sauer, Camper et al. 2002). During the maturation process, the constituent bacteria begin to excrete polysaccharides, such that the bacteria in the mature biofilm are encased in a matrix of exopolysaccharides that they produced (Costerton, Lewandowski et al. 1995; Ryder, Byrd et al. 2007). DNA and protein have also been shown to be components of *P. aeruginosa* biofilms (Parsek and Singh 2003; Bjarnsholt, Tolker-Nielsen et al. 2010). Finally, as the biofilm ages, a sub-population of the bacteria break away from the biofilm bulk, revert to the planktonic state (become motile), and disperse from the biofilm (O'Toole, Kaplan et al. 2000; Sauer, Camper et al. 2002; Kirov, Webb et al. 2007; Harmsen, Yang et al. 2010). Dispersion events appear to be influenced by the production of virulent bacteriophage from dormant prophage (Rice, Tan et al. 2009). While very few studies investigating *P. aeruginosa* biofilm formation on living tissue have been performed, it is thought that these steps are conserved during infection (Hoffmann, Rasmussen et al. 2005; Anderson, Moreau-Marquis et al. 2008; Moreau-Marquis, Bomberger et al. 2008; Woodworth, Tamashiro et al. 2008; Moreau-Marquis, Redelman et al. 2010)(Figure 1).

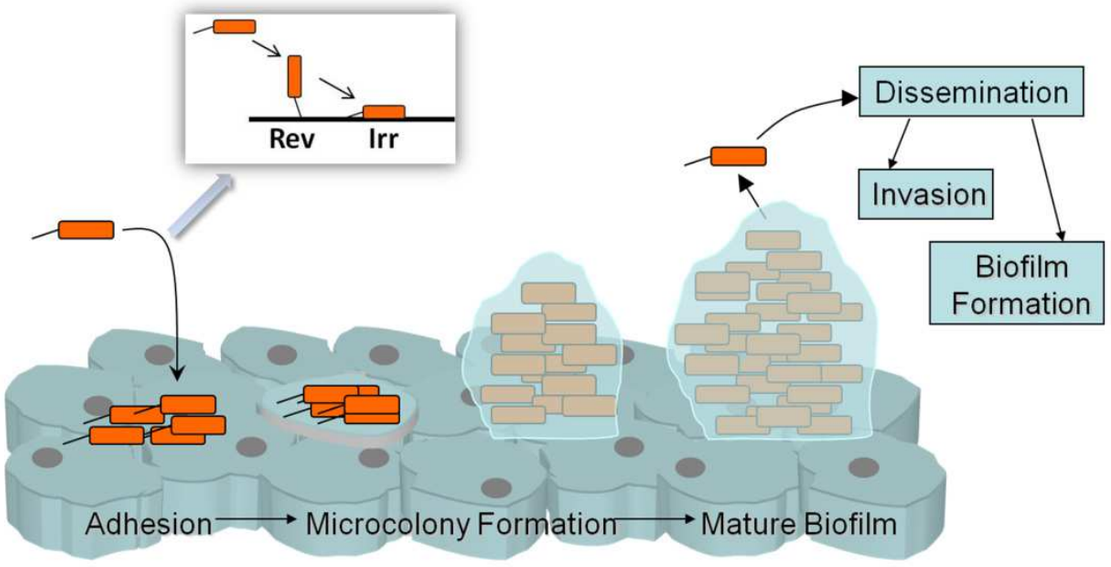

Fig. 1. Proposed biofilm formation cascade on living tissue. It is thought that many of the steps involved in bacterial biofilm formation on non-living surfaces also occur as bacteria form biofilms on human cells. Inset: Initially *P. aeruginosa* bind reversibly (Rev) by their flagellum. Irreversible binding (Irr) begins when the bacteria lay down on the surface along their long axis. See text for details.

2.2 Quorum sensing

As mentioned above, the production of quorum sensing (QS) molecules greatly influences biofilm maturation. QS is a method of self-recognition and cell density-dependent gene regulation (Cooley, Chhabra et al. 2008; Galloway, Hodgkinson et al. 2011). As a population of bacteria grows, small molecules (the QS signal) are produced and secreted. Once the density of the population is sufficiently high, the QS signal binds to an intracellular receptor that activates (or represses) a sub-set of genes (Galloway, Hodgkinson et al. 2011). Recognition of the QS signal is a stochastic process and the probability of a QS molecule binding to a receptor is low until the culture reaches a threshold density (Galloway, Hodgkinson et al. 2011). Hence, QS molecules allow bacteria to "sense the quorum", or the relative density of the population, and in this manner, they can coordinate their behaviors (Cooley, Chhabra et al. 2008). Because of the high bacterial density achieved in biofilms, QS plays a large role in regulating gene expression during biofilm development (Bjarnsholt, Tolker-Nielsen et al. 2010). *P. aeruginosa* contains 3 overlapping QS systems: Las, Rhl, and PQS (Wagner, Bushnell et al. 2003; Harmsen, Yang et al. 2010; Heeb, Fletcher et al. 2011). These systems regulate expression of virulence factors, exopolysaccharides, and other factors important for biofilm formation (Sauer, Camper et al. 2002; Wagner, Bushnell et al. 2003; Bjarnsholt, Tolker-Nielsen et al. 2010; Heeb, Fletcher et al. 2011). It has been found that QS systems are activated during biofilm maturation, and that mutation of QS genes leads to aberrations in overall biofilm architecture and sensitivity of the biofilm to stresses (Sauer, Camper et al. 2002; Bjarnsholt, Tolker-Nielsen et al. 2010; Harmsen, Yang et al. 2010; Heeb, Fletcher et al. 2011). As detailed below, much research is being devoted to the development of QS inhibitors for the disruption of biofilms.

2.3 The acute/chronic infection regulatory switch

There are numerous phenotypic changes that occur as planktonic *P. aeruginosa* transitions to the biofilm state, including a general decrease in expression of toxins and other tissue damaging virulence factors important for acute infections (Furukawa, Kuchma et al. 2006; Gooderham and Hancock 2009; Bjarnsholt, Tolker-Nielsen et al. 2010; Diaz, King et al. 2011). This suggests that *P. aeruginosa* displays 2 different infection phenotypes: an acute infection phenotype characterized by production of toxins, and a chronic infection phenotype characterized by biofilm formation and secretion of exopolysaccharides. In fact, recent evidence has revealed an inverse regulation of biofilm formation and virulence attributes associated with acute infections (Goodman, Kulasekara et al. 2004; Furukawa, Kuchma et al. 2006; Harmsen, Yang et al. 2010). For instance, expression of the AlgU alternative sigma factor leads to decreased expression of T3SS and increased production of the biofilm exopolysaccharide alginate (Wu, Badrane et al. 2004; Diaz, King et al. 2011). Similarly, the SadARS (also known as RocARS) three component regulatory system positively regulates biofilm maturation but inhibits the transcription of genes encoding components of the T3SS (Kuchma, Connolly et al. 2005; Kulasekara, Ventre et al. 2005). The LadS and GacS sensor proteins also enhance biofilm formation and exopolysaccharide production, but repress T3SS (Ventre, Goodman et al. 2006; Gooderham and Hancock 2009; Harmsen, Yang et al. 2010; Diaz, King et al. 2011). These sensors activate GacA, which, in addition to modulation of T3SS and exopolysaccharide production, can alter levels of pyocyanin, hydrogen cyanide, elastase, and lipase (Burrowes, Baysse et al. 2006; Gooderham and Hancock 2009). On the other hand, the regulatory protein RetS (also known as RtsM) inhibits the actions of GacA, and expression of RetS negatively influences exopolysaccharide production and biofilm formation but positively regulates T3SS gene expression and production of the toxins ToxA and LipA (Goodman, Kulasekara et al. 2004; Laskowski, Osborn et al. 2004). This alternate regulation of genes involved in acute toxicity and genes involved in biofilm formation suggests that during an infection, local environmental conditions might influence the infection phenotype of *P. aeruginosa*, producing an acute, toxic infection or a chronic, biofilm infection. Indeed, taking the human lung as an example, *P. aeruginosa* infection can lead to either acute pneumonia or, in the case of the CF lung, chronic colonization (Chastre and Fagon 2002; Furukawa, Kuchma et al. 2006). Depending upon the activity levels of the various acute and chronic regulators, a variety of intermediate bacterial phenotypes could occur on the acute to chronic spectrum. Investigation into this Acute/Chronic regulatory switch, and how it impacts human infections, is ongoing.

2.4 Evidence for biofilm formation in the CF lung

Numerous lines of evidence have confirmed that *P. aeruginosa* persists in CF lungs as biofilms. Perhaps most importantly, microscopic examinations of sputum samples and lung tissue sections have revealed the presence of microcolonies and large biofilm-like structures in the airways (Lam, Chan et al. 1980; Singh, Schaefer et al. 2000; Worlitzsch, Tarran et al. 2002; Bjarnsholt, Jensen et al. 2009; Hoiby, Ciofu et al. 2010; Hoiby, Ciofu et al. 2011). These biofilms can grow to larger than 100 µM in diameter (Worlitzsch, Tarran et al. 2002), and the bacteria within these biofilms have been identified as *P. aeruginosa* by fluorescent *in situ* hybridization (FISH) (Bjarnsholt, Jensen et al. 2009). Studies have suggested that in those individuals without sufficient antimicrobial therapy, these biofilms exist throughout the

lungs, whereas in those patients that have had aggressive antibiotic therapy, biofilms are confined to the conductive zone and are absent from the lower airways (Bjarnsholt, Jensen et al. 2009).

As further confirmation of biofilm formation in the CF lung, *P. aeruginosa* QS signaling molecules have been identified and characterized in CF patient sputum samples (Singh, Schaefer et al. 2000). Importantly, the ratios and relative proportion of the different QS molecules was similar to that of *P. aeruginosa* biofilms grown on abiotic surfaces. These data suggest that the bacteria within CF airways receive signals that induce them toward a chronic biofilm infection phenotype.

Indeed, *P. aeruginosa* isolates from CF airways display a number of characteristics indicative of biofilm formation. The conversion to mucoidy seen with chronically-infecting strains results from an overproduction of the biofilm exopolysaccharide alginate (Gibson, Burns et al. 2003; Ramsey and Wozniak 2005). Initially, steep hypoxic gradients in the mucus plugs of the CF airways stimulate the production of alginate (Worlitzsch, Tarran et al. 2002; Yoon and Hassett 2004). Over time, mutations in the gene *mucA*, encoding the membrane-localized anti-sigma factor MucA, result in constitutive expression of the alginate biosynthesis genes, through activation of the alternative sigma factor AlgU (Ohman and Chakrabarty 1981; Hughes and Mathee 1998; Hentzer, Teitzel et al. 2001; Ramsey and Wozniak 2005; Hoiby, Ciofu et al. 2010). It is thought that the constant oxidative stress encountered in the CF lung environment induces these mutations (Yoon and Hassett 2004; Hoiby, Ciofu et al. 2010).

CF lung isolates also accumulate mutations in T3SS genes (Dacheux, Attree et al. 2001; Jain, Ramirez et al. 2004; Smith, Buckley et al. 2006). Studies have shown an increasing number of T3SS defective isolates with increasing length of *P. aeruginosa* colonization (Jain, Ramirez et al. 2004). However, T3SS-competent bacteria have also been isolated from the lungs of CF patients (Dacheux, Toussaint et al. 2000; Jain, Ramirez et al. 2004; Jain, Bar-Meir et al. 2008), and it is possible that hyperactivation of AlgU might inhibit T3SS in these strains (Wu, Badrane et al. 2004; Diaz, King et al. 2011). Thus, both mutation and regulation appear to inhibit T3SS production in the CF lung during chronic infection, and this decrease in T3SS further supports the hypothesis that *P. aeruginosa* forms biofilms in the CF lung.

Intriguingly, many other mutations appear in genes involved in acute toxicity, including genes for lipopolysaccharide (LPS) biosynthesis, twitching motility, regulation of exotoxin A, pyoverdine synthesis, and QS factors (Smith, Buckley et al. 2006). A particular subset of chronic CF isolates, called small-colony variants (SCVs) due to their small colony morphology on agar plates, contains mutations in intracellular signaling proteins that lead to altered expression of polysaccharides, flagella, and type VI secretion (Starkey, Hickman et al. 2009). Thus, chronic CF *P. aeruginosa* isolates generally display a decrease in acute virulence. It is interesting to note that several studies have shown decreased virulence of chronic *P. aeruginosa* strains in mouse models of acute infection (Smith, Buckley et al. 2006; Bragonzi, Paroni et al. 2009). This adapted virulence of SCVs and other chronic CF isolates is thought to promote bacterial survival in the CF lung environment. T3SS toxins and other secreted factors are highly immunogenic, and mutation might protect the infecting bacteria from immune clearance. Furthermore, decreased bacterial toxicity would potentially inhibit the destruction of the biofilm habitat.

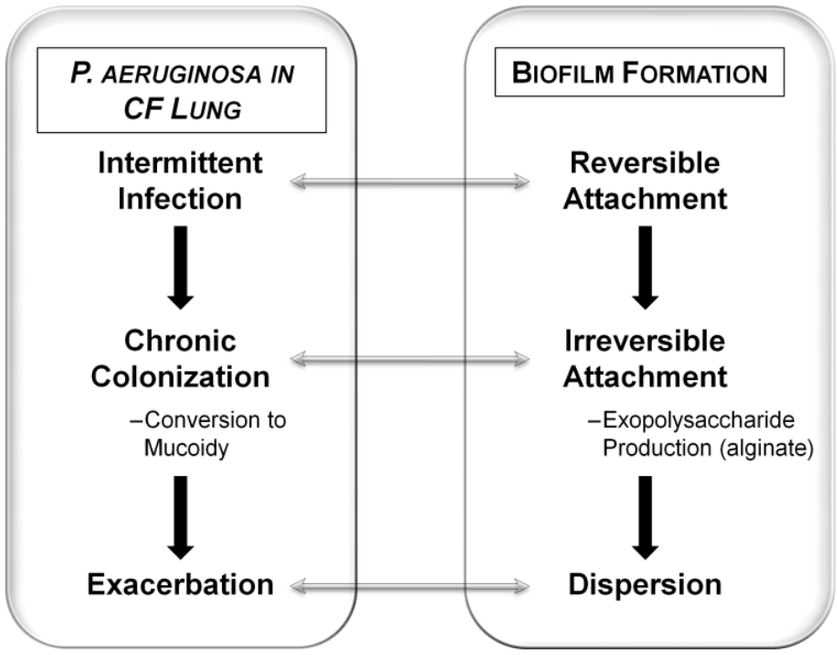

Fig. 2. Similarities between *P. aeruginosa* colonization of the CF lung and *in vitro P. aeruginosa* biofilm formation. The transition from reversible to irreversible attachment is mirrored in the transition from intermittent to chronic colonization. Similarly, just as bacteria in mature biofilms secrete exopolysaccharides, the conversion to mucoidy observed with CF isolates signals the overproduction of the exopolysaccharide alginate. Finally, it is possible that bacteria present during an exacerbation could represent bacteria that have dispersed from the biofilm and reverted to the planktonic phenotype expressing acute virulence factors.

Taken together, the presence of biofilm-like microcolonies and QS molecules, the increase in alginate production, and the decrease in T3SS and other acute infection phenotype factors strongly indicate that *P. aeruginosa* persists in the CF lung as biofilms. When comparing the biofilm formation cascade to the history of *P. aeruginosa* in an individual with CF, several intriguing parallels become apparent (Figure 2). Thus, the progression of intermittent infection, followed by chronic infection and conversion to mucoidy, is analogous to reversible binding, followed by irreversible binding and maturation of the *in vitro* biofilms, albeit chronic infection occurs on a much longer time scale than biofilms grown in the laboratory.

3. Consequences of biofilm formation in the CF lung

The formation of *P. aeruginosa* biofilms promotes chronic infection in the CF airways. As discussed in the following sections, several unique properties of biofilms contribute to this bacterial persistence, including antibiotic resistance, resistance to the activities of the immune system, and the high-frequency generation of bacterial mutants. Additionally, it has been found that *P. aeruginosa* contact with airway fluid leads to reduced flagella production (Wolfgang, Jyot et al. 2004), which potentially limits spread of the microorganism. Indeed, bacterial colonizers of the CF lung generally remain localized to the airways, and systemic

spread rarely occurs (Govan and Deretic 1996). The biofilms that result from the growth and accumulation of infecting bacteria confer several survival advantages.

3.1 Antibiotic resistance

Biofilm bacteria are more resistant to many stresses than their planktonic counterparts (Costerton, Stewart et al. 1999; Stewart and Costerton 2001; Donlan and Costerton 2002; Dunne 2002; Patel 2005). In fact, biofilm bacteria can display up to 1,000 fold greater antibiotic resistance than planktonic bacteria (Mah and O'Toole 2001). This increased antibiotic resistance is due to several factors, including reduced antibiotic diffusion through the biofilm exopolysaccharide matrix, reduced growth rates of biofilm bacteria, the development of dormant persister cells, and the production of specific antibiotic-resistance factors (Donlan and Costerton 2002; Mah, Pitts et al. 2003; Lewis 2005; del Pozo and Patel 2007; Anderson 2008). All of these factors appear to influence the antibiotic-resistant nature of *P. aeruginosa* biofilms. In particular, alginate has been shown to retard the movement of cationic antimicrobial peptides, quaternary ammonium compounds, and aminoglycosides (including tobramycin) through *P. aeruginosa* biofilms (Nichols, Dorrington et al. 1988; Campanac, Pineau et al. 2002; Chan, Burrows et al. 2005). Additionally, *P. aeruginosa* produces biofilm-specific antimicrobial inhibitors (Mah, Pitts et al. 2003). Antibiotic treatment of *P. aeruginosa* biofilms also stimulates increased production of resistance factors, such as β-lactamases and antibiotic efflux pumps (Whiteley, Bangera et al. 2001; Bagge, Schuster et al. 2004). Further compounding the problem of biofilm antibiotic resistance, chronic *P. aeruginosa* CF isolates can accumulate mutations in antibiotic resistance genes, resulting in increased expression and activity of the resistance factors. These mutations confer increased survival in the presence of particular antibiotics (Smith, Buckley et al. 2006). The combination of these activities enables *P. aeruginosa* biofilms to survive the intense, often daily, antibiotic treatment regime taken by individuals with CF.

3.2 Immune resistance

P. aeruginosa biofilms also persist despite the high level inflammatory reaction that occurs in the CF lung (Gibson, Burns et al. 2003; Boucher 2004). The biofilm matrix acts as a shield preventing opsonophagocytosis of biofilm bacteria (Worlitzsch, Tarran et al. 2002; Gibson, Burns et al. 2003; Parsek and Singh 2003). In sputum and lung samples, neutrophils have been seen surrounding *P. aeruginosa* biofilms, but they have rarely been observed within the biofilms (Bjarnsholt, Jensen et al. 2009; Hoiby, Ciofu et al. 2010). *P. aeruginosa* can also counteract the effects of harmful chemical species produced by immune cells. Alginate has been shown to protect biofilm bacteria from reactive oxygen species (Cochran, Suh et al. 2000; Battan, Barnes et al. 2004; Gomez and Prince 2007; Hoiby, Ciofu et al. 2010), and denitrification pathways expressed in *P. aeruginosa* can metabolize reactive nitrogen intermediates, such as nitric oxide (Davies, Lloyd et al. 1989; Yoon and Hassett 2004). Thus, neutrophils appear to be recruited to CF lung biofilms, but the bacteria are protected from attack. Of greatest concern, lysis of spent neutrophils has been shown to add to biofilm viscosity and volume due to the release of DNA and protein (Walker, Tomlin et al. 2005; Parks, Young et al. 2009).

Chronic biofilm growth might also inhibit immune recognition of *P. aeruginosa*. Reduced production of flagella, T3SS, and acute phase virulence factors in biofilms can lead to reduced antibody detection of these antigens (Adamo, Sokol et al. 2004; Jain, Ramirez et al. 2004; Wolfgang, Jyot et al. 2004; Smith, Buckley et al. 2006; Starkey, Hickman et al. 2009). Furthermore, some chronic *P. aeruginosa* isolates display a modified LPS, which can further contribute to immune evasion (Ernst, Yi et al. 1999).

3.3 The insurance hypothesis

Biofilm formation can also result in genetic diversity. In addition to mutations in virulence factors and antibiotic resistance factors, a host of other *P. aeruginosa* genes are mutated within biofilms (Boles, Thoendel et al. 2004; Smith, Buckley et al. 2006; Starkey, Hickman et al. 2009). It is thought that the constant stress encountered in the CF lung environment leads to DNA damage, and hence mutations. Several studies have found mutations in DNA mismatch repair genes in chronic CF *P. aeruginosa* isolates, which enhances the mutation rate (Smith, Buckley et al. 2006; Doring, Parameswaran et al. 2011). It has been suggested that this genetic diversification with *P. aeruginosa* biofilms supports the "Insurance Hypothesis", which states that diversity within a population provides protection for the community as a whole against a wide range of adverse or changing conditions (Boles, Thoendel et al. 2004). In other words, in the CF lung, mutation of individual *P. aeruginosa* cells within a biofilm will give rise to subpopulations with resistance to a wide range of different stresses and the ability to grow in a variety of different environments. In fact, it has been shown that genetic diversity within *P. aeruginosa* biofilms confers protection from oxidative stress (Boles, Thoendel et al. 2004). In this manner, genotypic changes, along with antibiotic resistance and immune evasion, promote *P. aeruginosa* survival and chronic infection in the CF lung.

3.4 Seed for recurring exacerbations?

Considering the presence of a large persistent population of *P. aeruginosa* in the lungs of individuals with CF, it is possible that biofilms serve as a reservoir of bacterial pathogens that emerge during a pulmonary exacerbation (VanDevanter and Van Dalfsen 2005). Indeed, during an exacerbation, lung function decreases while the symptoms of bacterial infection increase (VanDevanter, O'Riordan et al. 2010). Clinically, exacerbations have been defined as a sudden worsening of symptoms requiring physician intervention and the need for altered antibiotic treatment (Rogers, Hoffman et al. 2011), although some clinicians and researchers argue for more objective criteria (Bilton, Canny et al. 2011). This definition implies that bacterial activity is a large part of an exacerbation. However, the role of bacteria during an exacerbation remains a mystery. Some studies have shown that bacterial densities increase during an exacerbation (Mowat, Paterson et al. 2011), while others report similar bacterial levels before and during an exacerbation (Stressmann, Rogers et al. 2011). It has also been suggested that a virulent sub-population of bacteria emerge during an exacerbation, thus leading to symptoms of acute infection (Jaffar-Bandjee, Lazdunski et al. 1995; Stressmann, Rogers et al. 2011). In support of this hypothesis, researchers have found increased levels of *P. aeruginosa* exoenzyme S, exotoxin A, elastase, and alkaline protease in sputum samples during exacerbations (Grimwood, Semple et al. 1993; Jaffar-Bandjee, Lazdunski et al. 1995).

Moreover, it has been shown that high-dose antibiotic intervention for exacerbations decreases bacterial density and results in improved pulmonary symptoms, indication that bacterial activity plays a large role in initiation and progression of an exacerbation (Jaffar-Bandjee, Lazdunski et al. 1995; VanDevanter, O'Riordan et al. 2010; Tunney, Klem et al. 2011). Thus, during an exacerbation, it is possible that some fraction of the biofilm bacteria disperses from the biofilm and reverts to the acute planktonic phenotype, which will cause more tissue damage and lead to greater immune stimulation (Figure 2).

4. Treatment of *P. aeruginosa* infections of the CF lung: Triumphs and challenges

Treatment of *P. aeruginosa* lung infections remains challenging. The best course of action might be prevention of infection through aggressive infection control procedures. These procedures are meant to prevent person-to-person transmission as well as transmission from contaminated surfaces. It has been found that sputum-encased *P. aeruginosa* can survive on inanimate surfaces for up to 8 days (Saiman and Siegel 2004). Thus, thorough cleaning and sterilization of clinical rooms, apparatuses, and home respirators is recommended (Saiman and Siegel 2004; Saiman 2011). Furthermore, healthcare workers should practice good hand and respiratory hygiene (Saiman and Siegel 2004; Hoiby, Ciofu et al. 2011; Saiman 2011). Isolation and separation of individuals infected with particular pathogens, such as *P. aeruginosa* and multi-drug resistant bacteria, has also been suggested to reduce patient-to-patient spread. Many clinics also encourage re-gowning and re-gloving with each new patient contact. However, despite the best infection control protocols, most CF individuals still acquire *P. aeruginosa*, either from environmental sources or from other CF patients. As discussed below, there are a number of antimicrobial therapies implemented to control lung infection with *P. aeruginosa*.

4.1 Antibiotic treatments

Numerous antibiotics have been used to treat CF lung infection with *P. aeruginosa*, although the aminoglycoside antibiotic tobramycin has most often been used and has been studied the most (Gibson, Burns et al. 2003; Ryan, Singh et al. 2011). In order to achieve high concentration in the airways, tobramycin and other antibiotics are often inhaled in a nebulized form (Ryan, Singh et al. 2011). Studies have investigated the efficacy of a number of inhaled antibiotics, including tobramycin, colistin, gentamicin, ceftazidime, cephaloridine, aztreonam lysine, taurolidine, and a gentamicin/carbenicillin combination (Ryan, Singh et al. 2011). The use of inhaled antibiotics, can lead to increased lung function and decreased exacerbation frequency over placebo (Ryan, Singh et al. 2011). During stable periods, inhaled antibiotics such as tobramycin or colistin can be given as chronic suppressive therapies to maintain low bacterial levels within the airways (Hoiby, Ciofu et al. 2011). An economics study estimated that increased usage of inhaled tobramycin would lead to increased cost for medication but decreased physician and hospital visits. This would have a net decrease in healthcare costs (Woodward, Brown et al. 2010). It has also been suggested that this maintenance therapy be supplemented with 2-week courses of intravenous (IV) antibiotic combinations every 3 months for added anti-Pseudomonal pressure (Hoiby, Ciofu et al. 2011). In addition to antimicrobial therapies, other medications

such as DNase and hypertonic saline are widely used to increase airway clearance (Fuchs, Borowitz et al. 1994; Donaldson, Bennett et al. 2006; Elkins, Robinson et al. 2006; Parks, Young et al. 2009).

However, despite suppressive therapies, pulmonary exacerbations still occur. The types of antibiotics, dosing, and treatment schedules for exacerbation therapy vary greatly country-to-country and site-to-site. Synergy and a reduction in antibiotic resistance have been shown with thrice daily IV infusions of an aminoglycoside antibiotic and a β-lactam antibiotic (Bals, Hubert et al. 2011; Plummer and Wildman 2011). A recent study has found that twice daily treatments of tobramycin and ceftazidime are just as effective as thrice daily infusions, and this reduced treatment regimen can be safer and more convenient than a three times a day schedule. Studies have demonstrated that the bacterial response to antibiotic treatment is completed within 14 days (Adeboyeku, Jones et al. 2011), although in some cases, patients respond better to shorter or longer treatments (VanDevanter, O'Riordan et al. 2010; Plummer and Wildman 2011). Many more antibiotic treatment regimens are used in clinics and hospitals, and optimization of therapy for an individual exacerbation event often relies on symptoms and pulmonary function testing. Home-based IV antibiotic therapy of exacerbation has also been explored as an alternative to inpatient treatment. At-home therapy, while requiring specialized training for family members and friends, can reduce costs to families and hospitals, reduce incidence of hospital-acquired infections, improve disease manifestations, and can be more convenient for the affected individual (Balaguer and Gonzalez de Dios 2008).

4.2 Early colonization eradication

The period of intermittent infection, before the establishment of chronic *P. aeruginosa* biofilms, presents a unique opportunity for therapeutic intervention. Many studies have shown the efficacy of early aggressive antibiotic therapy to eradicate *P. aeruginosa* during this early colonization period. In the Copenhagen Model, which has been in place for over 20 years in the Copenhagen CF center, infected CF patients are given inhaled colistin and IV ciprofloxacin for 3 months (Hoiby, Frederiksen et al. 2005; Hansen, Pressler et al. 2008). 80% of patients treated with this regimen were free of chronic *P. aeruginosa* infection for up to 15 years, and the bacterial isolates recovered exhibited little resistance to colistin and ciprofloxacin (Hansen, Pressler et al. 2008). In a different study of other European CF centers, treatment with inhaled colistin and IV ciprofloxacin for 3 months was found to be 81% effective (Taccetti, Campana et al. 2005). In this study, treated patients were completely free from *P. aeruginosa* infection for an average of 18 months, and 73% of subsequent *P. aeruginosa* infections were found to involve a genotypically distinct strain, suggesting that the original isolate had been eradicated (Taccetti, Campana et al. 2005). This treatment was also associated with reduced overall treatment costs and little development of antibiotic resistance (Taccetti, Campana et al. 2005).

There are numerous variations on eradication therapies, and many studies evaluating the efficacy of these treatments (Stuart, Lin et al. 2010; Hayes, Feola et al. 2011). In an effort to develop standardized treatment guidelines for early eradication therapies, there have been 2 large, multicenter studies: the Early Inhaled Tobramycin for Eradication (ELITE) study in Europe, and the Early *Pseudomonas* Infection Control (EPIC) program in the United States. The

EPIC program, the results of which have yet to be published, is comparing standard culture-based therapy with twice daily inhaled tobramycin (300 mg) for 28 days every yearly quarter (Stuart, Lin et al. 2010; Hayes, Feola et al. 2011). The tobramycin-treatment group is further split into groups that additionally receive 14 days of either oral ciprofloxacin or oral placebo. The ELITE study treated participants for 28 days with twice daily inhaled tobramycin (300mg/5mL), and found that 93% of those treated were *P. aeruginosa*-free after 1 month (Ratjen, Munck et al. 2010). 66% of participants were free of *P. aeruginosa* infection for 2 years (Ratjen, Munck et al. 2010). Similar results were obtained with individuals treated for 56 days.

The early promise of eradication therapy studies demonstrates that these treatments will likely enhance overall patient health and reduce healthcare costs related to *P. aeruginosa* infection. Indeed, such early eradication protocols have dramatically increased the age at which chronic *P. aeruginosa* infection is established (Hoiby, Frederiksen et al. 2005; Hansen, Pressler et al. 2008). Furthermore, eradication can be achieved regardless of the age of the patient, provided there has been no evidence of prior *P. aeruginosa* infection (Hayes, Feola et al. 2011). However, even with constant monitoring and treatment, intermittent *P. aeruginosa* infection will eventually give way to chronic biofilm formation. It is well established that once chronic infection is initiated, eradication of *P. aeruginosa* from the CF lung is essentially impossible (Hoiby, Frederiksen et al. 2005; Hansen, Pressler et al. 2008).

4.3 Antibiotic resistance

Perhaps not surprisingly, treatment of CF lung infections with large doses of antibiotics leads to high levels of antibiotic resistance. Resistance of a CF lung isolate to a particular antibiotic is generally associated with treatment with that antibiotic (Emerson, McNamara et al. 2010). Greater resistance leads to longer therapies, which consequently induces more resistance (Plummer and Wildman 2011). The formation of *P. aeruginosa* biofilms plays a large role in the emergence of antibiotic resistance. In addition to the general resistance of biofilms and other stresses (Costerton, Stewart et al. 1999; Stewart and Costerton 2001; Donlan and Costerton 2002; Dunne 2002; Patel 2005), the high mutation rate and generation of diversity that occurs in *P. aeruginosa* biofilms results in variant strains that display increased resistance to antibiotics, through mutation of antibiotic targets and increased production and activity of multidrug efflux pumps (Smith, Buckley et al. 2006; Bals, Hubert et al. 2011; Mowat, Paterson et al. 2011). Moreover, the SCVs that develop in biofilms, and appear with increasing prevalence in CF patient sputum samples over time, are more innately resistant to a multitude of antibiotics (Starkey, Hickman et al. 2009; Bals, Hubert et al. 2011). The appearance of multidrug-resistant *P. aeruginosa* clones, which are associated with more severe lung disease and declining lung function (Plummer and Wildman 2011), has lead to an antibiotic dilemma. Novel antimicrobial strategies must be developed to combat these multidrug-resistant *P. aeruginosa* infections in the CF lung.

5. Hope for the future: Novel therapies and model systems

Recent investigations into anti-Pseudomonal treatments, with an eye toward inhibiting *P. aeruginosa* biofilm formation, have led to new drugs and novel implementation strategies of existing antimicrobials (Table 1). Likewise, advances in CF lung infection model systems are leading to new insights into the nature of chronic *P. aeruginosa* infection in the CF lung.

Strategy	References
Anti-biofilm Testing	
MBEC testing of current antibiotics	(Ceri, Olson et al. 1999; Keays, Ferris et al. 2009; Moskowitz, Emerson et al. 2011)
Quorum Sensing Inhibitors	
Furanones	(Steinberg, Schneider et al. 1997; Hentzer, Riedel et al. 2002)
Garlic extract	(Rasmussen and Givskov 2006; Harmsen, Yang et al. 2010)
Patulin	(Rasmussen, Skindersoe et al. 2005)
Penicillin acid	(Rasmussen, Skindersoe et al. 2005)
cis-2 decanoic acid	(Bjarnsholt, Tolker-Nielsen et al. 2010)
Salicylic acid	(Bjarnsholt, Tolker-Nielsen et al. 2010)
4-NPO	(Rasmussen and Givskov 2006; Bjarnsholt, Tolker-Nielsen et al. 2010)
Solenopsin A	(Bjarnsholt, Tolker-Nielsen et al. 2010)
Azithromycin	(Skindersoe, Alhede et al. 2008; Bjarnsholt, Tolker-Nielsen et al. 2010)
Clarithromycin	(Wozniak and Keyser 2004)
Ceftazidime	(Skindersoe, Alhede et al. 2008)
Ciprofloxacin	(Skindersoe, Alhede et al. 2008)
Inhaled Antibiotics	
Aztreonam lysine	(McCoy, Quittner et al. 2008; Retsch-Bogart, Burns et al. 2008; Parkins and Elborn 2010)
Levofloxacin	(Anderson 2010; Bals, Hubert et al. 2011)
Fosfomycin/Tobramycin	(Anderson 2010)
Tobramycin, inhalable powder	(Bals, Hubert et al. 2011)
Ciprofloxacin, inhalable powder	(Anderson 2010; Bals, Hubert et al. 2011)
Liposomally-encased Antibiotics	
Amikacin	(Anderson 2010; Bals, Hubert et al. 2011)
Tobramycin	(Bals, Hubert et al. 2011)
Polymyxin B	(Bals, Hubert et al. 2011)
New Antibiotics	
Tigecycline	(Parkins and Elborn 2010)
Doripenem	(Parkins and Elborn 2010)
Ceftibiprole	(Parkins and Elborn 2010)
Tomopenem	(Parkins and Elborn 2010)
CXA-101	(Parkins and Elborn 2010; Bals, Hubert et al. 2011)
NXL104/Ceftazidime	(Parkins and Elborn 2010)
ACHN-490	(Parkins and Elborn 2010)
CB182,804	(Parkins and Elborn 2010)
BLI-489/Pipericillin	(Parkins and Elborn 2010)
Disruption of Iron Metabolism	
Gallium	(Kaneko, Thoendel et al. 2007)
Desferasirox	(Moreau-Marquis, O'Toole et al. 2009)
Desferoxamine	(Moreau-Marquis, O'Toole et al. 2009)
Virulence Factor Modulation	
T3SS Inhibitors	(Aiello, Williams et al. 2010)
Vaccination	(Doring and Pier 2008)

Table 1. Novel and Emerging Therapies for *P. aeruginosa* Infection of CF Lungs

5.1 Novel therapies

5.1.1 Anti-biofilm strategies

Because biofilm formation plays an integral role in the persistence and antibiotic resistance of *P. aeruginosa* in the CF lung, many researchers have begun searching out ways to specifically destroy biofilms. The most obvious place to start in the development of anti-biofilm therapies is testing the efficacy of our current antibiotics against *P. aeruginosa* biofilms (Moskowitz, Emerson et al. 2011)(Table 1). In one retrospective analysis, the reported planktonic antibiotic susceptibilities of *P. aeruginosa* CF isolates were compared to the antibiotic susceptibilities of these strains grown as biofilms (Keays, Ferris et al. 2009). Those patients that were treated with antibiotics that could kill biofilm-state bacteria experienced lower treatment failure, decreased exacerbation risk, and decreased hospital stays (Keays, Ferris et al. 2009). Other studies have also shown that treatment tailored to biofilm susceptibility patterns can be effective (Moskowitz, Emerson et al. 2011). The recent development of the Minimum Biofilm Eradication Concentration (MBEC) Assay (also known as the Calgary Biofilm Device, distributed through Innovotech, Edmonton, CA), has permitted high throughput analysis of biofilm formation and biofilm susceptibilities of *P. aeruginosa* and other CF pathogens (Ceri, Olson et al. 1999; Tomlin, Malott et al. 2005; Davies, Harrison et al. 2007; Alfa and Howie 2009). Anti-biofilm therapies developed using the MBEC or other biofilm assays can thus be of great clinical benefit.

Another promising avenue of anti-biofilm research is the identification of molecules that interrupt QS signaling (Geske, Wezeman et al. 2005; Rasmussen and Givskov 2006; Galloway, Hodgkinson et al. 2011)(Table 1). By interfering with inter-bacterial communication and gene regulation, these compounds can lead to the dispersion of biofilm bacteria as well as alter virulence factor production. Generally, QS inhibitors fall into one of three categories: 1) those that block production of the QS signaling molecule, 2) those that degrade the QS molecule, and 3) those that prevent bacterial recognition of the QS signal (Rasmussen and Givskov 2006). Many large screens of natural compounds have been completed or are taking place to identify novel QS inhibitors, and several active compounds have emerged from these studies. For instance, halogenated furanones from the alga *Delisea pulchra* and synthetic furanones have been shown to block *P. aeruginosa* QS and biofilm formation, and they lead to increased *P. aeruginosa* killing when used in combination with traditional antibiotics (Steinberg, Schneider et al. 1997; Hentzer, Riedel et al. 2002; Bjarnsholt, Tolker-Nielsen et al. 2010). Likewise, garlic extract, patulin and penicillic acid from *Penicillium* species, *cis*-2-decanoic acid from *P. aeruginosa*, salicylic acid, 4-nitro-pyridine-N-oxide (4-NPO), and solenopsin A from fire ant venom have all demonstrated inhibition of *P. aeruginosa* QS, and some have shown direct biofilm disruption activity (Rasmussen, Skindersoe et al. 2005; Rasmussen and Givskov 2006; Bjarnsholt, Tolker-Nielsen et al. 2010; Harmsen, Yang et al. 2010). Some of these QS inhibitors, such as garlic extract, patulin, 4-NPO, and furanones, have also displayed a therapeutic effect in models of *P. aeruginosa* infection (Rasmussen and Givskov 2006). Importantly, it is thought that resistance to QS inhibitors will not develop because these compounds do not directly affect bacterial growth, and thus exert little selective pressure (Bjarnsholt, Tolker-Nielsen et al. 2010).

Intriguingly, it has been found that some traditional antibiotics can affect biofilm formation and virulence factor production through QS inhibition or other mechanisms. For instance, azithromycin, ceftazidime, and ciprofloxacin were all shown to affect *P. aeruginosa* QS and virulence factor production (Skindersoe, Alhede et al. 2008; Bjarnsholt, Tolker-Nielsen et al. 2010). Sub-inhibitory concentrations of clarithromycin can also alter biofilm morphology (Wozniak and Keyser 2004). Thus, treatment with these antibiotics has yielded unexpected consequences for *P. aeruginosa* infection.

5.1.2 Newer antimicrobial strategies

Development of traditional antibiotics, and novel delivery methods for antibiotics, has also yielded some successes (Table 1). Several new antibiotics have recently hit the markets in various countries, including tigecycline, doripenem, and the 5th generation cephalosporin ceftibiprole (Parkins and Elborn 2010). Many more antibiotics with potential efficacy against Gram-negative CF pathogens are in development, such as tomopenem, CXA-101, NXL104/ceftazidime, ACHN-490, CB182,804, and BLI-489/pipericillin (Parkins and Elborn 2010; Bals, Hubert et al. 2011). However, because many of these compounds are derivatives of existing antibiotics, resistance and toxicity might hinder further research on these novel therapies (Parkins and Elborn 2010).

On the other hand, the development of inhaled versions of existing antibiotics has been shown to improve delivery times and concentrate the antibiotic at the site of infection (Anderson 2010; Bals, Hubert et al. 2011; Ryan, Singh et al. 2011). Nebulized tobramycin has been used for years as an effective anti-Pseudomonal therapy, and many other inhaled antibiotic formulation have been studied (Ryan, Singh et al. 2011). Recently, Aztreonam Lysine for Inhalation has been approved in many countries for treatment of chronic CF lung infections, and studies have shown that use of this drug can improve quality of life and pulmonary function of CF patients, while decreasing *P. aeruginosa* burden and lower exacerbation severity (McCoy, Quittner et al. 2008; Retsch-Bogart, Burns et al. 2008; Anderson 2010; Parkins and Elborn 2010; Bals, Hubert et al. 2011). Work continues on other inhaled antibiotics, including aerosolized levofloxacin, fosfomycin/tobramycin, and inhalable dry powers of tobramycin and ciprofloxacin (Anderson 2010; Bals, Hubert et al. 2011). Inhalation of liposomally-encased antibiotics shows great promise for therapy of biofilm infections, as liposome delivery is thought to increase the penetration of biofilms (Smith 2005; Bals, Hubert et al. 2011). Patients treated with liposomally-encased amikacin showed improved lung function and reduction in sputum *P. aeruginosa* levels (Anderson 2010; Bals, Hubert et al. 2011). Similarly, liposomal encasement of tobramycin and polymyxin B might hold great promise as alternative treatments for chronic CF infections (Bals, Hubert et al. 2011).

Looking toward the future, there is great interest in identifying and developing novel chemical agents that disrupt bacterial metabolism, adhesins, virulence factor production, efflux pump activity, and bacterial intracellular signaling (Parkins and Elborn 2010; Bals, Hubert et al. 2011)(Table 1). Indeed, treatment with iron chelators (desferasirox and desferoxamine) or gallium, which is a non-reducible mimic for iron, can interfere with *P. aeruginosa* metabolism, resulting in biofilm disruption and protection against *P. aeruginosa* infection in animal models (Kaneko, Thoendel et al. 2007; DeLeon, Balldin et al. 2009; Moreau-Marquis, O'Toole et al. 2009). Inhibitors of *P. aeruginosa* T3SS have also been found

(Aiello, Williams et al. 2010). Further screening of chemical compound libraries will likely reveal many additional molecules with anti-Pseudomonal activity.

5.1.3 Vaccination

Vaccines against *P. aeruginosa* have also been proposed as a potential therapy for preventing chronic CF infections (Table 1). Researchers have explored vaccines against *P. aeruginosa* LPS, alginate, flagella, outer membrane proteins, pili, T3SS components, DNA, and killed whole bacteria (Doring and Pier 2008). Many of these vaccines have been tested in clinical trials, with moderate efficacy. It is thought that clearance and prevention of *P. aeruginosa* infection by aggressive early eradication programs masks the true effectiveness of these vaccines, and none of them have reached the market (Doring and Pier 2008). Passive immunotherapy with monoclonal antibodies or pooled immune serum has also been investigated for anti-Pseudomonal therapy (Doring and Pier 2008).

5.2 Novel model systems

5.2.1 Animal models

Researchers have tried for decades to develop an animal CF model that can maintain a chronic *P. aeruginosa* infection. Mice with various *CFTR* alleles and/or overexpression of βENaC have been tested as infection models, but *P. aeruginosa* is generally cleared from the lungs of these animals (Grubb and Boucher 1999; Mall, Grubb et al. 2004; Wilke, Buijs-Offerman et al. 2011; Zhou, Duerr et al. 2011). Mice and humans have very different lung physiologies, which most likely account for the inability to establish chronic infection in "CF" mice (Wilke, Buijs-Offerman et al. 2011). The recently developed pig and ferret CF animal models have been reported to develop spontaneous bacterial infections, and it is possible that chronic *P. aeruginosa* infection could be reproduced in these animals (Fisher, Zhang et al. 2011). However, these CF animals generally require surgery to correct the meconium ileus that develops in the young (Fisher, Zhang et al. 2011). Thus, the cost of these model systems is high.

5.2.2 Artificial sputum

In order to model the CF lung environment *in vitro*, several groups have created artificial sputum media. These media replicate the experimentally-determined composition of CF sputum samples, and they can support similar *P. aeruginosa* growth rates, gene expression patterns, nutritional preferences, and QS patterns as found in CF sputum (Sriramulu, Lunsdorf et al. 2005; Palmer, Aye et al. 2007). These media have also been useful for investigations of *P. aeruginosa* biofilm formation (Sriramulu, Lunsdorf et al. 2005; Garbe, Wesche et al. 2010). These models could lead to a better understanding of metabolic flux in *P. aeruginosa* biofilms in the context of CF lung infection.

5.2.3 Biofilms co-cultured with airway cells

Recently, several investigators have developed *P. aeruginosa* biofilms on cultured airway cells *in vitro* (Figure 3). These co-culture biofilm models were developed to more closely mimic the CF lung environment and potential signals that the bacteria receive from

mammalian cells during infection. In one model, *P. aeruginosa* bind to a monolayer or air-liquid interface differentiated layer of immortalized CF-derived (*CFTR* ΔF508 homozygous) bronchial epithelial cells (Moreau-Marquis, Bomberger et al. 2008; Moreau-Marquis, Redelman et al. 2010). Fresh culture medium then flows across the system and large bacterial aggregates form on the epithelial cells. The aggregates appear morphologically similar to biofilms formed on abiotic surfaces (Moreau-Marquis, Bomberger et al. 2008; Moreau-Marquis, Redelman et al. 2010). Furthermore, formation of these co-culture biofilms requires factors necessary for abiotic biofilm formation, and bacteria within co-culture biofilms display a pattern of gene expression consistent with that found in abiotic biofilms. In a different study, similar co-culture biofilms were formed by static incubation of *P. aeruginosa* and CF airway cells in the presence of arginine (Anderson, Moreau-Marquis et al. 2008; Moreau-Marquis, Redelman et al. 2010). Importantly, in both systems, the antibiotic resistance of the co-culture biofilms was greatly increased compared to both planktonic bacteria and abiotic biofilms (Anderson, Moreau-Marquis et al. 2008; Moreau-Marquis, Bomberger et al. 2008; Moreau-Marquis, O'Toole et al. 2009). It was also discovered that the co-culture biofilms displayed a different genetic response to tobramycin treatment than biofilms formed on plastic (Anderson, Moreau-Marquis et al. 2008). These data suggest that the surface upon which a biofilm forms can affect the properties of that biofilm, and they also offer clues into the high antibiotic resistance of *P. aeruginosa* biofilms that form in the CF lung. *P. aeruginosa* biofilms have also been shown to form on cultured mouse nasal septal epithelial cells (Woodworth, Tamashiro et al. 2008).

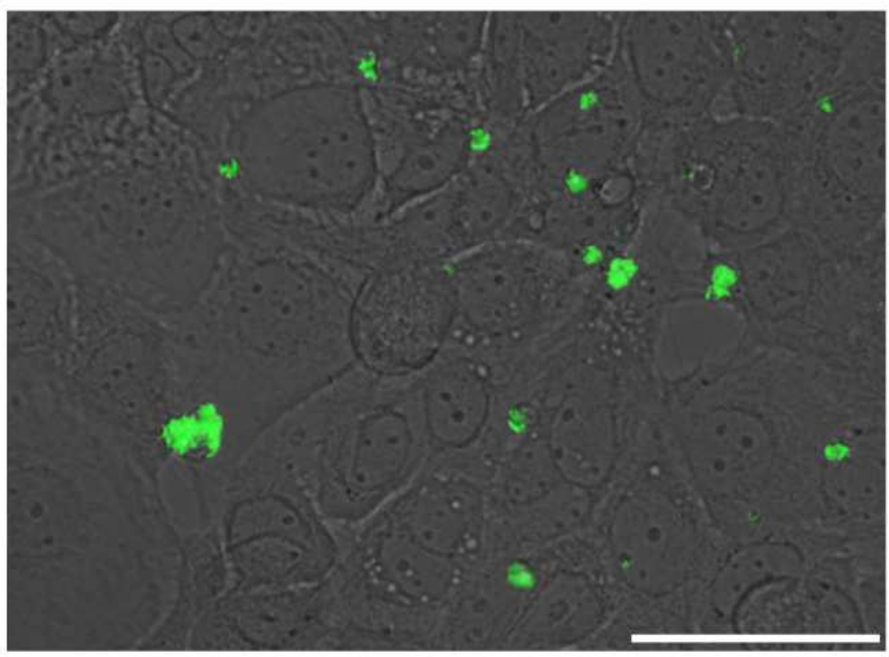

Fig. 3. *P. aeruginosa* biofilm microcolonies on cultured human CF-derived airway cells. Immortalized human bronchial epithelial cells, originally isolated from an individual with CF, were inoculated with *P. aeruginosa* constitutively expressing green fluorescent protein. Biofilm microcolonies (green) are attached to the surface of the cells. Bar = 50μm.

6. Conclusion

There have been a number of great advances in recent years in anti-Pseudomonal therapy of CF lung infections. In particular, early eradication treatments appear to show much promise in delaying the onset of chronic *P. aeruginosa* biofilm formation. The increasing arsenal against *P. aeruginosa*, including inhaled aztreonam and liposomal amikacin, will likely prove a benefit for *P. aeruginosa* treatment. Eradication of chronic *P. aeruginosa* may be possible, but it will take creative thinking. It is clear that new anti-biofilm treatments need to be discovered and implemented. The development of clinically-relevant models will further aid this process by providing appropriate systems for testing novel molecules. With renewed focus on the biofilm nature of the infection, much progress can be made toward eliminating chronic *P. aeruginosa* from the CF lung.

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8. References

Adamo, R., S. Sokol, G. Soong, M. I. Gomez and A. Prince (2004). Pseudomonas aeruginosa flagella activate airway epithelial cells through asialoGM1 and toll-like receptor 2 as well as toll-like receptor 5. *Am J Respir Cell Mol Biol* 30(5): 627-34.

Adeboyeku, D., A. L. Jones and M. E. Hodson (2011). Twice vs three-times daily antibiotics in the treatment of pulmonary exacerbations of cystic fibrosis. *J Cyst Fibros* 10(1): 25-30.

Aiello, D., J. D. Williams, H. Majgier-Baranowska, I. Patel, N. P. Peet, J. Huang, S. Lory, T. L. Bowlin and D. T. Moir (2010). Discovery and characterization of inhibitors of Pseudomonas aeruginosa type III secretion. *Antimicrob Agents Chemother* 54(5): 1988-99.

Alfa, M. J. and R. Howie (2009). Modeling microbial survival in buildup biofilm for complex medical devices. *BMC Infect Dis* 9: 56.

Anderson, G. G., S. Moreau-Marquis, B. A. Stanton and G. A. O'Toole (2008). In vitro analysis of tobramycin-treated Pseudomonas aeruginosa biofilms on cystic fibrosis-derived airway epithelial cells. *Infect Immun* 76(4): 1423-33.

Anderson, G. G., O'Toole. G. A. (2008). Innate and Induced Resistance Mechanisms of Bacterial Biofilms. *Bacterial Biofilms*. T. Romeo. Berlin, Springer-Verlag. 322: 85-105.

Anderson, P. (2010). Emerging therapies in cystic fibrosis. *Ther Adv Respir Dis* 4(3): 177-85.

Bagge, N., M. Schuster, M. Hentzer, O. Ciofu, M. Givskov, E. P. Greenberg and N. Hoiby (2004). Pseudomonas aeruginosa biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. *Antimicrob Agents Chemother* 48(4): 1175-87.

Balaguer, A. and J. Gonzalez de Dios (2008). Home intravenous antibiotics for cystic fibrosis. *Cochrane Database Syst Rev*(3): CD001917.

Bals, R., D. Hubert and B. Tummler (2011). Antibiotic treatment of CF lung disease: from bench to bedside. *J Cyst Fibros* 10 Suppl 2: S146-51.

Battan, P. C., A. I. Barnes and I. Albesa (2004). Resistance to oxidative stress caused by ceftazidime and piperacillin in a biofilm of Pseudomonas. *Luminescence* 19(5): 265-70.

Bilton, D., G. Canny, S. Conway, S. Dumcius, L. Hjelte, M. Proesmans, B. Tummler, V. Vavrova and K. De Boeck (2011). Pulmonary exacerbation: towards a definition for use in clinical trials. Report from the EuroCareCF Working Group on outcome parameters in clinical trials. *J Cyst Fibros* 10 Suppl 2: S79-81.

Bjarnsholt, T., P. O. Jensen, M. J. Fiandaca, J. Pedersen, C. R. Hansen, C. B. Andersen, T. Pressler, M. Givskov and N. Hoiby (2009). Pseudomonas aeruginosa biofilms in the respiratory tract of cystic fibrosis patients. *Pediatr Pulmonol* 44(6): 547-58.

Bjarnsholt, T., T. Tolker-Nielsen, N. Hoiby and M. Givskov (2010). Interference of Pseudomonas aeruginosa signalling and biofilm formation for infection control. *Expert Rev Mol Med* 12: e11.

Boles, B. R., M. Thoendel and P. K. Singh (2004). Self-generated diversity produces "insurance effects" in biofilm communities. *Proc Natl Acad Sci U S A* 101(47): 16630-5.

Boucher, R. C. (2004). New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur Respir J* 23(1): 146-58.

Bragonzi, A., M. Paroni, A. Nonis, N. Cramer, S. Montanari, J. Rejman, C. Di Serio, G. Doring and B. Tummler (2009). Pseudomonas aeruginosa microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am J Respir Crit Care Med* 180(2): 138-45.

Burrowes, E., C. Baysse, C. Adams and F. O'Gara (2006). Influence of the regulatory protein RsmA on cellular functions in Pseudomonas aeruginosa PAO1, as revealed by transcriptome analysis. *Microbiology* 152(Pt 2): 405-18.

Caiazza, N. C. and G. A. O'Toole (2004). SadB is required for the transition from reversible to irreversible attachment during biofilm formation by Pseudomonas aeruginosa PA14. *J Bacteriol* 186(14): 4476-85.

Campanac, C., L. Pineau, A. Payard, G. Baziard-Mouysset and C. Roques (2002). Interactions between biocide cationic agents and bacterial biofilms. *Antimicrob Agents Chemother* 46(5): 1469-74.

Ceri, H., M. E. Olson, C. Stremick, R. R. Read, D. Morck and A. Buret (1999). The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol* 37(6): 1771-6.

Chan, C., L. L. Burrows and C. M. Deber (2005). Alginate as an auxiliary bacterial membrane: binding of membrane-active peptides by polysaccharides. *J Pept Res* 65(3): 343-51.

Chastre, J. and J. Y. Fagon (2002). Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 165(7): 867-903.

Chiang, P. and L. L. Burrows (2003). Biofilm formation by hyperpiliated mutants of Pseudomonas aeruginosa. *J Bacteriol* 185(7): 2374-8.

Cochran, W. L., S. J. Suh, G. A. McFeters and P. S. Stewart (2000). Role of RpoS and AlgT in Pseudomonas aeruginosa biofilm resistance to hydrogen peroxide and monochloramine. *J Appl Microbiol* 88(3): 546-53.

Cooley, M., S. R. Chhabra and P. Williams (2008). N-Acylhomoserine lactone-mediated quorum sensing: a twist in the tail and a blow for host immunity. *Chem Biol* 15(11): 1141-7.

Costerton, J. W. (2001). Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. *Trends Microbiol* 9(2): 50-2.

Costerton, J. W., Z. Lewandowski, D. E. Caldwell, D. R. Korber and H. M. Lappin-Scott (1995). Microbial biofilms. *Annu Rev Microbiol* 49: 711-45.

Costerton, J. W., P. S. Stewart and E. P. Greenberg (1999). Bacterial biofilms: a common cause of persistent infections. *Science* 284(5418): 1318-22.

Dacheux, D., I. Attree and B. Toussaint (2001). Expression of ExsA in trans confers type III secretion system-dependent cytotoxicity on noncytotoxic Pseudomonas aeruginosa cystic fibrosis isolates. *Infect Immun* 69(1): 538-42.

Dacheux, D., B. Toussaint, M. Richard, G. Brochier, J. Croize and I. Attree (2000). Pseudomonas aeruginosa cystic fibrosis isolates induce rapid, type III secretion-dependent, but ExoU-independent, oncosis of macrophages and polymorphonuclear neutrophils. *Infect Immun* 68(5): 2916-24.

Davies, J. A., J. J. Harrison, L. L. Marques, G. R. Foglia, C. A. Stremick, D. G. Storey, R. J. Turner, M. E. Olson and H. Ceri (2007). The GacS sensor kinase controls phenotypic reversion of small colony variants isolated from biofilms of Pseudomonas aeruginosa PA14. *FEMS Microbiol Ecol* 59(1): 32-46.

Davies, K. J., D. Lloyd and L. Boddy (1989). The effect of oxygen on denitrification in Paracoccus denitrificans and Pseudomonas aeruginosa. *J Gen Microbiol* 135(9): 2445-51.

del Pozo, J. L. and R. Patel (2007). The challenge of treating biofilm-associated bacterial infections. *Clin Pharmacol Ther* 82(2): 204-9.

DeLeon, K., F. Balldin, C. Watters, A. Hamood, J. Griswold, S. Sreedharan and K. P. Rumbaugh (2009). Gallium maltolate treatment eradicates Pseudomonas aeruginosa infection in thermally injured mice. *Antimicrob Agents Chemother* 53(4): 1331-7.

Diaz, M. R., J. M. King and T. L. Yahr (2011). Intrinsic and extrinsic regulation of type III secretion gene expression in Pseudomonas aeruginosa. *Frontiers in Microbiology* 2.

Donaldson, S. H., W. D. Bennett, K. L. Zeman, M. R. Knowles, R. Tarran and R. C. Boucher (2006). Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 354(3): 241-50.

Donlan, R. M. and J. W. Costerton (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 15(2): 167-93.

Doring, G., I. G. Parameswaran and T. F. Murphy (2011). Differential adaptation of microbial pathogens to airways of patients with cystic fibrosis and chronic obstructive pulmonary disease. *FEMS Microbiol Rev* 35(1): 124-46.

Doring, G. and G. B. Pier (2008). Vaccines and immunotherapy against Pseudomonas aeruginosa. *Vaccine* 26(8): 1011-24.

Dunne, W. M., Jr. (2002). Bacterial adhesion: seen any good biofilms lately? *Clin Microbiol Rev* 15(2): 155-66.

Elkins, M. R., M. Robinson, B. R. Rose, C. Harbour, C. P. Moriarty, G. B. Marks, E. G. Belousova, W. Xuan and P. T. Bye (2006). A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 354(3): 229-40.

Emerson, J., S. McNamara, A. M. Buccat, K. Worrell and J. L. Burns (2010). Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. *Pediatr Pulmonol* 45(4): 363-70.

Ernst, R. K., E. C. Yi, L. Guo, K. B. Lim, J. L. Burns, M. Hackett and S. I. Miller (1999). Specific lipopolysaccharide found in cystic fibrosis airway Pseudomonas aeruginosa. *Science* 286(5444): 1561-5.

Fisher, J. T., Y. Zhang and J. F. Engelhardt (2011). Comparative biology of cystic fibrosis animal models. *Methods Mol Biol* 742: 311-34.

Fuchs, H. J., D. S. Borowitz, D. H. Christiansen, E. M. Morris, M. L. Nash, B. W. Ramsey, B. J. Rosenstein, A. L. Smith and M. E. Wohl (1994). Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N Engl J Med* 331(10): 637-42.

Furukawa, S., S. L. Kuchma and G. A. O'Toole (2006). Keeping their options open: acute versus persistent infections. *J Bacteriol* 188(4): 1211-7.

Gallagher, L. A. and C. Manoil (2001). Pseudomonas aeruginosa PAO1 kills Caenorhabditis elegans by cyanide poisoning. *J Bacteriol* 183(21): 6207-14.

Galloway, W. R., J. T. Hodgkinson, S. D. Bowden, M. Welch and D. R. Spring (2011). Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chem Rev* 111(1): 28-67.

Garbe, J., A. Wesche, B. Bunk, M. Kazmierczak, K. Selezska, C. Rohde, J. Sikorski, M. Rohde, D. Jahn and M. Schobert (2010). Characterization of JG024, a pseudomonas aeruginosa PB1-like broad host range phage under simulated infection conditions. *BMC Microbiol* 10: 301.

Geske, G. D., R. J. Wezeman, A. P. Siegel and H. E. Blackwell (2005). Small molecule inhibitors of bacterial quorum sensing and biofilm formation. *J Am Chem Soc* 127(37): 12762-3.

Gibson, R. L., J. L. Burns and B. W. Ramsey (2003). Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 168(8): 918-51.

Gomez, M. I. and A. Prince (2007). Opportunistic infections in lung disease: Pseudomonas infections in cystic fibrosis. *Curr Opin Pharmacol* 7(3): 244-51.

Gooderham, W. J. and R. E. Hancock (2009). Regulation of virulence and antibiotic resistance by two-component regulatory systems in Pseudomonas aeruginosa. *FEMS Microbiol Rev* 33(2): 279-94.

Goodman, A. L., B. Kulasekara, A. Rietsch, D. Boyd, R. S. Smith and S. Lory (2004). A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in Pseudomonas aeruginosa. *Dev Cell* 7(5): 745-54.

Govan, J. R. and V. Deretic (1996). Microbial pathogenesis in cystic fibrosis: mucoid Pseudomonas aeruginosa and Burkholderia cepacia. *Microbiol Rev* 60(3): 539-74.

Grimwood, K., R. A. Semple, H. R. Rabin, P. A. Sokol and D. E. Woods (1993). Elevated exoenzyme expression by Pseudomonas aeruginosa is correlated with exacerbations of lung disease in cystic fibrosis. *Pediatr Pulmonol* 15(3): 135-9.

Grubb, B. R. and R. C. Boucher (1999). Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiol Rev* 79(1 Suppl): S193-214.

Hansen, C. R., T. Pressler and N. Hoiby (2008). Early aggressive eradication therapy for intermittent Pseudomonas aeruginosa airway colonization in cystic fibrosis patients: 15 years experience. *J Cyst Fibros* 7(6): 523-30.

Harmsen, M., L. Yang, S. J. Pamp and T. Tolker-Nielsen (2010). An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. *FEMS Immunol Med Microbiol* 59(3): 253-68.

Hayes, D., Jr., D. J. Feola, B. S. Murphy, R. J. Kuhn and G. A. Davis (2011). Eradication of Pseudomonas aeruginosa in an adult patient with cystic fibrosis. *Am J Health Syst Pharm* 68(4): 319-22.

Heeb, S., M. P. Fletcher, S. R. Chhabra, S. P. Diggle, P. Williams and M. Camara (2011). Quinolones: from antibiotics to autoinducers. *FEMS Microbiol Rev* 35(2): 247-74.

Hentzer, M., K. Riedel, T. B. Rasmussen, A. Heydorn, J. B. Andersen, M. R. Parsek, S. A. Rice, L. Eberl, S. Molin, N. Hoiby, S. Kjelleberg and M. Givskov (2002). Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. *Microbiology* 148(Pt 1): 87-102.

Hentzer, M., G. M. Teitzel, G. J. Balzer, A. Heydorn, S. Molin, M. Givskov and M. R. Parsek (2001). Alginate overproduction affects Pseudomonas aeruginosa biofilm structure and function. *J Bacteriol* 183(18): 5395-401.

Hinsa, S. M., M. Espinosa-Urgel, J. L. Ramos and G. A. O'Toole (2003). Transition from reversible to irreversible attachment during biofilm formation by Pseudomonas fluorescens WCS365 requires an ABC transporter and a large secreted protein. *Mol Microbiol* 49(4): 905-18.

Hoffmann, N., T. B. Rasmussen, P. O. Jensen, C. Stub, M. Hentzer, S. Molin, O. Ciofu, M. Givskov, H. K. Johansen and N. Hoiby (2005). Novel mouse model of chronic Pseudomonas aeruginosa lung infection mimicking cystic fibrosis. *Infect Immun* 73(4): 2504-14.

Hogan, D. A. and R. Kolter (2002). Pseudomonas-Candida interactions: an ecological role for virulence factors. *Science* 296(5576): 2229-32.

Hoiby, N., O. Ciofu and T. Bjarnsholt (2010). Pseudomonas aeruginosa biofilms in cystic fibrosis. *Future Microbiol* 5(11): 1663-74.

Hoiby, N., O. Ciofu, H. K. Johansen, Z. J. Song, C. Moser, P. O. Jensen, S. Molin, M. Givskov, T. Tolker-Nielsen and T. Bjarnsholt (2011). The clinical impact of bacterial biofilms. *Int J Oral Sci* 3(2): 55-65.

Hoiby, N., B. Frederiksen and T. Pressler (2005). Eradication of early Pseudomonas aeruginosa infection. *J Cyst Fibros* 4 Suppl 2: 49-54.

Hughes, K. T. and K. Mathee (1998). The anti-sigma factors. *Annu Rev Microbiol* 52: 231-86.

Jaffar-Bandjee, M. C., A. Lazdunski, M. Bally, J. Carrere, J. P. Chazalette and C. Galabert (1995). Production of elastase, exotoxin A, and alkaline protease in sputa during pulmonary exacerbation of cystic fibrosis in patients chronically infected by Pseudomonas aeruginosa. *J Clin Microbiol* 33(4): 924-9.

Jain, M., M. Bar-Meir, S. McColley, J. Cullina, E. Potter, C. Powers, M. Prickett, R. Seshadri, B. Jovanovic, A. Petrocheilou, J. D. King and A. R. Hauser (2008). Evolution of Pseudomonas aeruginosa type III secretion in cystic fibrosis: a paradigm of chronic infection. *Transl Res* 152(6): 257-64.

Jain, M., D. Ramirez, R. Seshadri, J. F. Cullina, C. A. Powers, G. S. Schulert, M. Bar-Meir, C. L. Sullivan, S. A. McColley and A. R. Hauser (2004). Type III secretion phenotypes

of Pseudomonas aeruginosa strains change during infection of individuals with cystic fibrosis. *J Clin Microbiol* 42(11): 5229-37.

Kaneko, Y., M. Thoendel, O. Olakanmi, B. E. Britigan and P. K. Singh (2007). The transition metal gallium disrupts Pseudomonas aeruginosa iron metabolism and has antimicrobial and antibiofilm activity. *J Clin Invest* 117(4): 877-88.

Keays, T., W. Ferris, K. L. Vandemheen, F. Chan, S. W. Yeung, T. F. Mah, K. Ramotar, R. Saginur and S. D. Aaron (2009). A retrospective analysis of biofilm antibiotic susceptibility testing: a better predictor of clinical response in cystic fibrosis exacerbations. *J Cyst Fibros* 8(2): 122-7.

Kirov, S. M., J. S. Webb, Y. O'May C, D. W. Reid, J. K. Woo, S. A. Rice and S. Kjelleberg (2007). Biofilm differentiation and dispersal in mucoid Pseudomonas aeruginosa isolates from patients with cystic fibrosis. *Microbiology* 153(Pt 10): 3264-74.

Kuchma, S. L., J. P. Connolly and G. A. O'Toole (2005). A three-component regulatory system regulates biofilm maturation and type III secretion in Pseudomonas aeruginosa. *J Bacteriol* 187(4): 1441-54.

Kulasekara, H. D., I. Ventre, B. R. Kulasekara, A. Lazdunski, A. Filloux and S. Lory (2005). A novel two-component system controls the expression of Pseudomonas aeruginosa fimbrial cup genes. *Mol Microbiol* 55(2): 368-80.

Lam, J., R. Chan, K. Lam and J. W. Costerton (1980). Production of mucoid microcolonies by Pseudomonas aeruginosa within infected lungs in cystic fibrosis. *Infect Immun* 28(2): 546-56.

Laskowski, M. A., E. Osborn and B. I. Kazmierczak (2004). A novel sensor kinase-response regulator hybrid regulates type III secretion and is required for virulence in Pseudomonas aeruginosa. *Mol Microbiol* 54(4): 1090-103.

Lewis, K. (2005). Persister cells and the riddle of biofilm survival. *Biochemistry (Mosc)* 70(2): 267-74.

Lipuma, J. J. (2010). The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 23(2): 299-323.

Lyczak, J. B., C. L. Cannon and G. B. Pier (2000). Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. *Microbes Infect* 2(9): 1051-60.

Lyczak, J. B., C. L. Cannon and G. B. Pier (2002). Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 15(2): 194-222.

Mah, T. F. and G. A. O'Toole (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 9(1): 34-9.

Mah, T. F., B. Pitts, B. Pellock, G. C. Walker, P. S. Stewart and G. A. O'Toole (2003). A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. *Nature* 426(6964): 306-10.

Mall, M., B. R. Grubb, J. R. Harkema, W. K. O'Neal and R. C. Boucher (2004). Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med* 10(5): 487-93.

McCoy, K. S., A. L. Quittner, C. M. Oermann, R. L. Gibson, G. Z. Retsch-Bogart and A. B. Montgomery (2008). Inhaled aztreonam lysine for chronic airway Pseudomonas aeruginosa in cystic fibrosis. *Am J Respir Crit Care Med* 178(9): 921-8.

Miyata, S., M. Casey, D. W. Frank, F. M. Ausubel and E. Drenkard (2003). Use of the Galleria mellonella caterpillar as a model host to study the role of the type III secretion system in Pseudomonas aeruginosa pathogenesis. *Infect Immun* 71(5): 2404-13.

Monds, R. D., P. D. Newell, R. H. Gross and G. A. O'Toole (2007). Phosphate-dependent modulation of c-di-GMP levels regulates Pseudomonas fluorescens Pf0-1 biofilm formation by controlling secretion of the adhesin LapA. *Mol Microbiol* 63(3): 656-79.

Moore, N. M. and M. L. Flaws (2011). Epidemiology and pathogenesis of Pseudomonas aeruginosa infections. *Clin Lab Sci* 24(1): 43-6.

Moreau-Marquis, S., J. M. Bomberger, G. G. Anderson, A. Swiatecka-Urban, S. Ye, G. A. O'Toole and B. A. Stanton (2008). The {Delta}F508-CFTR mutation results in increased biofilm formation by Pseudomonas aeruginosa by increasing iron availability. *Am J Physiol Lung Cell Mol Physiol* 295(1): L25-37.

Moreau-Marquis, S., G. A. O'Toole and B. A. Stanton (2009). Tobramycin and FDA-approved iron chelators eliminate Pseudomonas aeruginosa biofilms on cystic fibrosis cells. *Am J Respir Cell Mol Biol* 41(3): 305-13.

Moreau-Marquis, S., C. V. Redelman, B. A. Stanton and G. G. Anderson (2010). Co-culture Models of Pseudomonas aeruginosa Biofilms Grown on Live Human Airway Cells. *J Vis Exp*(44): e2186.

Moskowitz, S. M., J. C. Emerson, S. McNamara, R. D. Shell, D. M. Orenstein, D. Rosenbluth, M. F. Katz, R. Ahrens, D. Hornick, P. M. Joseph, R. L. Gibson, M. L. Aitken, W. W. Benton and J. L. Burns (2011). Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. *Pediatr Pulmonol* 46(2): 184-92.

Mowat, E., S. Paterson, J. L. Fothergill, E. A. Wright, M. J. Ledson, M. J. Walshaw, M. A. Brockhurst and C. Winstanley (2011). Pseudomonas aeruginosa Population Diversity and Turnover in Cystic Fibrosis Chronic Infections. *Am J Respir Crit Care Med* 183(12): 1674-9.

Nichols, W. W., S. M. Dorrington, M. P. Slack and H. L. Walmsley (1988). Inhibition of tobramycin diffusion by binding to alginate. *Antimicrob Agents Chemother* 32(4): 518-23.

O'Toole, G., H. B. Kaplan and R. Kolter (2000). Biofilm formation as microbial development. *Annu Rev Microbiol* 54: 49-79.

O'Toole, G. A. and R. Kolter (1998). Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. *Mol Microbiol* 30(2): 295-304.

Ohman, D. E. and A. M. Chakrabarty (1981). Genetic mapping of chromosomal determinants for the production of the exopolysaccharide alginate in a Pseudomonas aeruginosa cystic fibrosis isolate. *Infect Immun* 33(1): 142-8.

Ouyang, L., S. D. Grosse, D. D. Amendah and M. S. Schechter (2009). Healthcare expenditures for privately insured people with cystic fibrosis. *Pediatr Pulmonol* 44(10): 989-96.

Palmer, K. L., L. M. Aye and M. Whiteley (2007). Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum. *J Bacteriol* 189(22): 8079-87.

Parkins, M. D. and J. S. Elborn (2010). Aztreonam lysine: a novel inhalational antibiotic for cystic fibrosis. *Expert Rev Respir Med* 4(4): 435-44.

Parkins, M. D. and J. S. Elborn (2010). Newer antibacterial agents and their potential role in cystic fibrosis pulmonary exacerbation management. *J Antimicrob Chemother* 65(9): 1853-61.

Parks, Q. M., R. L. Young, K. R. Poch, K. C. Malcolm, M. L. Vasil and J. A. Nick (2009). Neutrophil enhancement of Pseudomonas aeruginosa biofilm development: human F-actin and DNA as targets for therapy. *J Med Microbiol* 58(Pt 4): 492-502.

Parsek, M. R. and P. K. Singh (2003). Bacterial biofilms: an emerging link to disease pathogenesis. *Annu Rev Microbiol* 57: 677-701.

Patel, R. (2005). Biofilms and antimicrobial resistance. *Clin Orthop Relat Res* 437: 41-7.

Plummer, A. and M. Wildman (2011). Duration of intravenous antibiotic therapy in people with cystic fibrosis. *Cochrane Database Syst Rev*(1): CD006682.

Pressler, T., C. Bohmova, S. Conway, S. Dumcius, L. Hjelte, N. Hoiby, H. Kollberg, B. Tummler and V. Vavrova (2011). Chronic Pseudomonas aeruginosa infection definition: EuroCareCF Working Group report. *J Cyst Fibros* 10 Suppl 2: S75-8.

Rahme, L. G., E. J. Stevens, S. F. Wolfort, J. Shao, R. G. Tompkins and F. M. Ausubel (1995). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268(5219): 1899-902.

Ramsey, D. M. and D. J. Wozniak (2005). Understanding the control of Pseudomonas aeruginosa alginate synthesis and the prospects for management of chronic infections in cystic fibrosis. *Mol Microbiol* 56(2): 309-22.

Ran, H., D. J. Hassett and G. W. Lau (2003). Human targets of Pseudomonas aeruginosa pyocyanin. *Proc Natl Acad Sci U S A* 100(24): 14315-20.

Rasmussen, T. B. and M. Givskov (2006). Quorum-sensing inhibitors as anti-pathogenic drugs. *Int J Med Microbiol* 296(2-3): 149-61.

Rasmussen, T. B., M. E. Skindersoe, T. Bjarnsholt, R. K. Phipps, K. B. Christensen, P. O. Jensen, J. B. Andersen, B. Koch, T. O. Larsen, M. Hentzer, L. Eberl, N. Hoiby and M. Givskov (2005). Identity and effects of quorum-sensing inhibitors produced by Penicillium species. *Microbiology* 151(Pt 5): 1325-40.

Ratjen, F., A. Munck, P. Kho and G. Angyalosi (2010). Treatment of early Pseudomonas aeruginosa infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 65(4): 286-91.

Retsch-Bogart, G. Z., J. L. Burns, K. L. Otto, T. G. Liou, K. McCoy, C. Oermann and R. L. Gibson (2008). A phase 2 study of aztreonam lysine for inhalation to treat patients with cystic fibrosis and Pseudomonas aeruginosa infection. *Pediatr Pulmonol* 43(1): 47-58.

Rice, S. A., C. H. Tan, P. J. Mikkelsen, V. Kung, J. Woo, M. Tay, A. Hauser, D. McDougald, J. S. Webb and S. Kjelleberg (2009). The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. *ISME J* 3(3): 271-82.

Rogers, G. B., L. R. Hoffman, M. W. Johnson, N. Mayer-Hamblett, J. Schwarze, M. P. Carroll and K. D. Bruce (2011). Using bacterial biomarkers to identify early indicators of cystic fibrosis pulmonary exacerbation onset. *Expert Rev Mol Diagn* 11(2): 197-206.

Ryan, G., M. Singh and K. Dwan (2011). Inhaled antibiotics for long-term therapy in cystic fibrosis. *Cochrane Database Syst Rev*(3): CD001021.

Ryder, C., M. Byrd and D. J. Wozniak (2007). Role of polysaccharides in Pseudomonas aeruginosa biofilm development. *Curr Opin Microbiol* 10(6): 644-8.

Sadikot, R. T., T. S. Blackwell, J. W. Christman and A. S. Prince (2005). Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. *Am J Respir Crit Care Med* 171(11): 1209-23.

Saiman, L. (2011). Infection prevention and control in cystic fibrosis. *Curr Opin Infect Dis* 24(4): 390-5.

Saiman, L. and J. Siegel (2004). Infection control in cystic fibrosis. *Clin Microbiol Rev* 17(1): 57-71.

Salunkhe, P., C. H. Smart, J. A. Morgan, S. Panagea, M. J. Walshaw, C. A. Hart, R. Geffers, B. Tummler and C. Winstanley (2005). A cystic fibrosis epidemic strain of Pseudomonas aeruginosa displays enhanced virulence and antimicrobial resistance. *J Bacteriol* 187(14): 4908-20.

Sauer, K., A. K. Camper, G. D. Ehrlich, J. W. Costerton and D. G. Davies (2002). Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. *J Bacteriol* 184(4): 1140-54.

Shaver, C. M. and A. R. Hauser (2004). Relative contributions of Pseudomonas aeruginosa ExoU, ExoS, and ExoT to virulence in the lung. *Infect Immun* 72(12): 6969-77.

Singh, P. K., A. L. Schaefer, M. R. Parsek, T. O. Moninger, M. J. Welsh and E. P. Greenberg (2000). Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 407(6805): 762-4.

Skindersoe, M. E., M. Alhede, R. Phipps, L. Yang, P. O. Jensen, T. B. Rasmussen, T. Bjarnsholt, T. Tolker-Nielsen, N. Hoiby and M. Givskov (2008). Effects of antibiotics on quorum sensing in Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 52(10): 3648-63.

Smith, A. W. (2005). Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? *Adv Drug Deliv Rev* 57(10): 1539-50.

Smith, E. E., D. G. Buckley, Z. Wu, C. Saenphimmachak, L. R. Hoffman, D. A. D'Argenio, S. I. Miller, B. W. Ramsey, D. P. Speert, S. M. Moskowitz, J. L. Burns, R. Kaul and M. V. Olson (2006). Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. *Proc Natl Acad Sci U S A* 103(22): 8487-92.

Sriramulu, D. D., H. Lunsdorf, J. S. Lam and U. Romling (2005). Microcolony formation: a novel biofilm model of Pseudomonas aeruginosa for the cystic fibrosis lung. *J Med Microbiol* 54(Pt 7): 667-76.

Starkey, M., J. H. Hickman, L. Ma, N. Zhang, S. De Long, A. Hinz, S. Palacios, C. Manoil, M. J. Kirisits, T. D. Starner, D. J. Wozniak, C. S. Harwood and M. R. Parsek (2009). Pseudomonas aeruginosa rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *J Bacteriol* 191(11): 3492-503.

Steinberg, P. D., R. Schneider and S. Kjelleberg (1997). Chemical defenses of seaweeds against microbial colonization. *Biodegradation* 8(3): 211-220.

Stewart, P. S. and J. W. Costerton (2001). Antibiotic resistance of bacteria in biofilms. *Lancet* 358(9276): 135-8.

Stoodley, P., K. Sauer, D. G. Davies and J. W. Costerton (2002). Biofilms as complex differentiated communities. *Annu Rev Microbiol* 56: 187-209.

Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. Hancock, S. Lory and M. V. Olson (2000). Complete

genome sequence of Pseudomonas aeruginosa PA01, an opportunistic pathogen. *Nature* 406(6799): 959-64.

Stressmann, F. A., G. B. Rogers, P. Marsh, A. K. Lilley, T. W. Daniels, M. P. Carroll, L. R. Hoffman, G. Jones, C. E. Allen, N. Patel, B. Forbes, A. Tuck and K. D. Bruce (2011). Does bacterial density in cystic fibrosis sputum increase prior to pulmonary exacerbation? *J Cyst Fibros*.

Stuart, B., J. H. Lin and P. J. Mogayzel, Jr. (2010). Early eradication of Pseudomonas aeruginosa in patients with cystic fibrosis. *Paediatr Respir Rev* 11(3): 177-84.

Taccetti, G., S. Campana, F. Festini, M. Mascherini and G. Doring (2005). Early eradication therapy against Pseudomonas aeruginosa in cystic fibrosis patients. *Eur Respir J* 26(3): 458-61.

Tomlin, K. L., R. J. Malott, G. Ramage, D. G. Storey, P. A. Sokol and H. Ceri (2005). Quorum-sensing mutations affect attachment and stability of Burkholderia cenocepacia biofilms. *Appl Environ Microbiol* 71(9): 5208-18.

Tunney, M. M., E. R. Klem, A. A. Fodor, D. F. Gilpin, T. F. Moriarty, S. J. McGrath, M. S. Muhlebach, R. C. Boucher, C. Cardwell, G. Doering, J. S. Elborn and M. C. Wolfgang (2011). Use of culture and molecular analysis to determine the effect of antibiotic treatment on microbial community diversity and abundance during exacerbation in patients with cystic fibrosis. *Thorax* 66(7): 579-84.

VanDevanter, D. R., M. A. O'Riordan, J. L. Blumer and M. W. Konstan (2010). Assessing time to pulmonary function benefit following antibiotic treatment of acute cystic fibrosis exacerbations. *Respir Res* 11: 137.

VanDevanter, D. R. and J. M. Van Dalfsen (2005). How much do Pseudomonas biofilms contribute to symptoms of pulmonary exacerbation in cystic fibrosis? *Pediatr Pulmonol* 39(6): 504-6.

Ventre, I., A. L. Goodman, I. Vallet-Gely, P. Vasseur, C. Soscia, S. Molin, S. Bleves, A. Lazdunski, S. Lory and A. Filloux (2006). Multiple sensors control reciprocal expression of Pseudomonas aeruginosa regulatory RNA and virulence genes. *Proc Natl Acad Sci U S A* 103(1): 171-6.

Wagner, V. E., D. Bushnell, L. Passador, A. I. Brooks and B. H. Iglewski (2003). Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment. *J Bacteriol* 185(7): 2080-95.

Walker, T. S., K. L. Tomlin, G. S. Worthen, K. R. Poch, J. G. Lieber, M. T. Saavedra, M. B. Fessler, K. C. Malcolm, M. L. Vasil and J. A. Nick (2005). Enhanced Pseudomonas aeruginosa biofilm development mediated by human neutrophils. *Infect Immun* 73(6): 3693-701.

Whiteley, M., M. G. Bangera, R. E. Bumgarner, M. R. Parsek, G. M. Teitzel, S. Lory and E. P. Greenberg (2001). Gene expression in Pseudomonas aeruginosa biofilms. *Nature* 413(6858): 860-4.

Wilke, M., R. M. Buijs-Offerman, J. Aarbiou, W. H. Colledge, D. N. Sheppard, L. Touqui, A. Bot, H. Jorna, H. R. de Jonge and B. J. Scholte (2011). Mouse models of cystic fibrosis: phenotypic analysis and research applications. *J Cyst Fibros* 10 Suppl 2: S152-71.

Wolfgang, M. C., J. Jyot, A. L. Goodman, R. Ramphal and S. Lory (2004). Pseudomonas aeruginosa regulates flagellin expression as part of a global response to airway fluid from cystic fibrosis patients. *Proc Natl Acad Sci U S A* 101(17): 6664-8.

Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry and J. A. Bass (1980). Role of pili in adherence of Pseudomonas aeruginosa to mammalian buccal epithelial cells. *Infect. Immun.* 29(3): 1146-1151.

Woodward, T. C., R. Brown, P. Sacco and J. Zhang (2010). Budget impact model of tobramycin inhalation solution for treatment of Pseudomonas aeruginosa in cystic fibrosis patients. *J Med Econ* 13(3): 492-9.

Woodworth, B. A., E. Tamashiro, G. Bhargave, N. A. Cohen and J. N. Palmer (2008). An in vitro model of Pseudomonas aeruginosa biofilms on viable airway epithelial cell monolayers. *Am J Rhinol* 22(3): 235-8.

Worlitzsch, D., R. Tarran, M. Ulrich, U. Schwab, A. Cekici, K. C. Meyer, P. Birrer, G. Bellon, J. Berger, T. Weiss, K. Botzenhart, J. R. Yankaskas, S. Randell, R. C. Boucher and G. Doring (2002). Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. *J Clin Invest* 109(3): 317-25.

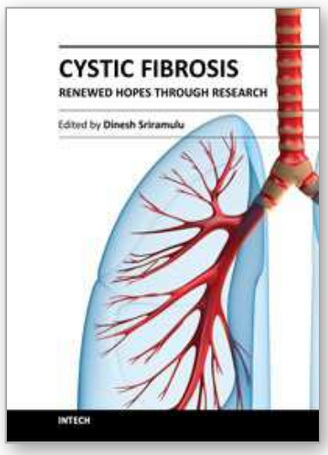
Wozniak, D. J. and R. Keyser (2004). Effects of subinhibitory concentrations of macrolide antibiotics on Pseudomonas aeruginosa. *Chest* 125(2 Suppl): 62S-69S; quiz 69S.

Wu, W., H. Badrane, S. Arora, H. V. Baker and S. Jin (2004). MucA-mediated coordination of type III secretion and alginate synthesis in Pseudomonas aeruginosa. *J Bacteriol* 186(22): 7575-85.

Yahr, T. L. and M. C. Wolfgang (2006). Transcriptional regulation of the Pseudomonas aeruginosa type III secretion system. *Mol Microbiol* 62(3): 631-40.

Yoon, S. S. and D. J. Hassett (2004). Chronic Pseudomonas aeruginosa infection in cystic fibrosis airway disease: metabolic changes that unravel novel drug targets. *Expert Rev Anti Infect Ther* 2(4): 611-23.

Zhou, Z., J. Duerr, B. Johannesson, S. C. Schubert, D. Treis, M. Harm, S. Y. Graeber, A. Dalpke, C. Schultz and M. A. Mall (2011). The ENaC-overexpressing mouse as a model of cystic fibrosis lung disease. *J Cyst Fibros* 10 Suppl 2: S172-82.



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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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