Persistent and aggressive bacteria in the lungs of cystic fibrosis children

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There have been enormous improvements in life expectancy of patients with cystic fibrosis, especially with improved nutrition and better understanding of the basic cellular defects. However, infection in particular with *Pseudomonas aeruginosa* and *Burkholderia cepacia*, has the greatest effect in decreasing life expectancy. Although infections can be prevented by rigorous infection control procedures, early aggressive antimicrobial chemotherapy and established infection managed by antibiotics, they are not completely effective. A greater understanding of how the bacteria evade the host defences and produce infection is needed.

Cystic fibrosis (CF) is an autosomal recessive disorder resulting from mutations in a gene on the long arm of chromosome 7[1]. The gene product is the cystic fibrosis transmembrane conductance regulator (CFTR) which regulates and facilitates transport of electrolytes across epithelial cell and other membranes. The mutations, (over 1000 described so far), can be by frameshift, deletion or by base substitution leading to amino acid substitution, however 60% of CF patients have ΔF508 (a three base [codon] deletion at phenylalanine 508). Although the mutations give abnormal electrolyte transport, how this explains the complete pathophysiology, especially in the lung, is unclear (Table 1). What is clear, however, is the mucus in the CF airways is highly viscid, sulphated and readily forms aggregates[2].

In the normal lung, the mucus layer acts to trap inhaled particles such as bacteria and is propelled upwards towards the pharynx by cilia (the mucociliary escalator), and then expectorated or swallowed. This defence mechanism is so potent that, despite heavy bacterial colonization of the upper airways (above the vocal cords), the lower airway is normally sterile. In the CF lung, the viscid mucous cannot be propelled so easily and the escalator fails, leading to an accumulation of mucus and trapped bacteria[3]. In addition, it has recently been shown that while extracellular fluid from cultured normal airways epithelia can kill bacteria, that from CF airways epithelia cannot[4]. A number of

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Table 1 Some factors promoting bacterial persistence and damage in the CF lung

IMPAIRED CLEARANCE	
Viscid mucus	Impaired mucociliary escalator
Altered composition of airways surface liquid	Poor microbial killing by β-defensins and cathelicidin
Increased expression of wrong receptor	Tetrasaccharide of asialoganglioside is receptor for *Ps. aeruginosa*
Decreased expression of correct receptor	CFTR is receptor for *Ps. aeruginosa* and binding leads to internalization then epithelial cell plus bacteria desquamated
DAMAGE	
Excessive neutrophil recruitment	Enhanced release of IL-8
Excessive neutrophil activation	Altered intraneutrophil pH regulation
Excessive release of neutrophil products	
Oxidative	Myeloperoxidase leading to protein oxidation
Non-oxidative	Elastase and other proteases leading to proteinase-anti proteinase imbalance
Malnutrition	Down-regulation of anti-inflammatory cytokine IL-10
Bacterial factors and products	Greatly augment damage

antimicrobial substances are excreted into the airways. These include lysozyme (which hydrolyses the peptidoglycan backbone of the bacterial cell wall), lactoferrin (which is an iron chelator), phospholipase A_2, proteases, complement and secretory IgA. A recent discovery has been that antibacterial cationic peptides are synthesized and secreted by epithelial cells of the airways and elsewhere. These peptides intercalate into bacterial membranes, permeabilise them and cause bacterial death. Among these are the β-defensins 1 and 2 and cathelicidins[6–8]. There is some evidence that, although these are expressed in the CF lung, they are not active in the airway surface liquid found there. However, if, for example, cathelicidin is over-expressed then this effect can be overcome[8]. There is, nevertheless, an intense inflammatory response in the CF bronchial tree with large amounts of neutrophils, macrophages and inflammatory mediators such as tumour necrosis factor-α (TNF), interleukin-1 (IL-1) and IL-8. Indeed secretion of IL-8, which is a neutrophil chemokine, seems to be triggered by exposure of bronchial submucous glands from CF patients to raised Cl^- ions. This causes accumulation of activated neutrophils which release α-defensins, reactive oxidants, and protease all of which potentiate lung damage. Although it appears that neutrophils from CF patients are not grossly deficient, there is evidence of altered intraneutrophil pH regulation[9] resulting in hypersecretion of granule contents including myeloperoxidase. CF sputum contains large amounts of myeloperoxidase and reactive oxygen and nitrogen intermediates which are toxic for tracheobronchial epithelial cells[10]. In an animal model, the malnutrition seen in CF contributes to poor bacterial clearance from the lungs and, by decreasing production of the anti-inflammatory cytokine IL-10, might result in excessive inflammation[11]. However, concentrations of one inflammatory mediator, nitric oxide (NO) which also has antibacterial activity, are low in the CF lung probably as a result of decreased inducible nitric oxide synthetase (iNOs) expression[12].

There do not appear to be any gross deficiencies in the specific (T- and B-cell) immune system; indeed, high levels of serum and sputum antibacterial antibodies are found in CF patients. However, there is evidence that some CF patients with chronic *Pseudomonas aeruginosa* colonization have more of a Th2 response than uncolonized CF controls[13]. Nevertheless, it does appear that the prolonged microbial colonization/infection that is characteristic of the CF lung results from defects in the innate or non-specific immune system. This is characterized by chronic infection with *Staphylococcus aureus* and non-capsulate *Haemophilus influenzae* in early life, followed by *Ps. aeruginosa* and *Burkholderia cepacia*, and much later *Stenotrophomonas maltophilia*[14], *Alcaligenes xylosoxidans*, non-tuberculous mycobacteria and some previously unidentified bacteria[15]. In this review, we will concentrate on mechanisms of persistence and aggression by the two most important CF lung pathogens – *Ps. aeruginosa* and *B. cepacia*.

Pseudomonas aeruginosa

In recent years, the pseudomonads have been subdivided into a number of new genera on the basis of the genetic sequences of their 16S-rRNA genes, and the number of new species has increased exponentially (Table 2). *Ps. aeruginosa* is the most important member of rRNA homology group I, and a major pathogen in the CF lung. It is a Gram-negative, oxidase positive rod that is motile by means of polar flagella (Fig. 1). It is ubiquitous in the moist environment, and can even grow in distilled water and disinfectant solutions.

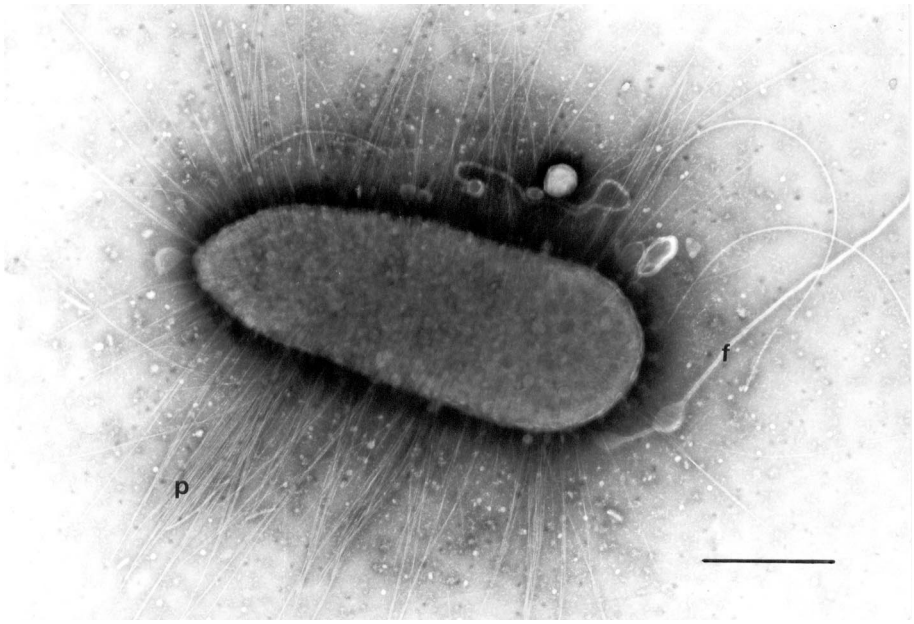

Fig. 1 Negative stain electron micrograph of *Pseudomonas aeruginosa* showing flagella (f) and pili (p). Bar = 500 nm.

Table 2 Medically important pseudomonads

rRNA homology group	Species	Infection in CF
I *Pseudomonas*	*Ps. aeruginosa*	Major pathogen
	Ps. fluorescens	Rare
	Ps. putida	Rare
	Ps. stutzeri	Rare
	Ps. alcaligenes	Very rare
	Ps. pseudoalcaligenes	Very rare
IIa *Burkholderia*	*B. cepacia* (genomovars I, III & VI)	Major pathogens
	B. multivorans (formerly genomovar II)	Common
	B. stabilis (formerly genomovar IV)	Common
	B. vietnamiensis (formerly genomovar V)	Common
	B. ambifaria (formerly genomovar VII)	Rare
	B. gladioli	Rare
	B. pseudomallei	Rare
	B. mallei	Not described
	B. thailandensis	Not described
	B. glathei	Not described
	B. glumae	Not described
	B. gramnis	Not described
	B. ubonensis	Not described
IIb *Ralstonia*	*R. pickettii*	Rare
	R. gillardii	Rare
	R. mannitolytica	Rare
	R. paucula	Rare
IIc *Oxalobacter*	*O. formigenes*	Absence in normal flora predisposes to nephrolithiosis
IId *Pandoraea*	P. pulmonicola	Rare
	P. pnomenusa	Rare
	P. apista	Rare
	P. sputorum	Rare
III *Comamonas*	*C. testosteroni*	Rare
	C. denitrificans	Not described
IV *Brevundimonas*	*B. diminuta*	Not described
	B. vesicularis	Not described
V *Stenotrophomonas*	*S. maltophilia*	Late in disease
	S. africana	Not described

Epidemiology

It is generally assumed that there is a hierarchy of colonization in the CF lung beginning with *H. influenzae* and *Staph. aureus* and subsequently with *Ps. aeruginosa* and *B. cepacia*. However, it is now clear that *Ps. aeruginosa* can affect the CF lung early in life; for example, 97.5% of children with CF in three centres in the US were infected by the age of 3 years[16]. In general, *Ps. aeruginosa* infection rates vary from 20–85% in most CF units, but with a

higher prevalence in adult units[17,18]. What affects the prevalence and age of onset of *Ps. aeruginosa* infection is not entirely clear, but there is evidence that continuous administration of antistaphylococcal antibiotics is associated with a higher rate of acquisition of *Ps. aeruginosa*[19]. There is little doubt, however, that infection with *Ps. aeruginosa* has a deleterious effect in terms of declining lung function, increased hospital admission rates, and increased and more rapid mortality[20–23]. This is particularly apparent if *Ps. aeruginosa* infection occurs early in life[21,23]. In some units, there is now a policy of early aggressive antipseudomonal therapy as soon as infection is detected, since it is impossible to cure the infection once it is established. This policy does seem to be effective both in preventing colonization and mortality and morbidity[24].

Ps. aeruginosa isolates can be typed for epidemiological purposes by phenotypic methods (such as pyocin typing, serotyping, phage typing, antibiogram) and genotypic methods such as pulsed field gel electrophoresis (PFGE) of macro-restricted chromosomal DNA, random amplified polymorphic DNA (RAP-D), ribotyping or flagellin gene polymorphisms[25–29]. In general, the genomic techniques are more sensitive and specific, but in reality no one method is completely reliable. During prolonged infection, the phenotype of *Ps. aeruginosa* can change from smooth, to rough, to highly mucoid colonial variants which may all be of the same genotype. During the early stages of disease, patients may be colonized intermittently and each patient has a unique genotype[16]. However, patients can be infected with two, three or more different genotypes concurrently or sequentially. The sources of the bacteria are many, and can include the inanimate environment both within and outside hospitals[18,30,31]. There is some evidence of cross-infection, especially between siblings, although the possibility of infection from a common source remains[28]. Outbreaks of infection with *Ps. aeruginosa* have been described in a number of CF units including Denmark[26], Liverpool[27], Manchester[32] and Melbourne[33]. Indeed, the Liverpool strain has been shown not only to cross-infect but also superinfect; that is, it colonizes patients already colonized by their own unique *Ps. aeruginosa* strain which it can displace[34]. Furthermore, this highly transmissible genotype was also able to cause pneumonia in the parents of a CF patient carrying the bacterium[35]. The complete genomic sequence of one strain of *Ps. aeruginosa* (PAO1) has now been published[36]. This is of great importance because it provides a point of reference with which to compare other strains including the highly transmissible lineages and will help our understanding of how they persist and cause disease.

Persistence

The initial stage in infection is attachment of bacteria to mucosal surfaces and/or the altered CF mucin. A confusing plethora of ligand-

receptor systems have been described for binding of *Ps. aeruginosa* to epithelial cells. These include pili (protein spikes that protrude from the bacterial surface), outer membrane proteins and even lipopolysaccharide on the bacterium[37–39] and gangliosides (asialo-GM-1), fucose residues, heparan sulphate proteoglycans or even the mutant CFTR itself[40–43] on the epithelial cell. In addition, *Ps. aeruginosa* binds to CF mucin via outer membrane proteins[44]. It has been demonstrated that CF epithelial cells express a greater density of an asialylated ganglioside receptor, GM-1, on their apical surface perhaps as a result of poor acidification of the Golgi where the gangliosides are processed[45]. It is suggested that binding of *Ps. aeruginosa* to this receptor might then, as a result of release of bacterial neuraminidase, expose more receptors. It has also been postulated that, in the normal lung, the first extracellular domain of CFTR (amino acids 108–117) acts as a receptor for *Ps. aeruginosa* (via lipopolysaccharide) and this binding results in internalization of bacteria[39,43]. This, it is proposed, is a mechanism for clearance of *Ps. aeruginosa* from the lung, since the epithelial cells die perhaps by apoptosis[46] and dead cells plus internalized bacteria are removed. In the CF lung, the mutant CFTR is not expressed (in the case of ΔF508) so there is no receptor for internalization and *Ps. aeruginosa* accumulates[43]. This hypothesis has been questioned by others, who found no correlation between expression of CFTR (human or murine) and binding or clearance of *Ps. aeruginosa* to or from epithelial cells *in vivo* or *in vitro*[47]. Heparan sulphate proteoglycans are expressed on the basolateral rather than apical surfaces of epithelial cells. It is postulated that the inflammatory process in the CF lung loosens the tight junctions between cells thus exposing the receptors and allowing greater adherence by *Ps. aeruginosa*[42]. From the above, it is clear that there is no one unifying hypothesis to explain how *Ps. aeruginosa* colonizes the CF airways. It is likely that the bacteria have a number of different strategies for attachment depending on the strain, stage of infection, and CFTR mutation.

Once established in colonization, *Ps. aeruginosa* must resist attempts by the immune system to dislodge it. It is already at an advantage in that three major components – the mucociliary escalator, peptide-mediated killing, and NO production – are impaired. However, the CF airway is a very harsh environment with large numbers of neutrophils, cytokines, chemokines, complement, T-cells, B-cells and specific antibody[9,10,13,48–50]. Indeed, attachment of *Ps. aeruginosa* to a lung pneumocyte cell-line or epithelial cells from CF airways itself induces release of a number of cytokines and regulatory proteins[51–54]. Pyocyanin, a phenazine redox active molecule that gives *Ps. aeruginosa* its greenish pigment, can also increase IL-8 expression in airway epithelial cells[55]. Nevertheless, the bacterium is not eliminated. This may result from alterations in neutrophil activity[12] and inhibition of opsonophagocytosis by digestion

of specific antibody by bacterial proteases such as elastase. Non-opsonic phagocytosis of *Ps. aeruginosa* involves at least two different receptors (CD14 and CR3) and it appears that mutants of *Ps. aeruginosa* can arise to escape this route of bacterial killing[56]. Thus, despite a florid inflammatory response, *Ps. aeruginosa* is able to persist in the CF airways.

During prolonged infection, the bacteria change tremendously, for example, changing from smooth to rough colonial morphology by loss of polysaccharide chains from lipopolysaccharide, by loss of flagella and thus motility, and production of a mucoid exopolysaccharide (alginate)[57]. The latter is particularly important in that it imparts further resistance to neutrophil-mediated killing[58], and contributes to the production of a biofilm[59]. This ability to evolve rapidly is a survival trait that enables *Ps. aeruginosa* to survive for years in the CF lung. For example, 36% of *Ps. aeruginosa* strains from 30 CF patients were found to be hypermutators, whereas this phenomenon was not found in 75 strains from non-CF patients[60]. Under normal circumstances, hypermutability carries a cost which limits survival; but, clearly in the CF lung, the cost of hypermutability is offset by the need to survive in such a harsh environment. Hypermutability often results from mutations in genes encoding DNA repair and error avoidance genes (*mut*S, *mut*Y) and this was so for the CF isolates. Thus, in this case, the ability to mutate rapidly in the harsh environment of the CF lung gives a survival advantage.

Another mechanism for survival is the production of a biofilm, and there are morphological and genetic data indicating biofilm production by *Ps. aeruginosa* in the CF lung[59,61]. At high densities, bacteria secrete high concentrations of a diffusible auto-inducer such as an N-acyl-homoserine lactone (HSL). This is produced by an enzyme which is a member of the LuxI family[62,63]. In the case of *Ps. aeruginosa*, two enzymes (RhlI and LasI) direct the synthesis of N-butyryl HSL and N-(3 oxododecanoyl)–HSL, respectively. These signal to all the other bacteria so as to co-ordinate expression of virulence factors, alginate production and formation of a biofilm. This process is called quorum sensing, and enables a pathogen to reach a critical mass and then release its virulence factors to produce a massive attack on the host. It is estimated that 4% of the ~6000 *Ps. aeruginosa* genes are controlled by quorum sensing. Following attachment to mucosal cells, the bacteria multiply and move together by twitching motility (mediated by type IV pili), to form microcolonies[64]. At this stage, quorum sensing induces alginate synthesis and biofilm formation occurs[61,62]. Within the biofilm, the bacteria are relatively well protected from the external environment including both host-produced microbicides and antimicrobial drugs. The latter explains why it is so difficult, if not impossible, to clear *Ps. aeruginosa* infection once it is established. Once the alginate-producing mucoid phenotype has been induced, it persists and, in addition to quorum sensing-

mediated conversion, it has been shown that hydrogen peroxide (an oxidant released by activated neutrophils), can induce mucoid *Ps. aeruginosa* in a biofilm *in vitro*[65].

Aggression

Infection by *Ps. aeruginosa* in the CF lung does not usually lead to immediate morbidity or mortality. Rather, it is a process of chronic infection with frequent exacerbations leading to a gradual decline in lung function. How much is a result of bacterial aggression or of the chronic inflammatory response to the bacterium is unclear. However, *Ps. aeruginosa* does have an impressive array of virulence determinants. It releases a variety of hydrolytic enzymes including proteases, elastase, lipase, phospholipase, alkaline phosphatase and mucin sulphatase. For some, release is apparently within vesicles formed from the bacterial outer membrane and release can be increased 3–5-fold by exposure to, for example, gentamicin[66]. *Ps. aeruginosa* is able to catalyze the breakdown of pulmonary surfactant, perhaps by phospholipase C activity although non-mucoid strains were more active than mucoid[67]. Most strains of *Ps. aeruginosa* produce a range of proteolytic enzymes active against a variety of substrates. Elastase degrades elastin and immunoglobulins. The mucin in the CF airways has sulphated terminal sugars and this prevents digestion by bacterial saccharidases. However, both *Ps. aeruginosa* and *B. cepacia* have mucin sulphatase activity[68] which allows further degradation of mucin and exposure of new receptors for pathogens. In addition, *Ps. aeruginosa* produces a number of other factors including pyocyanin[55], haemolysins, cytotoxins and siderophores all of which may contribute to aggression.

Two categories of *Ps. aeruginosa* isolates have been described that are invasive or cytolytic, but non-invasive for epithelial cells. Many pathogenic bacteria have type III secretion systems (TTS) that are assembled when the bacteria are in contact with epithelial cells[69]. TTS systems are used to transport effector molecules across the Gram-negative bacterial cell wall and have an apparatus for injecting them into host cells, by which they alter host cell activity. In *Ps. aeruginosa*, two of the TTS secreted effectors are exoenzyme S (ExoS) and ExoT, both of which are ADP-ribosyltransferases[70]. ExoS induces transcriptional expression of a number of pro-inflammatory cytokines and chemokines, thus contributing to pulmonary inflammation[71]. It appears that the invasion into epithelial cells is associated with defects in the TTS[72]. Recently, a genomic island, *Ps. aeruginosa* genomic island-1 (PAGI-1) which represents a 6729 bp region deleted from PAO1, has been found in 85% of clinical isolates including from CF patients[73]. As yet, it is unclear what roles the TTS system or PAGI-1 play in the pathogenesis of infection in the CF lung.

Burkholderia

Burkholderia spp. are in rRNA group II (Table 2), along with other CF lung pathogens such as *Ralstonia picketii* and *Pandoraea* spp[74]. However, *Burkholderia* spp., and in particular *B. cepacia*, are the most important pathogens in this group. They are Gram-negative, rod-shaped bacteria, motile by polar flagella (Fig. 2). They are unusual in that they do not have one circular chromosome but 2–4 circular replicons[75,76]. In addition, there are a number of insertion elements in the genome[77]. All of the above indicate that *Burkholderia* spp. have tremendous genomic plasticity.

There are a number of *Burkholderia* species, and *B. cepacia* has been subdivided into a number of genomovars by DNA–DNA and DNA–ribosomal RNA hybridization studies[57,78]. The term genomovar is used since, by taxonomic convention, bacteria cannot be given a species name unless identifiable by phenotypic characteristics. However, some of the genomovars have now been given species names (see Table 2) and rapid methods devised for their differentiation[79,80]. To date, only genomovars I, III and VI remain in the *B. cepacia* complex.

Epidemiology

B. cepacia is named after Burkholder who, in 1950, discovered it was the cause of onion soft rot (*cepia* is Latin for onion), and it is known that *Burkholderia* spp. are also widely distributed as saprophytes in the environment[78]. Prior to the 1980s, *B. cepacia* was regarded as a rare opportunist causing nosocomial respiratory, urinary tract or soft tissue

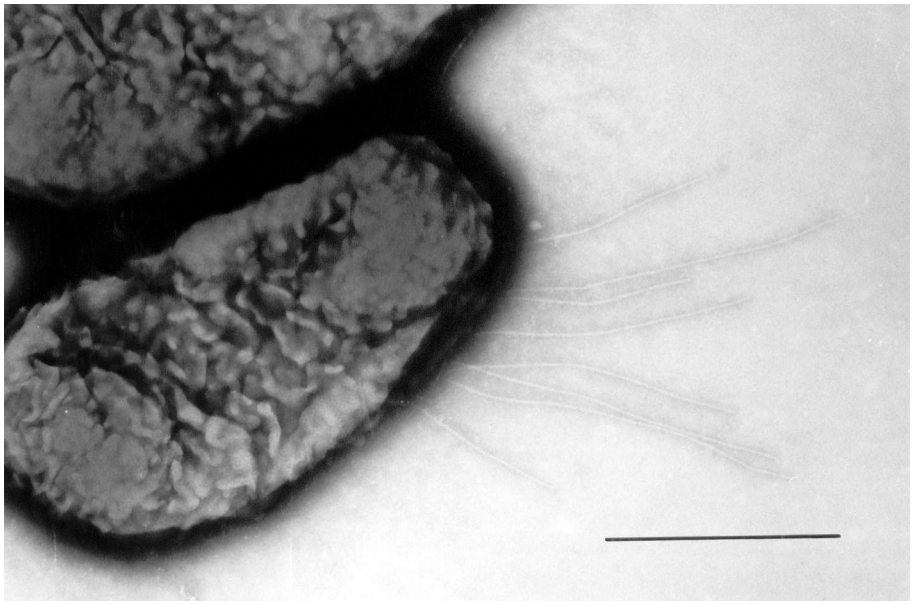

Fig. 2 Negative stain electron micrograph of *Burkholderia cepacia* showing pili. Bar = 500 nm.

infections, which was able to survive in disinfectant solutions[78]. Then, it became clear, that it was associated with infection in the CF lung[81] and widely distributed in the environment[57,78].

Subsequently, it emerged that certain strains of *B. cepacia* were highly transmissible and some could cause lethal infection in CF patients[78,82–84]. A variety of methods are available for typing *B. cepacia*. These include phenotypic methods such as pyrolysis mass spectroscopy[85] and lipopolysaccharide chemotyping[86], but genotypic methods such as PFGE, flagellin RFLP typing and ribotyping remain the gold-standard[57,78,82,83,87,88]. There is one highly transmissible lineage called ET-12 (Edinburgh-Toronto) which is in *B. cepacia* genomovar III. This possesses two markers of transmissibility cable pili (cbl)[89] and BCESM (*B. cepacia* epidemic strain marker)[90]. The presence of *cbl* genes seems to be limited to epidemic genomovar III strains, but BCESM is present in epidemic and non-epidemic strains of *B. cepacia* genomovars I and III as well as in *B. multivorans* and *B. stabilis*[91].

Certain strains of *B. cepacia* especially, but not only ET-12, are easily spread, person-to-person, directly presumably via respiratory secretions, (counts can exceed 10^8 cfu/ml) by, for example, kissing, or hands, or indirectly via spirometers or other medical equipment. Spread can occur both in hospital[82] and in a social setting[57,78,83]. One *B. cepacia* strain (not ET-12) has caused a nosocomial outbreak of infection in CF and non-CF patients[88]. The results of infection can vary from prolonged carriage with a gradual decline in lung function to fatal 'cepacia syndrome' with necrotizing pneumonia and bacteraemia[82]. Why such differences should occur is not clear, but might be related to other deficiencies unrelated to CFTR mutations, for example in mannose-binding lectin[92].

As with 'epidemic' strains of *Ps. aeruginosa*[34], it appears that *B. cepacia* ET-12 can super-infect CF patients already colonized with non-epidemic strains, displace them and result in fatal 'cepacia syndrome'[93]. This adds an extra layer of complexity for prevention of spread of *B. cepacia*[94]. Finally, although *B. cepacia* is described as a pathogen of onions and humans, there has been a recent outbreak of mastitis in dairy sheep predominantly due to genomovar III[95]. In addition, *B. cepacia* is being developed for use as a biopesticide to protect crops against fungi and for bioremediation to break down herbicides that are not easily biodegradable[96]. This could pose a further threat to CF patients and its use should be approached with extreme caution.

Persistence

Following transmission, the initial interaction between *B. cepacia* and the airways mucosa involves attachment. At least 5 morphologically different pili have been detected on epidemic and non-epidemic strains including: cable, filamentous, spikes and mesh forms[97]. Of these, the cable pili which are

associated with *B. cepacia* ET-12 are best characterized[89]. The receptor for cable pili is cytokeratin 13[98] which is enriched on the hyperplastic epithelia of CF airways[99]. Some cable pilus-negative *B. cepacia* appear to bind to asialo GM1[100]. *B. cepacia* is also able to bind respiratory mucin from CF patients[101]. Once established in colonization, *B. cepacia* must resist the bronchial killing and elimination mechanisms. Unlike *Ps. aeruginosa*, *B. cepacia* is resistant to epithelial derived antimicrobial peptides no matter what the salt concentration[102]. The ability to scavenge iron using a siderophore, ornibactin, is also important for the persistence of *B. cepacia*[103]. The CF airway also contains a number of reactive oxidants such as superoxide, hydrogen peroxide, hypochlorite and singlet oxygen released from activated neutrophils and macrophages. These are extremely toxic for bacteria, but virulent *B. cepacia* have evolved mechanisms for resisting attack. Such mechanisms include production of a melanin pigment[104] and expression of haem dimer binding proteins on the bacterial surface[105] which imparts catalase activity. Finally, *B. cepacia* does appear to have the ability to exist in a biofilm both in the CF lung and on plastic catheters[106,107], and there is recent evidence of a quorum sensing system mediated by N-octanoylhomoserine lactone[108]. This raises the intriguing possibility of cross-talk between *Ps. aeruginosa* and *B. cepacia* in the CF lung.

Aggression

It is not clear how *B. cepacia* produces such devastating infection nor why some patients have prolonged infection with gradual decline in lung function and others develop 'cepacia syndrome' with identical bacteria. *B. cepacia* produces an impressive array of potential virulence determinants including protease, lipase, haemolysins, mucin sulphatase and cytotoxins[57,68,78,109,110]. Of note, the haemolysin has also been shown to induce degranulation and programmed cell death of neutrophils[109] leading to both protection of bacteria and lung damage. Clinical isolates of *B. cepacia* also secrete greater amounts of cytotoxins than environmental strains. In the presence of ATP these cytotoxins induce macrophage and mast cell death[110]. *B. cepacia* is also able to penetrate into, and survive within, cultured macrophages and lung epithelial cells[111,112]. Isolates of *B. vietnamiensis* and *B. cepacia* genomovar VI were able to survive for at least 5 days in activated macrophages and bacterial entry stimulated the macrophages to release TNF and reactive oxidants[111]. Thus it is proposed that repeated cycles of phagocytosis and macrophage activation could promote chronic inflammation. It is noteworthy that *B. cepacia* can also survive and grow within free-living amoebae[113], a situation that parallels that of another lung pathogen *Legionella pneumophila*. How these phenomena are orchestrated is unclear, but the recent discovery of genes encoding a putative type III secretion system in *B. cepacia* ET-12[114] might help to provide an explanation. The recent description of a model of *B. cepacia*

infection in *Cftr*$^{-/-}$ mice will also help to advance our understanding of the pathogenesis of infection[115].

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