Adaptation and Evolution of Pathogens in the Cystic Fibrosis Lung

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As opposed to acute respiratory infections, the persistent bacterial infections of the lung that characterize cystic fibrosis (CF) provide ample time for bacteria to evolve and adapt. The process of adaptation is recorded in mutations that accumulate over time in the genomes of the infecting bacteria. Some of these mutations lead to obvious phenotypic differences such as antibiotic resistance or the well-known mucoid phenotype of Pseudomonas aeruginosa. Other mutations may be just as important but harder to detect such as increased mutation rates, cell surface changes, and shifts in metabolism and nutrient acquisition. Remarkably, many of the adaptations occur again and again in different patients, signaling that bacteria are adapting to solve specific challenges in the CF respiratory tract. This parallel evolution even extends across distinct bacterial species. This review addresses the bacterial systems that are known to change in long-term CF infections with a special emphasis on cross-species comparisons. Consideration is given to how adaptation may impact health in CF, and the possible evolutionary mechanisms that lead to the repeated parallel adaptations.

Key words: adaptation; cystic fibrosis; in host evolution; microbiota.

In the 1960s, Robert Doggett and colleagues [1] first described a strong association of unusual Pseudomonas aeruginosa isolates that produced copious amounts of a mucoid material with cystic fibrosis. The association was striking because P. aeruginosa was considered to be only rarely encapsulated [2]. This material was later identified as alginate [3, 4], a polysaccharide originally isolated from brown seaweeds [5]. In addition, there seemed to be a clear progression, with nonmucoid P. aeruginosa isolated in early disease and mucoid strains appearing later, sometimes present alongside nonmucoid strains and sometimes on their own. The crucial question was whether this observed “change” was due to the introduction of mucoid P. aeruginosa replacing or outcompeting existing nonmucoid strains, or whether it was possible that the infecting strains had gained, newly, the capacity to make alginate. The paucity of mucoid environmental strains led initially to the suspicion that strains were converting, rather than being replaced, which was confirmed with the advent of molecular typing techniques [2].

The finding that many mucoid strains were highly similar to nonmucoid strains from the perspective of sequence type, suggested that this was evidence of “intra–host” evolution, and the repeated (parallel) evolution in multiple patients argued that there were strong selective pressures at work that made mucoidy highly beneficial to the infecting organisms. The fitness benefits proposed focus on the physical barrier provided by alginate, with protection from immune cells such as neutrophils and alveolar macrophages, reduced penetration of antibiotics, and immune molecules such as complement and immunoglobulins [2, 6]. Adherence and production of a difficult to remove biofilm also may be important for persistence [7].

That a major phenotypic change could occur in P. aeruginosa opened up the possibility that other pathogens were adapting genetically to the CF niche, and that other phenotypic changes might be occurring. The recent development of whole genome sequencing as a major tool for understanding long-term infection in CF has helped to clarify the major patterns of genomic and phenotypic evolution, in addition to identifying new types of adaptations that had gone unnoticed before [8–10]. The critical quality of in-host evolution is that it occurs recurrently in multiple individuals with the same disease and even within the same patient in distinct infecting lineages, providing the strongest evidence we have that these specific changes are beneficial to infecting organisms [10–14].

WHAT IS THE CF RESPIRATORY TRACT LIKE?

To understand bacterial adaptation to the CF airway, it is important to understand what the niche is like from the bacterial perspective, and therefore what fitness obstacles need to be overcome. First, the CF respiratory niche is probably best
described as multiple niches that extend from the upper airway all the way through to the deepest alveolae, with multiple distinct environments and atmospheres. There are areas of high oxygen with nearby areas that, after a steep oxygen gradient, are effectively anaerobic. In this milieu, there is a lack of critical nutrients such as iron and zinc, and areas with high concentrations of oxygen radicals [15], varying levels of antibiotics, and immune molecules and cells. There are also other organisms replicating and occupying this space that compete for some of the same nutrients, and that may directly attack with bacteriocins or other toxic molecules. Areas of the lung with different amounts of mucus plugging, bronchiectasis, and aeration may present very distinct niches. The heterogeneity of the CF airway, the relative lack of movement between spaces, and the failure to clear bacteria quickly sets up the perfect environment for hundreds of local evolutionary experiments to play out. Indeed, multiple studies have shown phenotypic and genomic diversity in distinct parts of the lung upon explant [16–18].

**ANTIBIOTIC RESISTANCE AND TOLERANCE**

Antibiotic resistance is the most obvious clinical problem that arises from intra-host evolution. CF patients have very high levels of antibiotic exposure, which selects for pathogens that can evade antibiotic toxicity. Indeed, one reason that *P. aeruginosa* is a common pathogen in CF is likely because of its inherent antibiotic resistance to multiple drugs. Likewise, multidrug resistant (MDR) organisms are favored under standard CF treatment regimens, which not only consist of periodic treatments with broad spectrum antibiotics for pulmonary exacerbations but also long-term use of aminoglycosides (e.g., inhaled tobramycin and amikacin), monobactams (e.g., inhaled aztreonam), and oral azithromycin [19–21].

High exposure to antibiotics does not just lead to invasion of MDR pathogens. It can also lead to mutations in existing populations true antibiotic resistance or tolerance. Antibiotic tolerance is defined here as either an increased, but intermediate, minimal inhibitory concentration (MIC) or the ability to survive antibiotic challenge. The mechanisms of antibiotic resistance are as diverse as the mechanisms of resistance themselves. Mutations in genes for multidrug efflux pumps and associated proteins have been noted in *P. aeruginosa*, *Burkholderia* species, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans* [9, 12, 22–24]. These mutations often result in changes in MIC to multiple antibiotics at the same time. Mutations in porin genes, which diminish the diffusion of antibiotics into the bacterial cell, have been described for *P. aeruginosa* [9] and *Burkholderia* spp. [23]. In addition, point mutations for aminoglycoside, macrolide, and rifampin resistance have been described for nontuberculous mycobacteria (NTM) [25]. Point mutations in *ampD*, whose protein product controls the beta-lactamase AmpC expression, have been described for *B. multivorans* [23]. For *Staphylococcus aureus*, point mutations for linezolid and azithromycin have been described [26].

Several other adaptations seem to affect the ability of bacteria to weather an antibiotic challenge. Adaptations that enhance biofilm formation are the most recognized form of antibiotic tolerance in that they provide a barrier to diffusion of antibiotics [27, 28]. Biofilms also harbor distinct populations of cells, some of which may be less metabolically active and less susceptible since antibiotics preferentially kill metabolically active or dividing cells [29]. In general, bacteria with a less active metabolic state may be more tolerant to antibiotics, and this has led to the study of small colony variants (SCVs), metabolic auxotrophs, and, so-called persister cells in several organisms including *S. aureus* and *P. aeruginosa* [30–33].

**EXTRACELLULAR APPENDAGES AND CELL ENVELOPE CHANGES**

Bacteria present multiple molecules and appendages on their surface that can trigger an immune and inflammatory response [34], but these same surface factors (pili, flagella, LPS) are critical for bacterial motility, colonization, and interaction with their surroundings. Therefore, while they enable infection [35], they are also a liability for pathogens once they have established a stable infection. For *P. aeruginosa*, a recurrent and predictable change associated with adaptation to the host is the loss of flagella [9, 36], a major stimulator of the Toll-like receptor 5 (TLR5) [37], and type IV pili [9, 38, 39], for which an innate immune response is not well characterized. Loss of motility, due to the loss of flagella [40] has also been observed in *Burkholderia* spp., along with reduced TLR5 stimulation for nonmotile strains, although only a minority of the observed strains underwent this transition [41].

Changes in the surface exposed cell envelope is most well-known for lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria. *P. aeruginosa* strains have been observed that change the molecular conformation of their LPS, becoming less immunostimulatory [42–46]. Strains of *P. aeruginosa* isolated from CF lungs often do not express high molecular weight O antigen (displayed on LPS) which may make these strains more susceptible to killing in human serum [47, 48]. Loss of the LPS O antigen has also been noted to occur in *B. multivorans* infection through mutations in the *wbi* gene cluster [12]. Larger surveys have suggested that rates of loss of O antigen may be specific for each *Burkholderia* species with *B. cenocepacia* and *B. multivorans* having particularly high rates of conversion [49]. O antigen loss may lead to lower immunogenicity and evasion of the host immune response [50], or potential increased persistence through increased intracellular survival [51].

For gram-positive organisms such as *S. aureus*, innate immune responses linked to sensing by TLR2 of peptidoglycan
might be expected, but no such adaptive changes have been described. However, for nontuberculous mycobacteria (NTM) there have been changes noted in the glycopeptidolipids (GPL) of the outer cell envelope that lead to changes in immunostimulation as well as other phenotypic properties such as rough and smooth colony morphologies, biofilm formation, aggregation, and survival of macrophage killing [52].

While the pervasive changes in the superficial or exposed aspects of a bacterial cell in the CF lung are likely to be tightly linked to the immune response, it is worthwhile considering other possible advantages that losses or changes in these factors may have for bacteria. Indeed, some changes associated with LPS may arise simply because of selection for biofilms and CF-like nutritional regimes [53, 54]. Also, once established in a thriving community in the lungs, bacteria may no longer need to expend the energy for appendages that are primarily advantageous for colonization and motility. Other adaptations such as deep embedding in an alginate matrix may make these activities much less relevant. In general, reductive or degradative genomic evolution appears to be a theme for CF pathogens (see below). In this type of evolution, especially in bacteria with higher mutation rates or more active transposition or recombination, genes that are not needed are easily lost. It seems possible that many of the losses observed and taken as signs of adaptation are merely the consequence of random inactivation of unneeded systems.

**MUOIDY, BIOFILM FORMATION, AND QUORUM SENSING**

As noted above, mucoidy in *P. aeruginosa* was the first attribute that appeared to arise in a highly parallel fashion in CF patients. The mechanism of the transition to mucoidy has been deeply studied, and has been shown to originate from derepression of regulatory genes of alginate production, particularly *algU*, *algW*, *mucA*, *mucB*, *mucD* [55, 56]. These mutations have been seen repeatedly in multiple recent whole genome studies with loss of function mutations arising in multiple genes involved in the regulation of the production of the exopolysaccharide (EPS) alginate [14]. Organisms grown in an alginate biofilm are less susceptible than planktonic cells to antibiotic challenge and more protected from the immune system response [28, 57, 58].

While *B. cepacia* complex isolates have been shown to form biofilms [59–61], mucoid isolates were previously thought to be rare, especially in the environment [2]. However, some CF centers have reported that more than 80% of isolates from CF patients make EPS [62, 63], and culture conditions also greatly impact EPS production with environmental strains also producing EPS [64–66]. The most widespread *B. cepacia* EPS, cepacian [67, 68], is involved in the thickness and maturity of biofilms in some strains but not all [63]. Murine models show increased persistence of mucoid isolates compared to isogenic controls [69], and EPS has also been implicated in scavenging oxygen radicals and interfering with neutrophil chemotaxis [70]. However, the mucoid phenotype is complex, and in surveys from CF patients, *Burkholderia* species have highly variable biofilm-forming capacity, colony morphology, and levels of EPS production [63, 64]. Thus, while the impact of EPS remains unclear the virulent species *B. cenocepacia* has a high frequency of EPS producing isolates [64]. However, one study showed that patients with nonmucoid strains had more rapid lung function decline and worse outcomes [71]. Interestingly, unlike the pattern of change in *P. aeruginosa*, longitudinal *Burkholderia* isolates appear to switch both from mucoid to nonmucoid and vice versa, with the majority of phenotypic changes occurring from mucoid to nonmucoid [64].

Biofilms can take different forms and have distinct extracellular matrix components including exopolysaccharides other than alginate, and other important components such as pili or DNA [7, 72–74]. Another phenotypic switch noted for persistent *P. aeruginosa* infections in CF is the transition to rugose small colony variants (RSCVs), which is tied to the expression of nonalginate exopolysaccharides Psl and Pel [75–77]. RSCVs of *P. aeruginosa* form strong attachments to surfaces and pneumocytes, autoaggregate, and have been found in significant quantities in CF patients [78, 79]. Some are hyperpiliated and have increased twitching motility [78]. RSCVs have also been shown to be more resistant to phagocytosis [80, 81]. Multiple other phenotypes have been associated with *P. aeruginosa* RSCVs including both increases and decreases in cytotoxicity and virulence [31]. The original RSCV phenotype was described as the result of overproduction of cyclic-di-GMP and linked to the genes *pvrR*, encoding a cyclic-di-GMP phosphodiesterase, and *wspR*, encoding a diguanylate cyclase [82, 83]. Multiple additional naturally occurring mutations have been linked to the RSCV phenotype, the most common are loss-of-function mutations in genes whose products negatively regulate cyclic-di-GMP production (e.g., *wspF*, *fleQ*, *yfiN*, *yfiR*) [9, 80, 81]. The second messenger cyclic-di-GMP is involved in the control of multiple processes that are central to the adaptive processes of *P. aeruginosa* to the CF environment including elaboration of extracellular appendages, exopolysaccharide, adhesins, virulence and cytotoxicity systems among others [31]. Interestingly, recent work ties the mutations that lead to loss of flagella to Pel and Psl production, suggesting that the loss flagella may just be a “side effect” of the true fitness benefit of creating a Psl/Pel biofilm [75]. *Burkholderia cenocepacia* has also been noted to easily produce RSCVs in in vitro evolution studies that are due to *wsp* locus genes [84], and there are reports of cystic fibrosis patients with the SCV phenotype [85].

Bacteria living in close proximity are well known to communicate with each other through small molecules generally known as quorum sensing. In long-term infections in CF, *P. aeruginosa* has been noted to lose quorum sensing abilities
through mutation of lasR and rhl genes [38, 86–89]. The loss of quorum sensing has multiple impacts including modulation of expression of many other genes including those with an impact on virulence [90, 91]. It also may lead to increased growth rates due to more efficient use of amino acids, ability to use nitrate as an electron acceptor, and increased antibiotic resistance [87, 92]. While quorum sensing changes have been seen in *Burkholderia* spp. infections, inactivation may not be the norm in long-term infections [93].

*S. aureus* biofilms produce more heterogeneous biofilms in CF that involve both adhesive proteins, surface polysaccharides such as poly-N-acetyl-1,6 glucosamine surface polysaccharide (PIA/PNAG), wall teichoic acids, and DNA with the relative importance of each varying between strains [94]. Activation of the quorum sensing system *agr* in *S. aureus* leads to biofilm dispersal and it is notable that *agr* mutants are often found in CF samples. In biofilms, *agr* transcriptional activity is low, and *agr* mutants are known to be enhanced for biofilm formation [95].

**OTHER TYPES OF SMALL COLONY VARIANTS, AUXOTROPHS, AND METABOLISM**

Confusingly, the small colony phenotype (SCV) that arises in *S. aureus* from CF patients appears to have a very different biological underpinning than the RSCVs noted above for *P. aeruginosa* [96–99]. *S. aureus* SCVs have several critical advantages as residents of the CF lung including higher tolerance to antibiotic challenges, and increased intracellular survival [94]. These strains often have slower growth, decreased hemolysin production, less pigmentation, increased intracellular survival, and inactive *agr* systems all suggesting a quiescent, biofilm forming lifestyle [94]. The genetic basis of SCVs is heterogeneous, and the associated mutations cause defects in amino acid or nucleotide synthesis and electron transport. SCVs are often auxotrophs, meaning that they cannot synthesize critical molecules needed for growth, which they need to acquire from exogenous sources. *S. aureus* SCVs are most usually auxotrophs of the nucleic acid thymidine with mutations in the *thyA* gene [100, 101]. These isolates are associated with long-term trimethoprim sulfamethoxazole treatment, are found mostly in older patients, tend to have higher rates of antibiotic resistance, and have long-term persistence [98, 100, 102]. More recently, *S. aureus* SCVs have been found to be strongly associated with worse lung function [103, 104]. An interesting observation has been that interactions between *P. aeruginosa* and *S. aureus* may select for *S. aureus* SCVs and protecting them from toxicity of the very commonly used aminoglycoside tobramycin [105].

*S. maltophilia* has been reported to produce small colony variants that seem more similar to *S. aureus* SCVs than those reported for *P. aeruginosa* or *Burkholderia* spp. These are auxotrophs for hemin, methionine and thymidine and are associated with trimethoprim sulfamethoxazole exposure [106]. Auxotrophy also develops in *P. aeruginosa* isolates from CF, which mostly require amino acids (methionine, lysine, arginine) for growth [107–109]. It has been hypothesized that these forms can arise because the high levels of amino acids in the lung remove selection pressure [110]. Metabolomic studies have also shown evidence of metabolic adaptation with acetate production being negatively correlated with length of infection, and an increase in efficient uptake of amino acids that would be found in the human lung [111].

**REACTIVE OXYGEN SPECIES**

In addition to exogenous antibiotics, bacteria face an environment that is replete with damaging molecules released from neutrophils such as myeloperoxidase, elastase, leukocyte protease, and ROS [112, 113]. In general, bacteria must develop strategies to ameliorate this damage. Adaptive mutations for the oxidative stress (in *katG, yedY, moeA*) have been documented in *Burkholderia* cenocepacia [114]. One metabolic change noted in *P. aeruginosa* is mutations in the genes for the pyruvate dehydrogenase (*aceE* and *aceF*), which through reduction of glycolysis and the tricarboxylic acid (TCA) cycle can help resist reactive oxygen species [14, 115]. Because ROS can damage DNA, chronic ROS exposure can lead to mutations in many genes, which may happen to be in DNA repair genes that increase mutation rates even further [116]. Reactive oxygen stress has been shown to lead to mutations that create mucoid strains [117], and other experimental evolution studies done with chronic H2O2 have shown generation of mutants that result in a rough small colony variant phenotype (mediated by the *wspF* gene) [118]. However, some mucoid mutants have been shown to be even more susceptible to reactive oxygen stress [119].

**HYPERMUTATION AND GENOME DEGRADATION**

A recurrent theme in intra-host evolution is the generation of strains with higher rates of mutation. This phenomenon has been observed or inferred by sequence changes in *P. aeruginosa* [116, 120–125], *Haemophilus influenzae* [126], *Burkholderia* spp. ([12, 23, 127, 128] though see [129]) and *S. aureus* [26, 94] with the consequences being more rapid gain of mutations associated with antibiotic resistance [120] and other potentially beneficial traits. The genetic changes associated with hypermutability are associated with deletion or inactivation of DNA mismatch repair (MMR) genes (*mutLS, uvrD*) or the GO system (*mutMTY*), of oxidative damage repair and have been described in both gram-positive and negative CF pathogens [130–132].

Of course, while hypermutability offers a more rapid generation of beneficial point mutations, it is more likely to result in deleterious mutations. Therefore, the fitness cost of a
hypermutable phenotype is high [124] though this may be offset by other benefits in certain selective regimes [133]. It is interesting to speculate that a strategy that allows for a transient increase in mutation rate might be ideal. In this regard, it would be interesting to find evidence of restored MutLS activity, or antimutator phenotypes [134].

An area that has only started to be considered is the impact of recombination on rapid DNA change. If foreign DNA is available, then recombination is the fastest way to affect massive genomic change. One study of a single CF patient with chronic B. multivorans infection showed a significant genomic signal supporting recombination of more than 15% of the genomic sequence [23]. In P. aeruginosa, recombination as the dominant mode of sequence change has also been detected [135, 136].

Another mode of evolution is through insertion of transposable elements insertion elements (IS). Evolution through active IS elements in P. aeruginosa during CF infection has aids in the genesis of antibiotic resistance and the inactivation of genes [137–141]. In B. cenocepa, transposition of IS elements leads both to gene inactivation if inserted into a coding or regulatory sequence, and also as loci of sequence homology that can undergo recombination leading to large genomic deletions [114, 129].

The combined action of mutation, transposition, and recombination in CF leads to genome degradation that is often seen as organisms become specialists, adapting to a specific environment where a more diverse set of genes (and possible functions) is no longer needed [141].

DIVERGENCE AND THE MAINTENANCE OF DIVERSITY

Divergence of the initial infecting population and long-term persistence of that diversity is another common pattern in CF [12, 142, 143], which signals that the CF lung environment furnishes positive (or diversifying) selective forces [12, 144] as well as the potential that selective forces work differently on closely related bacteria. For P. aeruginosa, multiple studies have shown a large amount of phenotypic diversity in spumtum samples [16–18, 142, 143, 145–147], which has also been shown in other pathogens including Burkholderia [148, 149], and at least in one case of nontuberculous mycobacteria [150]. Diversity in S. aureus has been less well characterized with only a handful of studies characterizing within-host or longitudinal diversity [151–154].

What evolutionary forces generate this diversity? One possibility is that distinct changes occur in distinct biogeographical locations in the lung where populations experience different conditions as well as the stochastic effects associated with population bottlenecks [16, 155]. While anatomical regions can create subdivisions in bacterial populations, it is also possible that there are smaller subdivisions such as aggregates of bacteria or even in different regions of the airway lumen [141, 156]. In a subdivided population the existence of periodic selection that occurs with intermittent use of antibiotics, or recurrent periods of inflammation and immune response, might lead to selective sweeps, in which variants arise from mutation that dominate the population because of their increased fitness [157]. A subdivided population also favors diversification through genetic drift over selection [141].

An alternative explanation for increased and stable diversity is that the organisms with different phenotypes could be occupying different niches within the lung, however testing this hypothesis will require demonstrating differences in spatial organization or nutrient utilization.

Yet another possibility is that diversity arises from social interactions between organisms, which could take the form of mutualisms (divisions of labor) or unequal relationships such as with social cheaters who acquire benefits from the population while reducing any cost to themselves [158]. Interesting new work on P. aeruginosa populations suggests that genes for relatively cooperative functions are more likely to vary and have inactivating mutations than genes that largely function in the context of one bacterium, suggesting that social interactions between bacteria may be critical for diversification [159].

HOW DOES ADAPTATION AFFECT HEALTH AND TREATMENT?

Many studies use the word “pathoadaptation” to describe the process of adaptation to the host environment, but this term can be confusing since it also implies enhanced fitness that leads to worse disease [160, 161]. In the sense that the increased fitness of specific phenotypic changes make infections last longer than probably all in host adaptation can be considered pathoadaptation [162]. However, some phenotypic changes may decrease or change the overall virulence of the organism [163]. For instance, some hypermutator phenotypes have been directly tied to increased persistence but reduced or changed virulence [164].

The phenotypic differences that are well documented for P. aeruginosa can lead to significant differences in antibiotic susceptibility, which has an impact on susceptibility testing and likely treatment as well [165–168]. Similar diversity has been noted in nontuberculous mycobacteria isolated from the same individual [150] as well as in S. maltophilia [169], and it is probably a general feature of chronic lung infections.

A recent report [170] highlights the possibility that mutations can result in increased pathogenicity and antibiotic resistance. In this case report, whole genome sequencing revealed mutations in the exsD gene whose product acts to negatively regulate type III secretion (T3S), a major virulence factor for P. aeruginosa that is often lost earlier in infection and may be linked to worse outcomes [171, 172]. The authors note that this mutation could have led to hyperactivity of T3S that coincided with an acute
worsening in the patient’s status. Interestingly, mutation of the OprD porin was also observed in this report, which likely had an impact on the susceptibility of this strain to carbapenem antibiotics, suggesting that the exsD mutation may have genetically “hitch-hiked” along with an antibiotic resistance gene.

This example evokes themes that repeatedly confound our ability to gauge the impact of mutations and putative adaptation in cystic fibrosis. First, adaptations tend to occur later in disease when lung function is already declining, thus it is not always clear whether adaptation is causal or just a marker worsening disease. Second, mutations occur linked to one another in a genomic (and even microbial population) context. Therefore, it may be difficult to discern which mutations are beneficial to the organism, which are harmful to the host, and which are neutral or even beneficial to the host. As such, while phenotypes such as the mucoid phenotype in P. aeruginosa [6, 162, 173–183] or the SCV phenotype [103, 104] in S. aureus are strongly associated with worsening disease, it is difficult to assign them a causal relationship. Likewise, associations between P. aeruginosa auxotrophs, RSCVs, and quorum sensing and more severe disease remain associations [79, 102, 108, 184, 185].

It is also interesting to consider that some of the mutations that occur may actually lead to less inflammatory or destructive phenotypes. For instance, the loss of flagella might lead to less inflammation due to the immune response through TLR5 [37] or the differences in Lipid A acylation might decrease inflammation due to TLR4 signaling [42–46]. There have been no clinical studies to gauge the impact of these changes on disease progression.

WHAT IS NEXT?

As genomic and phenotypic data accumulate the next steps will be to clearly delineate the impact of bacterial adaptations on disease with the goal of using this information to make clinical decisions and interventions. The critical clinical studies will include careful determination of which adaptations are associated with clinical decline, and which are not. This will require large-scale studies that consider intra-host diversity and change over time. Once these associations are well established, they can be targeted with treatments and decision making. For instance, it may be possible to target specific phenotypes to aid in eradication of adapted strains. It may also be possible to monitor for specific adaptations to guide clinical decision making. Some adaptations may signal the need for more aggressive treatment, and some may change the risk benefit analysis of aggressive therapy leading to more judicious use of antibiotics or other drugs. These types of approaches will require truly translational research that includes rigorous genomic, basic, and clinical studies. There should also be more attention paid to emerging and less well-studied pathogens such as nontuberculous mycobacteria, S. maltophilia, and S. aureus, as well as other underappreciated members of the CF respiratory microbiome, such as anaerobes. It is important to note that microbial adaptations not only allow us to understand the process of intra-host evolution, but they also give a window into the underlying biology of the disease.

Notes

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