∂ Not Quite the Bully in the Schoolyard: *Staphylococcus aureus* Can Survive and Coexist with *Pseudomonas aeruginosa* in the Cystic Fibrosis Lung

Microorganisms often dwell and interact in complex environmental communities. These interactions may benefit multiple parties (e.g., protection in biofilms) or just one member (e.g., metabolite exchange). However, interspecies interactions can be antagonistic interactions in which one organism inhibits or kills its neighbor, especially involving nutrient and niche competition. In either case, polymicrobial interactions can alter the behavior, physiology, and persistence of microbes impacting human infections (1). Scientists have gained a greater appreciation for the constituents and causes of human polymicrobial infections, including respiratory tract infections (1). In addition to identifying the microbiota, studies have begun to define the interactions between microbes in these infections. The cystic fibrosis (CF) lung environment represents an ideal location for diverse microbes to interact.

Although diverse bacterial species colonize the airways of individuals with CF, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two key pathogens in terms of prevalence, sputum abundance, and associations with lung disease (2–4). *P. aeruginosa* has long been considered the dominant CF pathogen on the basis of circumstantial evidence. First, the U.S. CF Foundation Patient Registry reports an inverse relationship between the prevalence of both species; specifically, *S. aureus* declines during late teenage years, whereas *P. aeruginosa* increases (2) (Figure 1). Given the relatively high *S. aureus* prevalence in children and young adults (60–80%), *P. aeruginosa* is often believed to actively supplant *S. aureus* in secretions (5, 6). Second, *P. aeruginosa* outcompetes and suppresses *S. aureus* growth during *in vitro* coculture by producing antistaphylococcal compounds (7), which are induced by *S. aureus* (8) and are detected in CF sputum (9, 10). *P. aeruginosa* benefits from iron released after *S. aureus* lysis (11), potentially explaining why it targets *S. aureus*.

Based on this evidence, an assumption is that *P. aeruginosa* suppresses *S. aureus* in the CF lung, shifting prevalence with age. However, the Patient Registry figure depicts cross-sectional rather than longitudinal data of individuals and lacks quantitative culture results to measure changes in bacterial abundance (Figure 1). Rather than succumbing to *P. aeruginosa* antagonism, *S. aureus* may simply disappear with age, either in response to host physiological changes or with treatment, allowing other pathogens to occupy the space. *S. aureus* prevalence and coinfection rates with

P. aeruginosa have risen over the last decade (2), which also challenges the assumed *in vivo* dominance of *P. aeruginosa* over *S. aureus*. Finally, *in vitro* coculture models involve cells in a metabolically active state and in physical contact or immediate proximity to each other, both of which may not exist *in vivo*.

In this issue of the *Journal*, Fischer and colleagues (pp. 328–338) thoughtfully scrutinize the dynamic relationship between *P. aeruginosa* and *S. aureus*. The authors examined retrospective, longitudinal, and quantitative culture data from people with CF who regularly expectorated sputum (12). This large collection of culture data provided a unique resource to assess the presence and densities of these pathogens over an extended timeframe. Among patients with CF who provided ≥10 sputum or BAL quantitative cultures over 13 years, a majority of patients had cultures positive for each organism, high rates of simultaneous coinfection, and high bacterial densities of each (median $\log_{10}$ colony-forming units/ml of 6.52 for *P. aeruginosa* and 6.42 for *S. aureus*). This permitted longitudinal analyses of changes in culture abundance after acquisition of the competing species or during simultaneous coinfection. Contrary to the longstanding assumptions of *P. aeruginosa* dominance, the authors found that *S. aureus* had stable, long-term coexistence with *P. aeruginosa* in CF samples. Regardless of whether *S. aureus* preceded the introduction of *P. aeruginosa* or whether both organisms were cocultured early in the study, *S. aureus* bacterial densities did not decline with time in the presence of *P. aeruginosa*. Interestingly, coinfections actually increased rather than decreasing over time, and replacement of *S. aureus* by *P. aeruginosa* rarely occurred. In comparison, *Haemophilus influenzae*, another early CF pathogen, did not compete well against either organism. *S. aureus* is typically categorized according to methicillin susceptibility (methicillin-susceptible *S. aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA]). National surveillance has shown an increase in MRSA culture positivity in U.S. patients with CF from 2000 to 2010 (2). This increase was even more pronounced at the authors' center, raising doubts whether persistence was related to changes in *S. aureus* susceptibility rather than resilience to *P. aeruginosa* antagonism. To address this question, the authors repeated their analyses for MSSA and MRSA individually and found both subtypes had similar durations of infection and maintained high culture abundances when coinfecting with *P. aeruginosa*, although MRSA sputum densities were generally higher and persisted longer than MSSA.

Because these findings are provocative, a number of questions emerge. How generalizable are these single site findings to the broader CF population and other centers? The authors analyzed quantitative culture results from subjects who expectorated. These subjects represented ~40% of the clinic population, were relatively older, had worse lung disease, and had higher *P. aeruginosa* culture rates than the other patients with CF in the center. The interactions
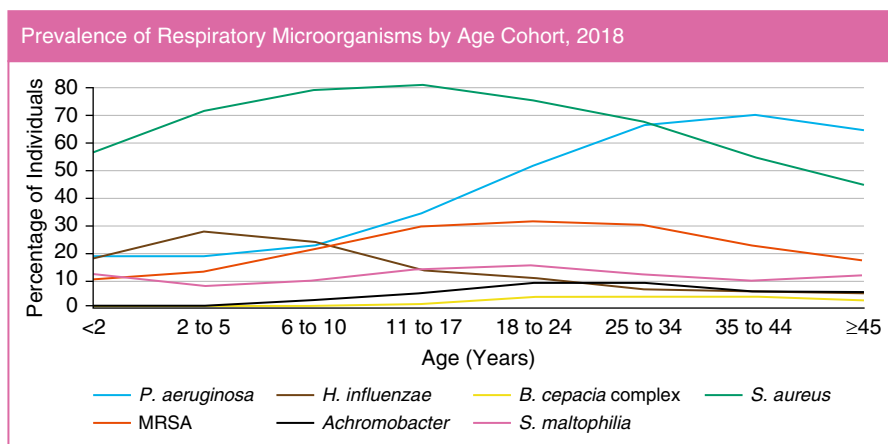
Figure 1. Data from the U.S. Cystic Fibrosis Foundation Patient Registry showing the prevalence of individuals with cystic fibrosis who had positive culture results for a bacterial species according to age group during 2018. The age cohort is presented on the *x*-axis, and the percentages of positive culture results are displayed on the *y*-axis for the following organisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus* (methicillin-susceptible), MRSA, *Haemophilus influenzae, Achromobacter* species, *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*. Although coinfections are not displayed, individuals may have been colonized with multiple organisms. Reprinted by permission from Reference 2. MRSA = methicillin-resistant *S. aureus*.

between *P. aeruginosa* and *S. aureus* may differ in younger, healthier patients with newly acquired bacteria. For example, persistent (late) CF *P. aeruginosa* isolates lose their competitive advantage *in vitro* over *S. aureus* compared with recently acquired (early) *P. aeruginosa* isolates (13, 14). In addition, the relatively high MRSA rates at this center raise doubts about whether treatment and antibiotic susceptibility alter outcomes between *P. aeruginosa* and *S. aureus*. A prospective, multicenter study encompassing a larger, younger, and healthier patient population with lower MRSA prevalence and including a longitudinal linkage between microbiology and antibiotic usage would alleviate these limitations.

Regardless of limitations, these results suggest that *S. aureus* is more resilient than previously believed, but how does *S. aureus* coexist with *P. aeruginosa in vivo*, given *P. aeruginosa's in vitro* dominance? These two species may be compartmentalized within the airways and only mix on expectoration. The concept of microbial compartmentalization in the CF airway has previously been demonstrated (15). Alternatively, *S. aureus* may adapt to the presence of *P. aeruginosa*, facilitating its *in vivo* coexistence. For example, *P. aeruginosa* and certain antibiotics select for *S. aureus* small colony variants *in vitro* that are tolerant to *P. aeruginosa* antagonism (9). The detection of small colony variants was not evaluated in this report.

Importantly, what does this information mean for the health and care of people with CF? Patients with CF coinfected with *P. aeruginosa* and *S. aureus* reportedly have worse respiratory outcomes than those with single infections (16). Characteristics associated with persistence, pathogenesis, and response to therapy of each species were affected by interactions between these bacteria *in vitro* (7). Fischer and colleagues (12) conclude that *P. aeruginosa* and *S. aureus* can coinfect for much longer than previously anticipated. These results suggest that concurrent treatments directed at both organisms may improve CF clinical outcomes. ∎

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The Lung Microbiome in Health, Hypersensitivity Pneumonitis, and Idiopathic Pulmonary Fibrosis: A Heavy Bacterial Burden to Bear

The microbiome regulates human health and immunity, contributing robustly to physiological homeostasis. The healthy lung is not sterile and consists of bacterial communities that exist in a relatively low biomass state and correlate with local immunity (1, 2). In the diseased lung, there is a growing recognition of the potential mechanistic role of alterations or "dysbiosis" of lung microbiota (3). In particular, the lung microbiome has been implicated in the pathogenesis of idiopathic pulmonary fibrosis (IPF).

Studies in patients with IPF demonstrate that an increased burden of lung microbiota, as well as specific taxa such as *Streptococcus* and *Staphylococcus*, is associated with an elevated risk of disease progression and/or mortality (4–6). Lung microbiota are associated with innate immune activation signatures in peripheral blood (7), and increased α diversity of lung microbiota correlates with lower alveolar inflammation (4). In animal models, lung dysbiosis precedes fibrosis and eradication of the microbiome significantly ameliorates fibrosis, suggesting a causal role for the lung microbiome, possibly through the activation of immune pathways (4, 8). Recently, Invernizzi and colleagues reported an

absence of correlations between key radiological markers and physiological features of IPF and lung bacterial burden, demonstrating that the increased bacterial burden reported in IPF is not simply the direct result of architectural distortion and parenchymal destruction (9). This addressed a key question in the field. However, it remains unknown whether these observations in IPF are universal to all interstitial lung disease (ILD).

Chronic hypersensitivity pneumonitis (CHP) is an enigmatic clinical syndrome and common form of ILD that frequently proves fatal. Both CHP and IPF share fibrotic remodeling of the lung parenchyma, may be indistinguishable by radiographic studies/histopathology, and respond to therapy directed at progressive fibrosis, supporting shared mechanistic pathways (10). Yet, patients with IPF fundamentally differ in prognosis (poorer in IPF, better in CHP), the presence of environmental antigen exposures (generally absent in IPF), and response to immunosuppression (detrimental in IPF, often beneficial in CHP), suggesting important differences between these diseases that are poorly understood.

In this issue of the *Journal*, Invernizzi and colleagues (pp. 339–347) report their findings on CHP and the lung microbiome in an elegant study (11). The authors compared key features of the healthy lung microbiome, the CHP microbiome, and the IPF lung microbiome. Patients with CHP exhibited a significantly lower lung bacterial burden compared with patients with IPF, although they still had greater lung bacterial burden compared with healthy subjects. However, bacterial burden was not associated with mortality in patients with CHP, unlike IPF, which is a fundamental new clinical observation. Furthermore, there were distinct differences in lung microbial composition between CHP