COMMENTARY

Cystic fibrosis and the war for iron at the host–pathogen battlefront

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Cystic fibrosis (CF) is an autosomal recessive disease caused by genetic mutations in the CF transmembrane conductance regulator (CFTR), an anion transporter normally expressed in secretory glands of the lungs, pancreas, digestive tract, liver, skin, and reproductive organs. When functioning properly, the CFTR protein conducts intracellular chloride anions across an epithelial cell membrane into the extracellular space. In CF, a dysfunctional or absent CFTR protein results in the production of abnormally thick, sticky mucus in the respiratory tract, as well as impaired secretion of bodily fluids, like digestive enzymes, bile, sweat, tears, and semen. Although the clinical manifestations of CF are protean, most people with two abnormal *CFTR* alleles suffer some degree of digestive and respiratory disease and, particularly in males, infertility; currently, in the developed world, the median life expectancy in CF is around 40 y (1). *CFTR* gene defects are particularly common among persons of European heritage; ~1 in 30 non-Hispanic whites is a carrier of a mutant *CFTR* allele, and 1 of every 2,300 white infants is born with CF (2).

One of the dreaded complications of CF lung disease is infection with *Pseudomonas aeruginosa*, which is an independent risk factor for excess morbidity and mortality in children with CF (3). In PNAS, Hendricks et al. (4) explore respiratory virus infection as a potential facilitator of *P. aeruginosa* acquisition in the CF airway.

Environmental isolates of *P. aeruginosa* are responsible for initial infection in infancy or early childhood, and subsequent transient infections are common in CF (Fig. 1). However, over time *P. aeruginosa* undergoes genetic adaptations to the CF airway, including increased production of alginate, an exopolysaccharide that confers a slimy mucoid coat; decreased synthesis of flagellin, a protein that enables bacterial movement and navigation; and modifications to the cell wall component lipopolysaccharide (LPS). In general, these genetic modifications decrease the virulence of the organism but enhance its ability to survive in the infected host. The mucoid alginate coating protects the bacterium from phagocytosis, and alterations in flagellin and LPS, potent Toll-like receptor ligands, prevent induction of protective innate immune responses (3, 5). These genetic adaptations occur independently in different isolates over time; however, eventually, clonal selection of a specific genotype ensues, which then predominates in the chronic phase of infection (3, 5, 6).

During persistent infection, *P. aeruginosa* also transitions to a communal mode of growth in biofilm, a sticky matrix of proteins, polysaccharides, and nucleic acids, secreted by surface-adherent bacteria, within which they replicate. Biofilm formation confers structural integrity to the bacterial colony and protects it from host defenses and antibiotics alike, in part by

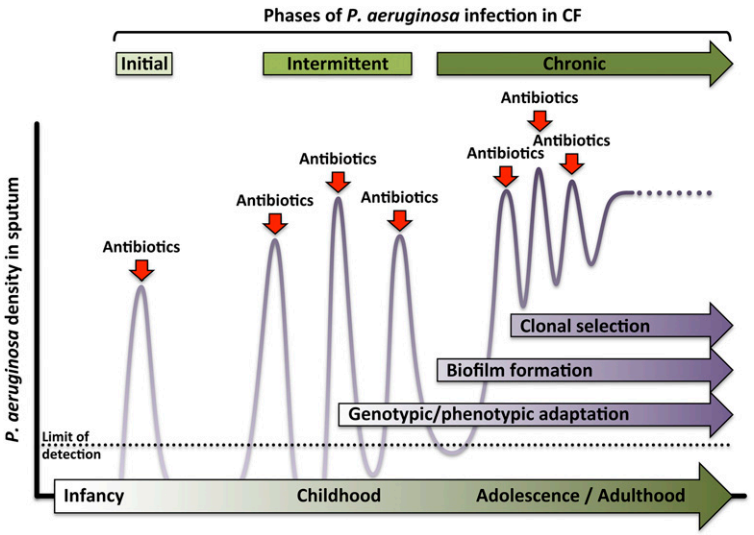

Fig. 1. Typical time course of *P. aeruginosa* colonization of the CF respiratory tract. Initial acquisition of a wild-type environmental isolate of *P. aeruginosa* is treated with antibiotics and eradicated. During subsequent intermittent infections, genetic adaptations to the CF airway result in impaired bacterial clearance. These adaptations include: a transition to a biofilm mode of growth, production of a mucoid coating to elude phagocytosis, altered expression of virulence factors like flagellin and LPS, and enhanced resistance to antibiotics. Eventually a dominant genotypic clone emerges, which continues to adapt to its host.

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blocking their diffusion into the biofilm interior. Antibiotic concentrations of 1,000–10,000 times greater than those needed to kill a free-floating bacterium are required to kill bacteria in biofilms (7); thus, during repeated or prolonged antibiotic therapy, drug-resistant genotypes can be selected in biofilm-embedded bacteria exposed to subinhibitory antibiotic concentrations. Current practice favors early, aggressive treatment of *P. aeruginosa* infections in children with CF, with a goal of delaying the onset of chronic colonization and the clinical complications that follow (1, 5). In the past decade, the percentage of CF patients infected with *P. aeruginosa* has declined overall; however, ~20% of adults with CF are colonized with multidrug-resistant isolates, likely resulting from cumulative antibiotic exposures (1).

Epidemiological studies have linked wintertime (8), and viral respiratory tract infections in particular (9, 10), with the acquisition of *P. aeruginosa* by CF patients. Viral-bacterial synergism in human disease has been well described between influenza virus and bacterial species that cause pneumonia, particularly *Streptococcus pneumoniae* and *Staphylococcus aureus* (11, 12). Previous research demonstrated that antecedent respiratory syncytial virus (RSV) infection promotes the adherence of *P. aeruginosa* to lung epithelium in vitro (13) and enhances bacterial replication in mice in vivo (14).

In PNAS, Hendricks et al. (4) extend these observations mechanistically, describing a dysregulation of nutritional immunity—particularly iron homeostasis—that accompanies respiratory virus infection and promotes *Pseudomonas* biofilm growth. Because most microbes require iron for metabolism and replication, humans have evolved elaborate iron-withholding defenses to keep it from invading pathogens. Iron stores are scrupulously maintained, primarily by intracellular sequestration in complex with hemoglobin or ferritin. Free iron is scarce; what little there is becomes rapidly bound up by extracellular transferrin or lactoferrin. As a consequence, nearly all bacteria use iron-snatching counter-measures, such as erythrocyte-destroying hemolysins or siderophores that chelate iron with greater affinity than host storage proteins (15). In acute infection, *P. aeruginosa* produces a major siderophore, pyoverdine, which scavenges ferric iron and imparts a characteristic green tint to its colonies in culture. In this arms race for iron, CF confers a distinct disadvantage to the host side. Clinical data suggest that iron and iron-binding proteins are inherently elevated in the sputum of CF patients, regardless of their bacterial colonization status (16), and in vitro experiments imply that defective CFTR underlies this baseline dysregulation of iron homeostasis (17). Now, Hendricks et al. (4) show that a respiratory virus infection can further sabotage host iron reserve mechanisms, enabling the transition to a biofilm growth mode in concurrent *P. aeruginosa* infection.

In an immortalized human CF bronchial epithelial cell line, homozygous for the *CFTR* mutation ΔF508, Hendricks et al. (4) demonstrate that infection with RSV, human rhinovirus 14, or human adenovirus 5 promotes biofilm formation upon subsequent *P. aeruginosa* infection. RSV-induced biofilm development was also observed in primary bronchial epithelial cells from both healthy donors and those with CF. The authors go on to show that *Pseudomonas* biofilm formation on CF epithelial cells is enhanced by RSV-induced type III IFN secretion at the apical cell membrane, accompanied by apical release of both iron and transferrin. Finally, in a neonatal mouse model, they demonstrate that levels of both iron and transferrin are higher in bronchoalveolar lavage fluid (BALF) from RSV-infected pups, compared with that from mock infections, and that BALF from the RSV-infected mice also supports in vitro *P. aeruginosa* biofilm formation. From these data, the authors suggest that the host innate immune response to viral respiratory infection, particularly a dysregulation of iron homeostasis, creates a microenvironment particularly favorable for biofilm production by *P. aeruginosa*.

Other respiratory pathogens—particularly *S. aureus* and *Haemophilus influenzae*—are commonly acquired by children and adolescents with CF. However, as CF patients age, *Pseudomonas* persists and predominates in the lung, often to the exclusion of other bacteria. Approximately two-thirds of all adults with CF are chronically colonized with *P. aeruginosa* (1). Why this occurs with *P. aeruginosa* in particular, and less so with the iron-requiring, biofilm-producing *S. aureus*, is not clear. Interestingly, Hendricks et al. (4) observed that RSV infection promotes *P. aeruginosa* biofilm growth on both primary CF and non-CF human bronchial epithelial cells. Although *CFTR* mutations are independently associated with excess iron secretion (16, 17), BALF from RSV-infected BALB/cJ mouse pups, which express wild-type CFTR, was also enriched in iron and able to promote in vitro biofilm formation by *P. aeruginosa*. Thus, respiratory virus-induced dysregulation of iron homeostasis appears to occur regardless of host *CFTR* genotype. Perhaps the iron-releasing effects of CFTR dysfunction and RSV infection are additive during acute infection with *P. aeruginosa*, promoting biofilm formation and thus persistence in CF airways in particular. However, viral respiratory infections do not appear to affect the presence or density of *Pseudomonas* in the sputum of CF patients already chronically colonized with it (18, 19).

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If respiratory virus infections do promote *P. aeruginosa* acquisition in CF, the question arises as to what to do about it. As every parent knows, viral respiratory infections are all too common in childhood, with or without CF. RSV infects most infants during their first year of life, with nearly all children infected at least once by the age of 2 y (20). We lack vaccines or antiviral treatments for most respiratory viruses; for RSV, a monoclonal antibody directed against the surface fusion (F) protein, palivizumab, is given monthly during RSV season to premature infants and others at high risk for severe RSV disease (20). However, very limited clinical data have as yet failed to show a benefit with palivizumab prophylaxis in preventing either RSV infection or *Pseudomonas* colonization in infants with CF (21, 22). More antiviral vaccines and therapeutics are clearly needed for all children; in the meantime, compounds that help sequester iron away from bacteria might have clinical application in CF, as Hendricks et al. suggest (4). Indeed, gallium, an iron mimetic that is taken up by *P. aeruginosa* but is metabolically unable to substitute for it, is bactericidal in animal models and is in clinical trials in CF (23). Ultimately, in the absence of vaccines against the myriad of respiratory viruses that children face, antimicrobial therapies that give the host side an edge in the arms race for iron may help to level the battlefield for patients with CF.

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