Clinical Implications of Basic Research

Biofilms, Antimicrobial Resistance, and Airway Infection

THE use of susceptibility testing to identify appropriate antimicrobial agents has long been an important element of the practice of infectious-disease medicine. In most clinical settings, the finding that a strain of bacteria is susceptible to a specific antibiotic is a reliable indicator of the effectiveness of that drug. An exception is the treatment of pulmonary infection in patients with cystic fibrosis. Once chronic *Pseudomonas aeruginosa* infection is established in the lungs of these patients, the bacteria are rarely, if ever, eradicated despite treatment with combinations of antimicrobial agents with demonstrated potency in vitro. Even direct administration of aerosolized antibiotics and the use of optimal dosing regimens fail to clear all the organisms from the lung.

Most patients with cystic fibrosis have a response to appropriate antimicrobial therapy even if sputum cultures continue to be positive for *P. aeruginosa*. However, some have no clinical or bacteriologic response, and experienced clinicians may resort to the use of empirical therapy in these patients and even prescribe chloramphenicol and polymyxin B sulfate — drugs that are generally avoided by the rest of the medical community but that occasionally induce clinical responses. The failure to eradicate susceptible bacteria from the lungs of patients with cystic fibrosis, who already have a large bacterial burden (10^8 to 10^9 colony-forming units per milliliter), can result not only in selection for and persistence of multidrug-resistant organisms but also in infection with exotic opportunists, such as *Achromobacter xylosoxidans, Stenotrophomonas maltophilia,* and *Burkholderia cepacia* complex.

A new appreciation for the complexity of the biologic makeup of *P. aeruginosa* has provided important insights into lung infection in patients with cystic fibrosis. These opportunistic bacteria thrive in a free-living, planktonic form or in biofilms, which are highly structured communities that coat rocks in streams, faucets, catheters, and the mucosal surfaces of airways. In addition to the mucoid (alginate-producing) organisms that are typically present in the lungs of patients with cystic fibrosis, nonmucoid *P. aeruginosa* form biofilms. The coordinated expression of diverse groups of genes within this community of bacteria is directed by small, diffusible molecules called quorum sensors. The quorum sensors produced by *P. aeruginosa* include several different homoserine lactones that are highly diffusible both within and outside the organisms.

In the normal host, inadvertently inhaled bacteria, perhaps from showers, faucets, hot tubs, puddles, or other aqueous environments, are cleared by the mucociliary escalator and the innate defenses of the airway. Under such conditions of low bacterial density, bacterial expression of quorum sensors is negligible and planktonic growth predominates (Fig. 1). A recent study demonstrated that in the normal lung, lactoferrin, a component of the innate defense system, actively protects against the formation of a biofilm by blocking the primitive motility system of *P. aeruginosa*.[1] If, however, the number of organisms increases greatly, as would occur in airways that are occluded by mucin plugs, quorum sensors are secreted by the bacteria and achieve a critical density. These small signaling molecules then diffuse back into the organisms, where along with transcriptional activators (LasR and RhlR), they coordinate the expression of virulence genes that allow the bacteria to evade the host's immune system and thus foster the survival of the bacterial community. The bacterial products regulated by these genes include proteases, hemolysins, iron-scavenging pigments, catalase, and superoxide dismutase, which protect the bacteria from the host's phagocytic cells and facilitate colonization of the lung.

For many reasons, bacteria in biofilms are much harder to eradicate than those growing in the planktonic form. Within biofilms — and especially within biofilms in the airways of patients with cystic fibrosis, which are organized behind mucin plugs — the low oxygen tension and the limited availability of iron foster the anaerobic growth of *P. aeruginosa* and slow the rates of cell division. Under these conditions, the activity of β-lactam antibiotics is poor, since they target actively dividing bacteria, and the aminoglycosides have limited efficacy, since they act on aerobically growing organisms.

A fuller explanation of the resistance of *P. aeruginosa* in biofilms to antibiotic therapy was recently provided by Drenkard and Ausubel.[2] These investigators determined that a single genetic locus in *P. aeruginosa* is associated with both the ability to form biofilms and antimicrobial resistance. Aminoglycoside-resistant, small-colony variants have long been observed among the multiple phenotypes of *P. aeruginosa* isolated from sputum cultures of patients with cystic fibrosis. Because of their slow rate of growth and the high rate of reversion to the wild type, their clinical significance was not appreciated. Drenkard and Ausubel noticed that these variants had an increased ability to attach to glass and plastic surfaces and were especially proficient in forming biofilms. These colonies were also resistant to several classes of antibiotics, including aminoglycosides, β-lactams, and tetracyclines. Using classic bacterial genetic techniques, they identified a locus that they called *pvrR* (phenotype vari-

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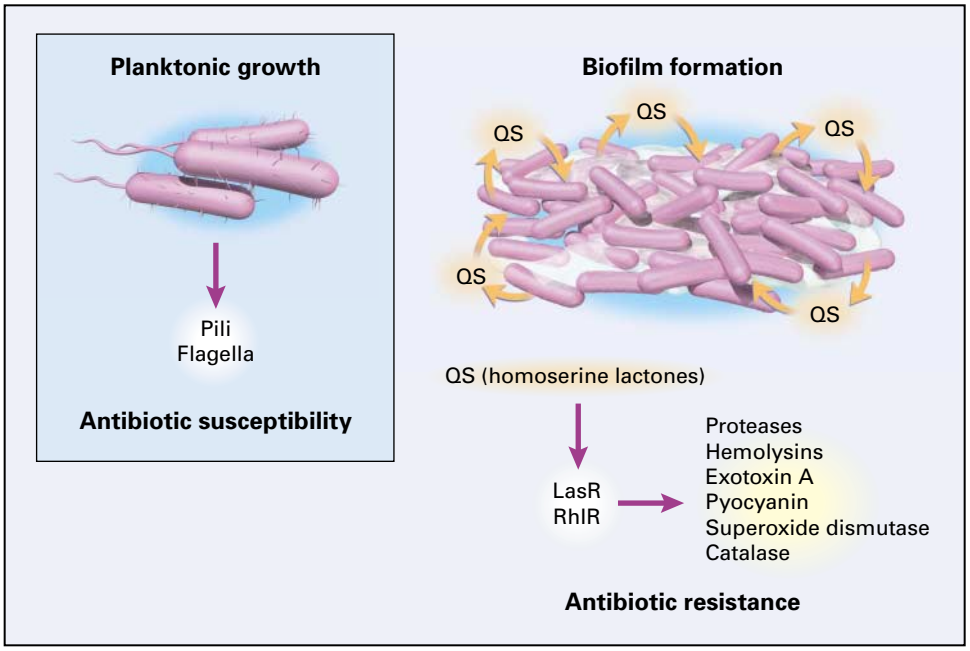

Figure 1. The Formation of a Biofilm by *Pseudomonas aeruginosa.*

In the normal host, inadvertently inhaled bacteria are cleared by the innate defenses of the airway. Under such conditions of low bacterial density, bacterial expression of quorum sensors (QS), which are diffusible homoserine lactones, is negligible and planktonic growth (left-hand panel) predominates. Lactoferrin, a component of the innate defense system, actively protects against the formation of a biofilm by blocking the primitive motility system of *P. aeruginosa*. Under conditions of high bacterial density (right-hand panel), quorum sensors are secreted by the bacteria and freely diffuse within the bacterial community. Quorum sensors interact with transcriptional activators LasR and RhlR to direct the expression of several factors that facilitate the persistence of bacteria in the lung, such as proteases, hemolysins, exotoxin A, pyocyanin, superoxide dismutase, and catalase, and thus enable the organisms to evade the effects of antibiotics.

ant regulator), apparently a two-component response regulator in *P. aeruginosa* that directs gene expression in response to specific environmental conditions. The *pvrR* locus was found in all seven bacterial samples isolated from patients with cystic fibrosis. Overexpression of this gene resulted in antibiotic-susceptible strains with a decreased propensity to form biofilms. Although these studies did not identify the mechanism associated with both the increased ability to form biofilms and multidrug resistance, the finding that a single locus is associated with both phenotypes suggests that they may be related.

Thus, the bacteria in a biofilm formed in the lung are inherently resistant to antibiotics. This characteristic is not evident when the same clones are grown planktonically in vitro. For this reason, the susceptibility of *P. aeruginosa* to antibiotics in the laboratory, even when susceptibility testing is performed under ideal in vitro conditions, may not reflect the true susceptibility of the organisms in vivo.

These studies provide some justification for selecting antimicrobial therapy for patients with cystic fibrosis on the basis of their clinical response and not just on the basis of the results of susceptibility testing. Unfortunately, organisms within biofilms lining the airways of patients with cystic fibrosis are likely to remain less susceptible to antibiotics than are the bacteria isolated from sputum cultures. It is intellectually satisfying, however, to have bacteriologic justification for therapeutic decisions that are based on clinical acumen.

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