Evolving stealth: Genetic adaptation of *Pseudomonas aeruginosa* during cystic fibrosis infections

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Most patients with the genetic disease cystic fibrosis (CF) succumb to chronic airway infection caused by the bacterium *Pseudomonas aeruginosa*. CF lung disease follows a characteristic pattern. Early in life, patients suffer from transient airway infections with *P. aeruginosa* that resemble bronchitis. Genetic fingerprinting studies show that these infections are distinct events; infection develops and is subsequently cleared, and the next bout is caused by a new strain acquired from the environment (1). Unfortunately, this stage is temporary as the airways of nearly all patients become permanently colonized by *P. aeruginosa*. From this point forward, the same bacterial lineage can persist continuously in the lungs for years or even decades and cannot be eradicated by any known therapy (2).

Much research in CF has focused on how chronic infection affects the patient because inflammation from infecting bacteria causes persistent respiratory symptoms and an inexorable decline in lung function. However, the onset of chronic infection is also transformative for the bacteria because an environmental *P. aeruginosa* strain (that may have been living in, say, a water pipe) must adjust to the alien conditions of the lung and live long-term within the host. Hope for developing new treatments rests in part on understanding how bacteria adapt to the airway and resist host defenses and antibiotics. The innovative work by Smith *et al.* in this issue of PNAS (3) explores this question by using a powerful tool: whole-genome sequencing.

Smith *et al.* (3) sequenced the genome of a *P. aeruginosa* strain early in a CF infection and the descendent of that strain, still present in the patient's lungs, 7.5 years later. Sixty-eight mutations were found in the late isolate. Most were single-base pair changes, and many were predicted to result in a change or loss of protein function. To construct a timeline of the strain's evolution, Smith *et al.* genotyped strains collected at intermediate times. They also investigated whether the identified mutations were common in CF by genotyping paired (early and late) *P. aeruginosa* isolates from 29 other patients and found some genes that were mutated in most CF patients.

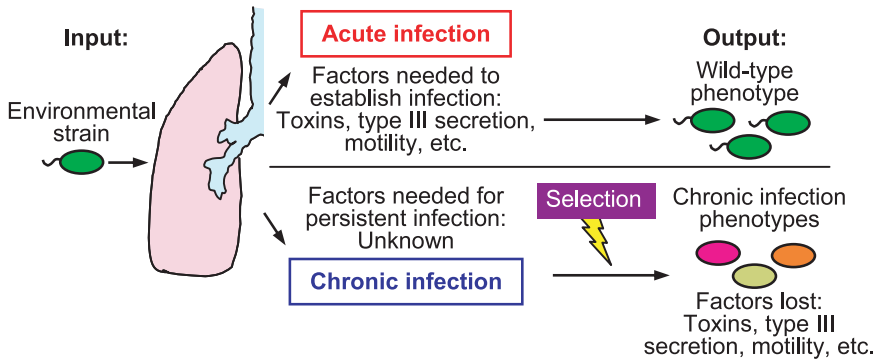

Fig. 1. The factors needed for acute infections are generally well understood, whereas those needed for chronic infection are not. Bacterial functions needed for acute infection are selected against in chronic CF infections.

Genes can evolve by two general mechanisms: natural selection and neutral evolution which occurs when mutations that do not affect fitness accumulate because the individuals harboring them increase in number by chance (4). These two mechanisms can be distinguished by measuring the ratio of mutations that do not change protein sequence (synonymous mutations) to those that do (nonsynonymous mutations). In neutral evolution, synonymous mutations are frequent as these changes are selectively neutral, or nearly so. Selection increases nonsynonymous mutations as they can affect protein function. The relative importance of selection and neutral evolution is debated and depends on the population size, strength of selection, and other factors, all of which are uncharacterized *in vivo*. Distinguishing between the two mechanisms is important because knowing which genes evolve by selection tells us about conditions in the lung and how bacteria adapt.

Smith *et al.* (3) found a remarkably high ratio of nonsynonymous to synonymous mutations during the evolution of *P. aeruginosa* in the lungs of CF patients. This high ratio was seen in the whole-genome sequence comparison of the early and late isolate from the index patient and also when comparing candidate genes from early and late isolates from 29 other CF patients. These results strongly point toward selection; in other words, many of the genetic changes observed were advantageous for life within the host.

An almost universal finding in the CF infections studied by Smith *et al.* (3) was that selection favored the loss of functions used by bacteria to invade and injure the host. Examples include motility, type III secretion, O antigen biosynthesis, exotoxin, protease, and phenazine production, among others. These findings are consistent with earlier work examining the phenotypic changes that develop during CF infections (5–13).

One of the genes most commonly mutated in the present study was the principal quorum-sensing regulator, *lasR*. *P. aeruginosa* uses quorum sensing to coordinate the expression of several invasive functions. Mutants with inactivated central regulators like *lasR*, and others (such as *vfr*) may have a particularly strong selective advantage as many invasive functions are eliminated with one fell swoop.

Why are these functions so disadvantageous during chronic infection? Smith *et al.* (3) suggest that their loss could help bacteria evade host defenses because many invasive factors are also ligands for the immune system. This idea is appealing as infecting bacteria are subject to an intense immune response. However, immune evasion seems likely to be advantageous in both acute and chronic infections; so why are these factors required for acute infections but detrimental in chronic infections? Part of the explanation

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may lie in differences in the host environment that alter the cost–benefit balance of expressing invasive functions.

The conditions present in acute infections may force pathogens to "injure the host or die." If nutrients were scarce, for example, host injury may then be critical for growth and survival. The value of host injury could also increase in the face of an intense acute immune response. In contrast, many chronic infections occur in damaged tissues where nutrients may be more available and immune responses blunted. In these environments, the benefits of invasive functions may be diminished, leading to their loss over time. The actual situation is likely to be far more complex because the relative value of various functions may change during the course of infection; invasive functions may be needed initially but become burdensome once infection is established.

The finding that invasive functions are selected against in CF infections points out a paradox; many of these functions are considered to be "virulence factors," a designation that suggests that they are needed for infection. Historically, functions have been defined as virulence factors if they are important in acute infections. However, the results from Smith *et al*. (3) and other investigators (14–16) suggest that we may need to expand the concept of virulence to distinguish between factors required for acute and chronic infection.

A more complete view may be to designate functions as "invasive factors" if they promote acute infection or dissemination within the host and as "persistence factors" if they are needed for chronic infection (Fig. 1). Both groups are important for virulence, but in very different ways. Currently, much more is known about the first category than the second. The work by Smith *et al*. (3) is an important step forward in this regard. However, it tells us more about what is disadvantageous in chronic infection as opposed to what is needed. Progress will require the development of sophisticated chronic infection models (like those for acute infections) that allow us to directly determine what functions are required for bacterial persistence. These functions may be targets of future drugs designed specifically for action against chronic infections.

Perhaps the most intriguing finding from this study relates to the bacterial diversity that may exist in chronic infections. For most of the study, investigators examined only one bacterial isolate from each airway specimen, but at a few time points, more than one isolate was genotyped. When multiple isolates were studied, the investigators found that various bacterial genotypes coexist within the lung. For example, the specimen obtained when the patient was 3 years of age contained five different *P. aeruginosa* genotypes, all related to the initial isolate.

This finding may be best understood if one views chronic infections from an ecological perspective. Population diversity increases in ecosystems when environmental conditions vary in time and space (17). Heterogeneous environments tend to maintain diversity because different genotypes are often better suited for different local conditions. Furthermore, as conditions change, new niches can arise, further increasing diversity. Environmental heterogeneity surely exists at sites of chronic infection. For example, a course of antibiotics, the obstruction of a lung segment by impacted secretions, or a bout of pulmonary bleeding all will dramatically alter local conditions. Such heterogeneity could maintain diversity in the infecting bacterial community (Fig. 2), and this diversity could have important consequences.

Ecologists have long recognized that diversity can enhance both the productivity and stress resistance of many types of biological communities. This has been explained by the "insurance hypothesis," which posits that the presence of diverse subpopulations increases the range of environmental conditions in which some community members will survive, or even thrive (18). "Insurance effects" could be of great benefit to infecting bacterial populations because, like other communities, their long-term persistence depends on their ability to withstand environmental stress. If the degree of bacterial diversity detected at the 3-year time point in this CF patient reflects a common situation, insurance effects may contribute to the ability of chronic infections to persist in the face of host defenses and antibiotics.

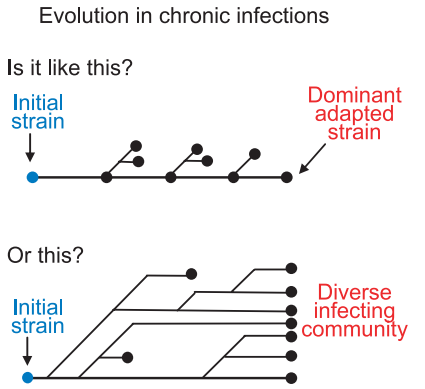

Fig. 2. Hypothetical phylogenetic trees of bacterial evolution in chronic infections. The blue dot represents the initial infecting strain, and the black dots represent genetic variants descendent from the initial strain. Evolution could produce a dominant adapted strain or a diverse community of infecting bacteria.

The work by Smith *et al*. (3) and other investigators studying CF *P. aeruginosa* isolates has opened a window to complex questions of adaptation, evolution, and diversity in chronic bacterial infections. The challenge ahead is to expand these findings, determine their generality, and develop tractable models so that we can target the functions required for bacterial persistence.

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