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Bacterial communication and group behavior

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Perspective Series

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SERIES INTRODUCTION

Bacterial communication and group behavior

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The existence of species-specific and interspecies bacterial cell-cell communication and group organization was only recently accepted. Researchers are now realizing that the ability of these microbial teams to communicate and form structures, known as biofilms, at key times during the establishment of infection significantly increases their ability to evade both host defenses and antibiotics. This Perspective series discusses the known signaling mechanisms, the roles they play in both chronic Gram-positive and Gram-negative infections, and promising therapeutic avenues of investigation.

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The past decade has seen the emergence of a new field in basic microbiology. The new basic knowledge has led to interest and activity in developing therapeutic agents to treat certain kinds of persistent bacterial infections. Scientists had long held the view that bacterial cells behaved as self-sufficient individuals, unable to organize themselves into groups or communicate. During infection, the bacterial mass was considered nothing more than the sum of these individuals. I have called this the lone-wolf view of a bacterial cell (1). The idea that bacteria could function as groups and that individuals within the group could respond to the group as a whole seemed almost ludicrous. This sort of peer pressure was thought to be restricted to "higher organisms," like humans. Through hard work and creativity, a small band of microbiologists chipped away at this viewpoint, and now it is generally accepted that bacteria produce, and respond as groups to, chemical signals and that this interaction can lead to the coordination of group bacterial activities. This phenomenon has become known as quorum sensing (2–4). We also understand that groups of bacteria can form physical structures with unique characteristics, so-called biofilms (5, 6). Quorum sensing and biofilm biology have become very active areas in microbiology, and a large group of investigators is working on these fascinating aspects of bacterial biology, hoping to develop new therapeutic agents to treat associated persistent bacterial infections.

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The problem of bacterial group behavior in medicine

Of particular relevance to this Perspective series, we now understand that the ability of bacteria to function as groups is crucial in the development of a number of infectious diseases (6). In fact, biofilms cause a variety of persistent infections, including chronic middle ear infections, bone infections, heart valve infections, infections related to implanted medical devices, and lung infections in people with the autosomal recessive inherited disease cystic fibrosis (6). A recent report also indicated that the chronic nature of some urinary tract infections is related to the ability of the infectious agent *Escherichia coli* to form a biofilm (7). The armament of therapeutic agents available to treat bacterial infections today is restricted to antibiotics developed specifically to kill or stop the growth of individual bacteria. The development of these agents did not take into account the unique biology of bacterial groups. This is a problem for a number of reasons, not the least of which is that when bacteria are growing within a biofilm they lose their sensitivity to antibiotics. Thus biofilms result in persistent infections that cannot be resolved with standard antibiotic treatments (6). Because we have not considered the problem of group biology in bacteria until recently, good therapeutic strategies to treat biofilm infection are not available. One might imagine that bacterial communication systems represent an Achilles' heel, a fragile target for potential new anti-infective drugs. This idea has not escaped the notice of both academic investigators and scientists in the biotechnology industry. The reviews in this Perspective series will cover many interesting aspects of our basic knowledge about the diversity of quorum sensing systems and the relevance of these systems to bacterial diseases. Several of the reviews will also address issues surrounding the targeting of bacterial group behavior as a therapeutic approach for the treatment of certain persistent infections.

Beyond the small talk: quorum sensing controls virulence

The idea that bacteria can make species-specific extracellular chemicals, which signal the development of important traits, can be traced back to the 1960s (8, 9). Certain species of marine bacteria were shown to luminesce only when cultures reached sufficient cell density. The delay in luminescence in early culture growth was correlated to the bacterial production of an extracellular signal, later shown to be an acyl-homoserine lactone (10).

A pneumococcal phenomenon termed "natural competence" was also the subject of considerable scrutiny. This ability of the pneumococcus to take up DNA occurs in the late logarithmic stage of culture growth when cell density is high. DNA uptake has been shown to be dependent on the "competence factor," an extracellular signaling molecule that accumulates in the growth medium at high cell density (9). We now know that the competence factor is a peptide signal (11). These first prescient reports (8, 9) were, at best, ignored by most microbiologists. Subsequent identification of the chemical nature of these bacterial signals and identification of the signal receptors gradually led to acceptance of the idea that communication among bacterial cells was possible. Yet most researchers considered bacterial communication to be isolated to only a few specific bacterial strains or species, and not important to bacterial virulence. Approximately 15 years ago, several independent studies reported that acyl-homoserine lactone–mediated signaling was not restricted to luminescent bacteria. Several different Gram-negative bacterial species were shown to make acyl-homoserine lactones (12), and genes encoding homologs of the acyl-homoserine lactone receptor were identified in plant and human pathogens (13, 14). In this Perspective series, Roger Smith and Barbara Iglewski discuss acyl-homoserine lactone signaling in the opportunistic pathogen *Pseudomonas aeruginosa* (15). Morten Hentzer and Michael Givskov discuss some of the known signaling mechanisms and potential antipathogenic drugs that specifically target these systems in a manner unlikely to pose a selective pressure for the development of resistant mutants — an increasing consequence of antibiotic treatment (16). One promising approach includes the production of synthetic agents that mimic endogenous anti–quorum sensing compounds produced by certain algae that successfully inhibit bacterial surface colonization.

Unlike Gram-negative bacteria, which use acyl-homoserine lactone signals, pneumococcal quorum sensing circuits use a small peptide signal, and we now understand that Gram-positive bacteria commonly use peptide signals in communication. Examples of these peptide-based signaling systems in pathogenic bacteria will be described by Dennis Cvitkvitch and colleagues in their review of *Streptococcus* quorum sensing (17), and by Jeremy Yarwood and Patrick Schlievert in their review of the control of virulence by quorum sensing in *Staphylococcus* (18).

A theme that will emerge from this series of reviews is that quorum sensing often controls genes involved in virulence. Quorum sensing allows a bacterial pathogen to coordinate the synthesis of extracellular virulence factors so that they are not expressed early in infection when the bacterial load is low. One can use a military analogy: the bacterial army does not display its weapons until the troops have amassed and are prepared to attack the host.

Now that it is generally accepted that bacteria can communicate and function as groups, new types of so-called quorum sensing systems are being described frequently. One particularly interesting example is the Autoinducer-2 system described in the Perspective by Michael Federle and Bonnie Bassler (19). In 1979, we reported that one particular marine luminescent baterial species could respond to signal molecules produced by other marine bacteria by activation of its luminescence genes. We called this nonspecific signaling "alloinduction" (20) and pursued it no further. Bassler and her colleagues have further investigated this signal and receptor and have recognized the implications of this phenomenon. They and others have shown that many different bacteria can sense and respond to this signal, and that this signaling mechanism also governs the expression of specific virulence factors. In their series Perspective, Federle and Bassler discuss their view that many bacteria use this common signal to monitor the general level of the surrounding microbial population and activity and that making a general measurement may afford some advantage to the group as a whole. As an aside, we have placed the review by Federle and Bassler first in this series because it provides a general overview of the mechanisms of several specific signaling systems as a way to introduce the non–species specific system on which they focus their work. So this review serves to introduce the reviews that follow.

The biofilm problem

Many of the Perspectives in this series on quorum sensing will touch on the subject of biofilms. Microbiologists have become increasingly interested in biofilms and their importance in medicine. Biofilms are groups of bacteria encased in a self-produced extracellular polymeric matrix. Modern imaging technology has revealed that biofilms are organized into heterogeneous groups of individual organisms. Even when the biofilm consists of

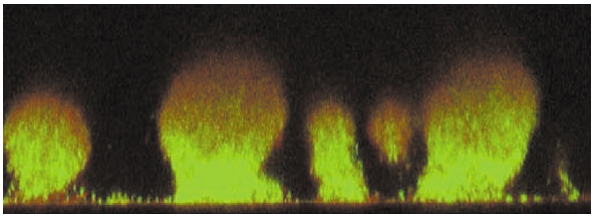

Figure 1
An example of differentiated structures in a single-species biofilm. Scanning confocal microscope image of a *P. aeruginosa* biofilm growing under a flow on a glass surface. The large (about 100 μm in height) differentiated mushroom-like structures are labeled with the green fluorescent protein expressed from a promoter controlled by quorum sensing and the red fluorescent protein expressed from a constitutively active promoter. The quorum-controlled product is found mostly in the base of the structures. Image provided by Yannick Lequette, University of Iowa.

a single bacterial species, elaborate structures are formed and bacteria within specific regions of the biofilm exhibit different activities when compared with bacteria in other regions (Figure 1). I have already discussed several examples of biofilm-based infections and some of the reasons why biofilms cause persistent infections not readily resolved by antibiotic treatment.

Investigators working on biofilms and those working on quorum sensing have a common interest in how bacteria function as a group. Our finding that, in certain bacterial species, quorum sensing can control how biofilms develop has served as a catalyst to bring the two fields of research together. A connection between quorum sensing and biofilm-pattern development in several bacterial species has been demonstrated. In some bacteria, including *P. aeruginosa* and the emerging pathogen *Burkholderia cepacia*, quorum sensing seems to play a role in the development of normal biofilm structures (21–23). In other bacteria, quorum sensing may play a role in the dispersal of individual organisms from the biofilm. This appears to be the case for the photosynthetic bacterium *Rhodobacter sphaeroides* (23). Although the connection between quorum sensing and biofilm development might entice one to think that biofilm infections can be controlled by interference with quorum sensing, this has yet to be established. Organisms like *P. aeruginosa* are still able to form biofilms, albeit abnormal ones, without quorum sensing. Hentzer and Givskov (16) discuss recent studies suggesting that quorum sensing might be a suitable target for anti-*Pseudomonas* biofilm therapy.

The quorum sensing–biofilm connection helped to establish that there is a genetic component to biofilm development. This, and a direct classical genetic-screening approach (24), have introduced new ways in which to study biofilm biology. The link between quorum sensing and biofilm development has brought together a large group of biologists interested in bacterial group dynamics. Later in this Perspective series, William Costerton and colleagues discuss the special biology of biofilms (25). Bacteria residing within biofilms can resist host defenses, and they also demonstrate a tolerance to antibiotics at concentrations that would kill them outside of the biofilm environment. The coalescence of scientists interested in bacterial group behavior into a common research field represents a potentially powerful force in the development of suitable therapeutics for cystic fibrosis lung infections and other biofilm-related diseases.

Conclusions

As with any new area in science, there is a risk of being naive in our enthusiasm. This emerging area in bacterial pathogenesis, communities, and cell-to-cell communication will benefit from the input of population biologists and ecologists, scientists who traditionally have not been deeply involved in research on infections. It has become clear that the special behavior of microbial groups can influence disease processes. We now need to investigate what selective pressures drive group behavior and to develop drugs designed to modulate bacterial group behavior, as opposed to bacteriocidal therapies. In developing these therapies for clinical application, we must overcome many hurdles. How would a clinical trial be designed? What sort of infections should be targeted as we gain the technical expertise to control bacterial group activities in a clinical setting? This Perspective series will address these challenges and others in the emerging field of bacterial communication and group behavior.

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