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Interrupting Bacterial Chatter to Thwart Infection

Interfering with communication among bacteria can prevent them from mounting a unified and perhaps deadly assault on their host organism, research by Howard Hughes Medical Institute (HHMI) investigators shows. The finding suggests a different kind of medicine that could be less likely than traditional antibiotic to promote the development of drug-resistant bacteria.

The new research, published July 30, 2009, in *Molecular Cell*, targeted a bacterial communication process known as quorum sensing, which triggers bacteria to act collectively only once they reach sufficient numbers to make their common activity worthwhile. In the case of disease-causing bacteria, that collective action is often the release of toxins.

Virulent bacteria do not want to begin secreting toxins too soon, or the host's immune system will quickly eliminate the nascent infection, explains HHMI investigator Bonnie Bassler, who led the research. So they use quorum sensing to count themselves, and launch their attack only when they reach a sufficiently high number. This way, the bacteria are more likely to overpower the immune system.

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- **Bonnie L. Bassler**

Quorum sensing is much like coordinating an army, says Bassler, who is at Princeton University. "These are the lines of communication from the generals to the crowd that make something happen. If you cut off those lines of communication, you have just individuals acting and you don't get the benefit of the collective, coordinated behavior."

The process gives bacteria some of the same advantages that multicellular organisms have, she adds. “You have all these cells doing the same thing at the same time, and the whole organism—the collective—benefits.”

To measure their own numbers, bacteria produce, release, and detect chemical signals called autoinducers. As a population of bacteria grows, it releases more autoinducer into its environment. When individuals detect that a threshold level of autoinducer is present, they change their behavior – by releasing a toxin, for example.

Bassler and her colleagues disrupted these lines of communication by interfering with molecules called acyl-homoserine lactone (AHL) autoinducers, which drive quorum sensing among a kind of bacteria known as Gram-negative bacteria. Gram-negative bacteria include *Pseudomonas*, *E. coli* and *Salmonella*, and other disease-causing microbes. In the study, the team focused on *Chromobacterium violaceum*, which rarely infects human, but can be lethal to other organisms. *C. violaceum* lends itself to studies of quorum sensing because it produces a readily detected, bright purple dye when it detects that its population has reached a critical mass.

Gram-negative bacteria have two types of receptors that detect AHL, ultimately triggering changes in the activity of the microbes’ virulence genes. One receptor type, LuxR, binds with the partner AHL autoinducer inside the cell; the other, LuxN, binds with its cognate AHL molecule outside the cell. The researchers developed inhibitors that each interfered with both receptor types.

Bassler says the team based its work on earlier research from her lab that screened 35,000 chemicals at the Broad Institute in Cambridge, Mass., co-founded by HHMI investigator Stuart L. Schreiber. That screen identified 15 chemicals that could interfere with LuxN-type receptors, inhibiting AHL quorum sensing.

Team member Lee Swem then had what Bassler called an inspired idea: to determine whether these same chemicals could also interfere with the LuxR-type receptors, even though the two types of receptors are evolutionarily unrelated and work by very different means and reside in different sub-cellular locations.

The researchers took one promising candidate from the Broad Institute screen and tweaked the chemical’s molecular structure to yield a host of similar chemicals, including two even stronger inhibitors, chlorothiolactone (CTL) and chlorolactone (CL).

“Lo and behold, they worked,” Bassler says of the chemicals’ impact on LuxR-type receptors. “That was quite remarkable because we didn’t have any evidence that they [the inhibitors] could get inside of the cell. So Lee’s work showed that the inhibitors could work from both the outside and the inside.”

The most potent of these inhibitors, CL, protected the roundworm *Caenorhabditis elegans* from death due to *C. violaceum* infection, without itself causing any apparent ill effects, the researchers found.

The experiment shows that interfering with quorum sensing may provide an alternative to traditional antibiotics, Bassler says, and circumvent the problem of resistance that antibiotics foster by killing off susceptible bacteria but allowing resistant ones to survive and propagate.