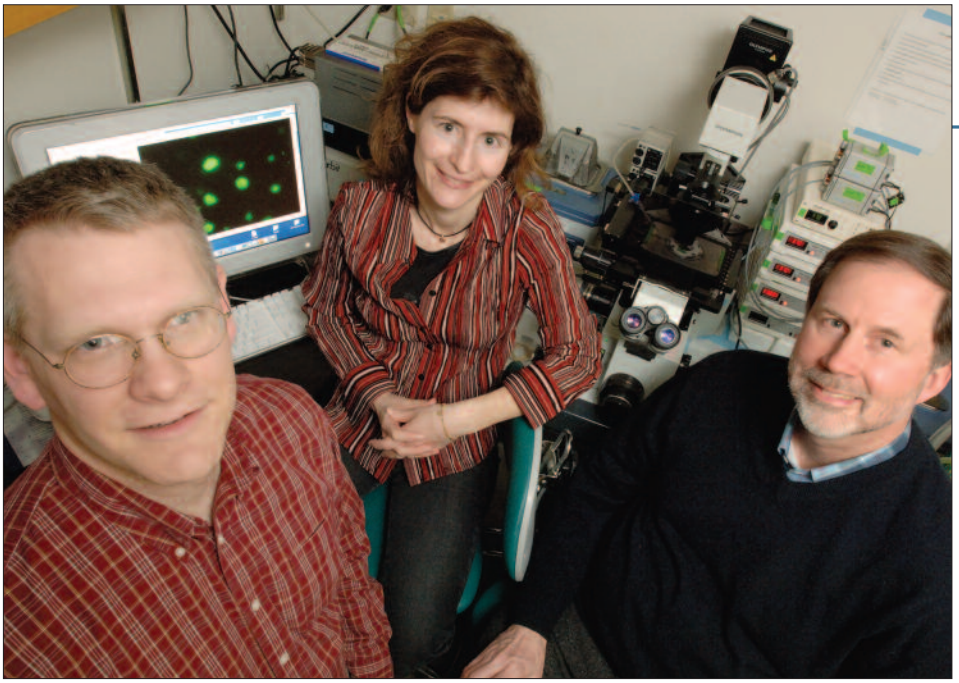

JON GILBERT FOX

Stanton, right—as well as postdocs Anderson, left, and Moreau-Marquis, center—study bacteria in biofilm form.

For a WEB EXTRA with a movie, through a confocal microscope, of *P. aeruginosa* in action, see dartmed.dartmouth.edu/spring08/html/disc_tornadoes_we.php.

Hunting down gray tornadoes in the lungs

They look like tiny gray tornadoes: little *Pseudomonas aeruginosa* bacteria spinning around on their flagella, corkscrewing down, and attaching to the surface of human airway epithelial cells.

"This is very early in the process," explains postdoctoral researcher Sophie Moreau-Marquis, Ph.D., as she watches the drama unfold on her computer monitor. She had filmed the free-swimming planktonic bacteria as they clustered together under the microscope to form biofilms on airway cells from a cystic fibrosis (CF) patient. Bacteria in biofilm communities are far more resistant to antibiotics than are free-swimming bacteria.

Glands: And that's a problem, especially for people with CF, an inherited disease of the mucous and sweat glands that affects the respiratory, digestive, and reproductive systems. *P. aeruginosa* colonize airway cells in the lungs of CF patients. The disease makes their mucus thick and slimy, so it builds up and blocks the airways, making it easy for bacteria to get a foothold and cause infections.

"One of the major problems in cystic fibrosis is that the patients have a persistent infection with *Pseudomonas*," says CF researcher Bruce Stanton, Ph.D. "That bacterial infection is what causes the lung disease. . . . You can give patients antibiotics, [but you] can never get rid of the infection."

So Stanton and other DMS scientists—in the Departments of Physiology and of Microbiology and Immunology—are trying to figure out what triggers the transformation of *P. aeruginosa* from the free-swimming to the biofilm form. Two recent papers highlight their latest work—one on how iron affects the formation of *P. aeruginosa* biofilms, and another on how antibiotic therapy actually facilitates the formation of biofilms.

In order "to outsmart bacteria, you really have to get up early."

A mutation in the gene responsible for CF "causes the CF airway cells to release more iron," which bacteria use to develop increased resistance to antibiotics, says Stanton. He's the principal investigator of that study, which was published in the *American Journal of Physiology: Lung Cellular and Molecular Physiology* and highlighted in its Editorial Focus section. "The bacteria try to trick the airway cells into allowing them to grow and to be maintained. And the airway cells secrete substances [including iron] that facilitate the development and the growth of the bacteria, as well as antibiotic resistance." In fact, bacteria are 50-fold more resistant to antibiotics in the lungs of CF patients versus in a petri dish.

"This is obviously not good news for CF patients, because it shows that current therapies are unable to eradicate biofilms in *Pseudomonas* infection," says Moreau-Marquis, a member of Stanton's lab. "And now we're starting to understand why." Next, they plan to explore whether iron chelators used in conjunction with antibiotics have a synergistic effect in killing the bacteria.

Tool: Moreau-Marquis and another postdoc—Gregory Anderson, Ph.D., who works in the lab of microbiologist George O'Toole, Ph.D.—helped develop a new tool to study the interaction between *P. aeruginosa* and human airway cells. Previously, biofilms were observed either indirectly—in sputum coughed up by CF patients—or on lab-grown airway cells that had already died. But the postdocs created a flow chamber apparatus, so a constant flow of nutrients feed cells as bacteria nestle in contact-lens-size "pools" on glass slides. The researchers now can observe, under a microscope, live interactions between airway cells and bacteria.

The second study, published in *Infection and Immunity* and highlighted in the journal's Spotlight section, showed that the antibiotic tobramycin downregulates bacterial virulence but promotes biofilm formation. "There's a growing idea that *Pseudomonas* has two different lifestyles," says Anderson. The free-swimming form causes acute pneumonia, and the biofilm form causes chronic infection. Tobramycin may just shift the balance between the two forms. "So we're decreasing the virulence," he adds, but "enhancing the persistence of the bacteria in the lung."

Scary: Making such discoveries "is very exciting as a scientist, but also scary," says Moreau-Marquis. "If you want to outsmart bacteria, you really have to get up early. They are so resistant and so able to bypass everything we know. . . . There's a lot of work to do, that's for sure."

LAURA STEPHENSON CARTER