n squadron of jet bombers can be seen swooshing below the ceilings of maternity wards. There are no echoes of antiaircraft fire reverberating off baby cribs, either. But the appearance of tranquility is deceiving. There is an air battle of epic proportions taking place.

The enemy, in the form of flying bacteria, is everywhere, though invisible. Ground zero is the lining of the lungs, where the defense is situated.

Starting with every baby’s first breath, the combat begins. Inhaled bacteria seek to colonize the lungs by invading...
Patients may dramatically improve because of Robert J. Bridges, professor and vice chair of the University of Pittsburgh School of Medicine’s Department of Cell Biology and Physiology. Bridges, whose PhD is in physiology, and his collaborators have discovered a pharmacologic treatment designed to allow CF patients to mount a defense against bacteria and viruses inhaled as they take routine breaths.

This dramatic encounter occurs practically every time people swallow or clear their throats, except for the 30,000 children and young adults in the United States who have cystic fibrosis (CF). The bacteria eventually win the battle in their bodies. They die young—90 percent directly from pulmonary problems. Thirty-one is the median age of survival for people with this genetic disorder.

What looks like the next generation of Pac Man is really Robert Bridges’s clever approach to treating cystic fibrosis. Sodium (Na) flows from the fluid lining the lungs (pale yellow) across the cell membrane into the cell (hot pink). Excessive sodium absorption because of too many active channels dehydrates the lining, which is a deadly trait of CF. Bridges has found a compound (purple wedge) that prevents a protease (green Pac Man) from opening too many channels.

As the bacteria home in on the epithelium, the cilia stand ready with their secret weapon: mucus. The layer of sticky goo—supported above the epithelium by the cilia—traps the unsuspecting bacteria. Then the cilia, swaying in the sodium chloride fluid, propel the mucus with its prisoners out of the airways. Tragedy is averted.
ride secretion and its implications concerning life-threatening diarrhea.

In 1983, CF was characterized as a chloride transport disorder. Raymond A. Frizzell—who was the director of the CF center at the University of Alabama at Birmingham (UAB)—took notice. He sought out additional chloride transport experts to join his team. (Years earlier, as a postdoctoral fellow with Pitt’s physiology department, Frizzell had published his own breakthrough papers on chloride transport.) Bridges came on board to continue his own chloride channel research. In July of 1989, his research changed, thanks to the discovery of the CF gene (CFTR), by Lap-Chee Tsui, at the Hospital for Sick Children in Toronto, who worked with Francis Collins, of the Human Genome Project, and John Riordan. (Tsui happened to earn his PhD in biological sciences from Pitt in 1979.)

“Before the gene had been discovered,” Bridges recalls, “the chloride channel that I was working on in Germany was thought to be the most important chloride channel in CF. But as it turned out, when the CF gene was discovered in ’89, it wasn’t the right channel.”

Naturally, he switched channels. Not everyone in the CF community followed suit, however. Now there were two ways to deal with CF: Try to devise a treatment to minimize its effects, as Bridges was. Or, try to fix the gene. Most researchers opted for the gene therapy approach—by a landslide.

“Before the gene was discovered,” says Bridges, “with the publicity around it, some parents took their children off the lung transplant program, the waiting list, in anticipation that gene therapy was around the corner.”

Orenstein remembers the jubilation: “When the gene for CF was discovered in 1989, a lot of people said, ‘Well, we’re going to have a cure right away. We’re going to have gene therapy, and CF is going to be a thing of the past.’ But in CF animal models and in some patients, it looks like the efficiency of gene transfer has been lower than you would like, and the inflammatory response has been higher than you would like.”

Frizzell says it must have been during one of those curry-inspired discussions that he mentioned to Bridges an interesting paper he’d just read.
Penland notes that the tricky part of gene therapy is, as he puts it, “getting the gene of interest into the cells of interest, and keeping it there.” He thinks overcoming these hurdles will take “quite a few more years.”

Bridges didn’t pursue the gene therapy approach, but he did consider the implications of the malfunctioning or missing protein.

To use a nonmilitary analogy, the role the CFTR protein plays can be compared to the job of a guy who takes care of a pool. If the pool man is dependable, he’ll do such a good job regulating the chemistry, the owners will notice nothing but cool lapping water. Likewise, a dependable CFTR protein controls the sodium-chloride fluid (pool) that coats our lungs, keeping them nicely hydrated. To push the analogy further, if the CFTR pool man is doing his job, that means a big one-way water volleyball game is on—the cilia being the teams that flick away the bacteria- and virus-laden mucus.

If the pool guy is sloppy or doesn’t show up, the pool chemistry gets strange. In the case of CF, what actually happens is this: The chloride is not secreted properly through its channels within the epithelium, so it isn’t able to use its chemical properties to pull sodium and water along to the lining of the lungs. That means the pool can’t fill up properly in the first place. To make matters worse, the cells’ sodium channels (imagine a bunch of drains in the pool) start working overtime, sucking the sodium back into the epithelium; the chloride and water follow. That empties the pool.

When the lungs are dehydrated, the cilia lose their ability to spike the mucus. Coughs, sneezes, and swallows are of little help; the cilia are doomed to be crushed by the weight of the goo. Eventually, bacteria colonize in the lungs, and lethal pulmonary conditions are likely to develop.

Researchers take lung cells from transplant patient tissue, grow them on filters, then place them in the above chamber to test the cells’ response to various compounds.

Bridges searched for a pharmacological answer to the dehydration problem. For years, he continued to focus on the chloride channel in hopes of developing a compound that would assist its secretion.

Then, in the early 1990s, a team of Swiss researchers pinpointed the type of channel, ENaC, responsible for sodium transport. That got Bridges to switch channels again. He shifted his focus, delving into the complexities of the sodium channel. (Remember the drains that work overtime?) Yet, as with any lung cell biology experiment, he and the other UAB investigators needed a rare commodity, lung tissue. The University of Pittsburgh performs on average 47 lung transplants annually, seven on CF patients. Largely on the basis of that kind of tissue availability, Frizzell, Bridges, and 15 others from the CF center at UAB relocated to Pitt’s Department of Cell Biology and Physiology in 1995. Within two years, the school had established its Cystic Fibrosis Research Center. Frizzell and Bridges serve as codirectors.

Since making the trek north, the researchers from the UAB group have remained close, meeting almost daily for lunch. They often treated themselves to outings at a student-friendly Tibetan restaurant along Forbes Avenue in the heart of Oakland (which has since closed). For this crowd, the backdrop of minced mutton, steamed buns, and sweet tea was preferable to any water cooler for swapping stories. The café also lent a relaxed air to musings on their research, what an outcome might mean, where to go next. . . . Frizzell says it must have been during one of those curry-inspired discussions that he mentioned to Bridges an interesting paper he’d just read. It had been published in Nature (10/9/97) by those same Swiss researchers who’d identified ENaC. In that paper, the Swiss described how they controlled sodium channels in renal epithelial cells with a compound made from cows, called Aprotinin. Aprotinin is a protease inhibitor, which means it can stop enzymes from splitting proteins (and in the case of CF, stop them from letting fluid pass through).

From that article, Bridges deduced it might be possible for such a compound to control the movement of sodium in the pulmonary epithelial cells and, importantly, the fluid that follows closely behind.
With this microscope, researchers can video-record the voyage of pencil shavings (substituting for bacteria) as cilia transport them along lung tissue.

When Bridges began his Aprotinin experiment, it was clear that particular compound would never be a remedy for CF patients.

“Aprotinin is antigenic,” he explains, “so you can only use it once on a patient because the patient will develop antibodies to it.” If a patient is treated with it more than once, it’s likely to induce shock. And CF patients will need more than one dose, whatever the drug. Genetic disorders such as CF call for ongoing treatments, since the medical condition is embedded in the DNA.

Aprotinin couldn’t be the answer, but it could lead to an answer if Bridges’s experiment demonstrated that the protease inhibitor controlled the sodium channels in the epithelial cells lining the lungs.

It did. Bridges remembers that feeling of quiet euphoria in his lab as he monitored the results: “More than anything else, it confirmed that I was thinking properly.”

Next, he reasoned, he would have to find a protein similar to Aprotinin that wouldn’t elicit antibodies. He tried another protease inhibitor compound, but it didn’t control the sodium channels. He tried another. He tried quite a few. None worked.

“Finally,” he says, “I asked myself, ‘What makes Aprotinin so special?’”

He scoured the most esoteric of medical literature, hoping to discover the magic of Aprotinin. Three months later he found that magic in an amino acid sequence that distinguishes Aprotinin from the other protease inhibitors. The sequence is known as the Kunitz domain.

Bridges immediately did a PubMed search looking for a protease inhibitor that wasn’t antigenic, but contained the Kunitz domain. From that, he was able to identify an existing compound that looked like it fit the bill.

The compound is a human-recombinant protein equivalent to Aprotinin. Bridges garnered a sample; then conducted a test on transplant patient lung tissue. It worked. The compound inhibited the sodium channels, and fluid remained atop the lining of the lungs for several hours. (Other researchers had been working with another sodium inhibitor, the effects of which lasted just a few minutes.) Bridges had found a way to keep the pool filled and the volleyball game on.

This meant that perhaps one day, by periodically inhaling this compound—much as one might take asthma aerosol medication—CF patients would breathe more freely. The compound holds the most promise for CF patients who can begin the treatments early in their lives, or those with moderate pulmonary damage, since the approach still relies on the cilia to be strong enough to flick off the mucus.

Clinical studies will begin later this year. Orenstein—whose CF center at Children’s Hospital has about 400 patients—hopes Pittsburgh is chosen as a site for the trials.

“The buzzword in medicine these days is bench-to-bedside. We have the perfect setup for doing that. Those guys [at Pitt’s CF Research Center] are great at the bench, and we have a lot of experience at the bedside.”

No matter where the trials are held, Penland will be watching enthusiastically from his CF Foundation office in Bethesda: “With the caveat that inhibiting sodium absorption is going to have a positive effect on the lung functioning of CF individuals, there is no question the compound has real potential.

“If the clinical studies go well, I don’t see why it can’t be a viable treatment option within seven to 10 years.”

PROTEIN POWER

If you don’t have cystic fibrosis, the more you learn about the disorder, the more you’ll be amazed by how deceptively simple it seems to be to keep your airways clear.

Neil Bradbury, assistant professor of cell biology and physiology, is one of several at Pitt delving into the intricacies of the disorder. He made a splash when he figured out the pathway by which the CF protein, called CFTR, is moved out of the plasma membrane of cells lining the lungs and the intestinal tract. He determined that the protein has sort of a zip code, defined by a sequence of amino acids that helps it be recognized and removed from the plasma membrane. (This is during the process that cells use to obtain and pass material through a cell, known as endocytosis.) This zip code, however, tells not only where the protein should go, but when it should. What does that matter? you ask. Well, how long CFTR decides to hang around the plasma membrane can make you feel a lot better or a lot worse. The protein is the means by which the membrane is hydrated; in the case of your lungs, it allows cilia to stay buoyant and flexible enough to repel mucus that would otherwise become fertile ground for bacterial growth.

And if you don’t have as many CFTR proteins in your plasma membrane as your body needs, that spells trouble. The protein creates a critical chloride channel that siphons the hydrating fluid from the cells onto the outer membrane. In some CF patients, these channels are prematurely removed from the plasma membrane. Bradbury thinks he knows why. He has discovered that sometimes the CF gene is mutated so that the body alters the rate at which the protein is removed. By understanding how cells recognize the zip code, he hopes to hide the code from the cell and keep CFTR in the plasma membrane, where it’s needed. —EL