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FROM THE CLINIC TO THE LAB AND BACK AGAIN, HARD WORK YIELDS FRESH INSIGHTS

BY CHUCK STARESINIC

CLOSING THE LOOP ON ASTHMA

Sally Wenzel will never forget the patient who felt he had to ask her, his 28-year-old physician, whether it was okay for him to go fishing on the Chesapeake Bay. He was her elder—a nice man who worked maintenance on a college campus in Virginia. His name was unforgettable because he shared it with a celebrity—we'll call him Ray Charles. His problem was asthma, and he had it bad.

We all take some things for granted. For most people, breathing is one of them. No matter what happens in the course of a day—good day, bad day, sick day, birthday—one expects to move air in and out of one's lungs without even thinking about it. Those who live with severe asthma think about it every day.

Severe asthma is not the same as the mild condition that sent Pittsburgh Steelers halfback Jerome Bettis to the sideline for his inhaler so that he could get back on the field for the fourth quarter. For Charles, an attack felt like he was suddenly breathing through a too-small straw or even a coffee stirrer—an accurate approximation of what happens during an attack.

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The bronchial passages constrict and limit the airflow both in and out of the lungs. A bad attack could come on quickly—and without prompt medical attention, it would asphyxiate him.

Nevertheless, fishing on the Chesapeake is not the type of activity associated with asthma attacks. It's not exactly strenuous. Asthma triggers like pollen, dust, and other pollutants are rare on the open water. Wenzel, who was then a fellow in pulmonary medicine, knew that her patient had been doing well in the past year. Moreover, she thought it would be a terrible precedent for her to tell this man that he should not go fishing. She believed that an important part of her job was to enable her patients to enjoy a better quality of life.

But the Chesapeake is a big body of water. So Wenzel told Charles to carry his medications and stay relatively close to shore where he could get medical help in a crisis. It was the early 1980s, and inhaled steroids to reduce inflammation in the lungs were still a decade away. Charles' meds were all slower acting oral anti-inflammatories.

"We didn't really understand the whole steroid thing back then," Wenzel says now.

In fact, the field of asthma was littered with unknowns. Nobody—with the possible exception of pathologists who examined lung tissue postmortem—even knew much about what the inside of an asthmatic's lungs looked like. Living asthmatics were in something of a blind spot for pulmonologists.

The reasons are complex. But they start with perspective—asthma was largely seen as an allergic disease, and allergists did not look inside the lungs of their patients. They pricked the skin and exposed it to pollen, dust, peanuts, and other allergens. They tried to determine what set off their patients' inflammatory responses.

For pulmonologists, severe asthma patients were, at best, a puzzle and, at worst, a compliance problem. If a patient didn't get better, or if he got worse, it was often assumed that he wasn't following doctor's orders. Pulmonologists blamed emotional disturbances—which they were unable to treat—for exacerbating symptoms. (There is evidence that emotions can exacerbate asthma symptoms, but it's far from definitive.)

Wenzel is now a professor of medicine in the University of Pittsburgh School of Medicine and director of the pulmonary division's Asthma and Allergy Center. Her peers say that she has contributed as much to our understanding of severe asthma in the past 20 years as any pulmonologist. But back when she was physician to Ray Charles, she knew as little as everyone else. She recalls pestering her mentors with questions:

"I would go to my faculty members when I was a fellow, and I'd say, 'These people can get so sick so fast. What's happening, and why?' And no one could give me an answer. They'd say, 'Well, there's probably some inflammation, some swelling of the airways, and muscle spasm…' And that was it!

"This is a disease that is the most common respiratory disease in young people," she says now of asthma. "It afflicts up to 10 percent of the people in the country, and all you can tell me is that there is a little inflammation, swelling, and some twitchy airways? That's not a good enough answer! So I developed this little interest in asthma."

Wenzel applied for her first National Institutes of Health (NIH) grant to study asthma at the Medical College of Virginia, which is now Virginia Commonwealth University.

"No one gave me a chance in—excuse me—*hell* of getting it because the Medical College of Virginia, and certainly the pulmonary division, wasn't known as a research powerhouse," she says. "'Oh sure, write it,' they said, 'but we don't think you'll get funded. It will be a good exercise.'"

She did get funded, and other residents and fellows began sending asthmatic patients her way. One such patient was Ray Charles. Wenzel got to know him quite well over the course of a year. He told her stories about his wife and three children, and he really seemed to be doing better when he asked her about going fishing with his buddy.

The next Monday at work, she ran into the chief of the pulmonary division. "So, Sally," he said, "was Ray Charles your patient?"

"What do you mean, *was* Ray Charles my patient?" she replied.

Charles had died fishing on the Chesapeake. He had gone a little too far from shore, and he had an asthma attack. His buddy motored him to the eastern shore, which was arguably less medically equipped than the western shore. By the time anyone could attempt to resuscitate him, it was too late.

Wenzel doesn't ascribe magical properties to earthly events. She's a scientist and a pragmatist. Nevertheless, she says that when she heard of Charles' death, "There was something that said, 'You know this is a problem. And you've been given an

opportunity to help figure it out, because it shouldn't have happened.'

"It probably hit home as hard as anything I could have imagined."

Inflammation is good, within reason. It is a defense mechanism. It is how we stay alive despite the onslaught of infection and injury. Inflammation protects the body from invasion by foreign organisms, and it helps to repair damaged tissue.

The inflammatory process begins when cells react to an injury or infection by producing chemicals that signal the immune system. This starts a cascade of events. Blood flow to the area increases. White blood cells arrive in large numbers and consume foreign material and injured cells. In a powerful feedback loop, the white blood cells release more and more chemical signals that amplify and perpetuate the inflammatory response. They also produce toxic chemicals such as oxygen radicals, nitric oxide, and enzymes, all of which help to kill invading microorganisms. All of this is good, so long as the process can be switched off and so long as the inflammation is a reaction to a genuine threat.

Lung inflammation occurs as a result of bacterial and viral infections and because of exposure to air pollution and allergens. Inflammation in the lungs, as in any other part of the body, helps to destroy these foreign invaders and irritants.

In asthma, however, inflammation ceases to be a primarily beneficial process. Perhaps it is an overreaction to something in the air that poses no real danger. Perhaps the body fails to dial back the inflammation as it should following an injury, infection, or exposure to allergens. The persistence and extent of the inflammation begin to erode the person's quality of life.

To make matters worse, the lungs of a person who has weathered repeated attacks of severe asthma begin to show permanent structural changes. Doctors call it remodeling. The thin layer of epithelial cells lining a healthy lung thickens. The smooth muscle cells beneath become a bit spastic, and they fail to smoothly expand and contract the bronchial passages with each breath. These are ominous developments—even if doctors were to find an antidote to the inflammation, the structural transformations would remain, resulting in narrower bronchial passages that no longer function properly.

The chemical pathways that lead to inflammation are fascinatingly complex and poorly understood. Environmental triggers are many and they affect each person differently. Depending upon a person's genetic makeup and general health, a dust storm could trigger a life-threatening asthmatic episode, a brief coughing and sneezing fit, or nothing at all. For the most part, scientists don't know why.

For a physician like Wenzel, the questions from the start were: How do we close the loop on asthma? How do we make the observations in patients that will tell us why one person has a severe asthma attack when another does not? And how do we find a treatment that will help that one patient?

Nandini Krishnamoorthy (PhD '08) came to the University of Pittsburgh School of Medicine to pursue a PhD in immunology. In the same way that every creative writing student imagines someday having a book reviewed in *The New York Times*, Krishnamoorthy arrived in Pittsburgh in 2004 imagining her research publications someday appearing in a top-notch journal like, say, *Nature Medicine*. In her case, however, it actually happened, and it happened about as quickly as these things are possible.

Krishnamoorthy's doctoral dissertation led directly to a May 2008 *Nature Medicine* paper that identified a molecule in immune cells essential to the sort of faulty immune response that results in asthma and allergies. Theoretically, identifying a molecule that is critical to the chain of events that leaves a person gasping for breath and waiting for an ambulance can lead directly to new drugs and therapies that target this molecule. (It's important to note, however, that the research subjects here are all mice, not humans. More on that later.)

It started like this: Krishnamoorthy had the good fortune to land in the laboratory of Prabir Ray, an associate professor in Pitt's

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medicine and immunology departments. Ray has a 20-year track record of delving into the cellular and molecular mechanisms of immune response.

In 1997, Anuradha Ray—now a Pitt professor of medicine and immunology, but then at Yale University with husband Prabir—had published vital insights into the mechanisms responsible for creation of the Th2 cells that orchestrate asthma both in mice and in humans. When Krishnamoorthy began looking for a dissertation topic, the Rays encouraged her to explore the molecular mechanisms that induce asthma in the lung. By that time, Anuradha Ray's group had developed a mouse model of Th2 response in the lung. Timothy Oriss, a Pitt research assistant professor of medicine in the group, published their mouse model in the January 2005 issue of *Immunology*. Oriss had exposed mice to some rather typical allergens and toxins—egg white and cholera toxin, in this case. He wasn't interested in cholera or egg allergies, per se; he was interested in the immune response.

Oriss had found that combining the egg white and cholera toxin significantly altered how the mice reacted. Mice exposed to the cocktail produced a great number of white blood cells called myeloid dendritic cells, which locate foreign material and present it to other immune cells to prime them to attack. The presence of these cells was associated with greater inflammation, including lung inflammation. Mice exposed to egg white alone produced larger amounts of a different type of dendrite—plasmacytoid dendritic cells—that is known to curb inflammation. Predictably, these mice had less lung inflammation. Ray suggested that Krishnamoorthy explore exactly how and why cholera toxin produced its effect on dendritic cells.

Krishnamoorthy took up the challenge. She exposed dendritic cells to cholera toxin, then examined a microarray profile of the genes activated by the toxin. The microarray led her to a gene for a molecule called c-Kit, and her dendritic cells produced a lot of it when exposed to cholera. Most people aren't exposed to cholera, however. Krishnamoorthy wondered what would happen if the cells were exposed to an allergen that people do commonly encounter, like house dust mites, a notorious asthma trigger.

"We found the same result," says Krishnamoorthy. "This was when things got exciting."

C-Kit wasn't new to scientists, but it hadn't previously been so directly implicated in asthma and wasn't typically associated with dendritic cells. It is a signaling molecule that triggers other events in biochemical cascades. If it were present in dendritic cells, the team hypothesized, it probably had an important function in the inflammatory chain of events.

Working with a team of colleagues including Prabir Ray, Anuradha Ray, and Oriss, Krishnamoorthy was able to show that in some instances c-Kit was the very first molecule triggered when allergens were present.

This is not the only immune response in which c-Kit is involved. Neither is c-Kit involved in every allergic response. But Krishnamoorthy, who is now a postdoc in Prabir Ray's lab, believes that her findings open a whole new area of investigation for understanding and limiting inflammation in asthma as well as in other diseases.

Here's one simple reason why it could be a long time before a drug that inhibits c-Kit goes into clinical trials for asthma patients: Krishnamoorthy's research subjects are all mice, and all of Sally Wenzel's patients are humans.

Wenzel says that one of her recurring professional frustrations is "knowing that I will probably never have my papers published in *Nature* or *Nature Medicine* or any of the top journals, because I, in my human systems, can't close the loop."

In this sense, closing the loop means having a theory about what a particular molecule or gene does, then running an experiment to support or disprove that theory. In mice, you have a shot at closing the loop in one paper because you can easily test drugs in mice. It's more difficult in humans, for obvious ethical and legal reasons. Scientists can even eliminate whole genes in mice. That's what Krishnamoorthy and her colleagues did, knocking out the c-Kit gene and creating a population that could not produce the molecule. (When they exposed these mice to allergens, the knockouts lacked the vigorous inflammatory response that normal mice exhibit, lending credence to their theory that c-Kit was important to the allergic response. Loop closed. Paper published.)

Despite the obstacles to closing the loop that a human-focused researcher encounters, Wenzel sells herself short when she says that she "thinks" she has contributed to our understanding of asthma. Back when she was a young doc with her inaugural NIH grant, she was one of the first pulmonologists to investigate asthma using a bronchoscope. It's hard to believe, but until the early 1980s, there were no invasive studies of asthma patients. No one looked at their lungs.

When she started down this path, Wenzel had herself bronchoscoped so that she would know exactly what she was asking of her patients. She learned that she could observe an asthma attack in a small section of lung tissue by introducing a tiny bit of allergen through the scope; she would then rinse the area with saline and study what came out in the wash. Over time, she gravitated toward the severe asthma patients, who needed the most help.

As a result of this work, Wenzel was able to point scientists to the importance of one particular immune cell—the neutrophil—in asthma inflammation. No one had ever looked closely enough to find it in the lungs of a person with severe asthma. Their attention had been on other immune cells seen in milder asthma. Wenzel now believes the neutrophil may be involved in a significant number of asthma cases, and this has important implications for treatment.

Wenzel also was one of the first to biopsy the distal lung in severe asthmatics, taking tissue samples from so deep in the organ that the process had to be guided by X-ray. She has now been doing this work for 20 years and her laboratory has a unique database of clinical, physiological, genetic, and pathologic data from more than 400 people with severe and milder forms of asthma. Using this extensive resource, her lab has been key to documenting and describing the various types and presentations of asthma, which has proven to be both heterogeneous and, nevertheless, comprised of only a handful of phenotypes.

Behind much of these data are patients Wenzel has known for decades. One mom rigged her laundry basket with a homemade bungee-cord harness so that she could drag it around as needed. Lifting and carrying simply took too much effort. When her asthma was bad, her lung function would drop to 30 percent of its usual capacity.

"In the 10 years that I knew her, she had been intubated and put on a breathing machine about six times," says the doctor.

The woman's children had seen their mother lose consciousness and get rushed to the hospital. In elementary school, one of

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them successfully petitioned to take a CPR class offered to the high school students—on the grounds that he believed he might need to resuscitate his mom someday.

"The seventh time she had an attack, she died, at age 46," Wenzel says. "Never did anything to bring this on." Like Charles, Wenzel calls the woman, "one of the main reasons I'm doing this today."

According to Serpil Erzurum, codirector of the Cleveland Clinic's asthma center and chair of the pathobiology department there, Wenzel has informed much of what we know about the architecture of the lung and the changes that occur with asthma and the progression of the disease. "She has been able to describe very specific changes that are now accepted as paradigms of the disease but weren't completely understood or documented before," she says.

Wenzel was the person who organized the American Thoracic Society's workshop on severe asthma in the late 1990s. In a culmination of two years' work, the committee drafted the definition of severe asthma that now serves as the standard worldwide. The group's efforts also led to recognition by the NIH of the subset of dangerously ill asthma patients who were not well served by existing medications.

The NIH subsequently dedicated significant resources to a severe asthma research program, for which Wenzel is a principal investigator. Pitt is one of eight centers in the country funded as a part of the program. Also through Wenzel's work, a new program in pediatric severe asthma has been initiated.

The side-by-side programs will offer lifelong management of asthma, Wenzel says. Researchers will study the progression of the disease from childhood to adulthood so as to understand the relationship between the two. (Severe asthma in childhood does not necessarily lead to severe asthma in adulthood.) They also will try to determine the role of viral infection and other physiological events in the development of severe asthma. (The woman whose son learned CPR experienced a drastic worsening of her asthma after childbirth.)

An enduring frustration for Wenzel has been the difficulty in closing the loop between the mouse studies in the labs of researchers like the Rays and the asthmatic patients she treats. Just as she arrived at the University of Pittsburgh in 2006, Wenzel wrote an editorial for the *American Journal of Respiratory and Critical Care Medicine* titled "The Mouse Trap—It Still Yields Few Answers." Chronic asthma is a disease unique to humans, she argued, and while scientists have probed and enhanced their understanding of the mouse immune system, too few basic science discoveries in that species have led to asthma treatments in humans.

"Mice do not have asthma," she wrote.

Pulmonologist Steven Shapiro—then of Harvard University and now, coincidentally, chair of Pitt's Department of Medicine—penned an opposing editorial. Perhaps, he suggested, the trick to animal modeling lies in knowing how far to take the analogy.

Wenzel, along with Prabir and Anuradha Ray, acknowledges that exploring the inflammatory pathways of mice does yield important insights. It may be one-hundredth of all the data from animal models that transfers over, says Wenzel, but it has enabled these colleagues to close the loop at times.

In October 2007, Wenzel and others published a study in *The Lancet* in which they tested a drug designed to block the inflammatory molecule interleukin-4 and its closely related cousin, interleukin-13. Over the years, data from animal models documented in the labs of Prabir and Anuradha Ray had indicated that production of interleukin-4 might be responsible for kick-starting all sorts of immune activity that is ultimately not good for people with asthma. This was thought to be a major pathway. Many of these studies were in mice, but Wenzel used a drug that blocked both interleukin-4 and interleukin-13 in human volunteers. To test the efficacy of the drug, the volunteers then inhaled allergens known to trigger asthma attacks. The drug offered a clear benefit over the placebo.

Loop closed. Paper published. Further trials are needed for this drug and many others to cure this complex, varied disease. But all sides agree that, whether mice get asthma or not, this is what the work is all about. ■