Strain on the Lungs

UB PHYSICIANS ARE INTERNATIONAL LEADERS IN COPD RESEARCH

"In the early 1990s it wasn't really clear what bacteria were doing in COPD. People thought that maybe they weren't causing the infection—that bacteria just were along for the ride. The jury was out." —TIMOTHY MURPHY, MD

By Lois Baker

Donald Reitz walks slowly down the corridor of the Buffalo Veterans Affairs Medical Center (VAMC) on his way to the hospital's respiratory clinic to pick up a steroid inhaler. Reitz, 78, has chronic obstructive pulmonary disease, or COPD. Cigarette smoking is the major cause of COPD, and Reitz had a four-pack-a-day habit for decades. He quit for good in 1980 after spending a week in the hospital battling pneumonia, but the damage was done.

In a sense, Reitz was lucky. The Buffalo VAMC, where he was diagnosed, is home to the longest running clinical investigation of COPD in the United States. Five years ago, Reitz volunteered to take part in the study. "If my contribution helps someone down the road," he says, "I'm tickled pink."

Timothy Murphy, MD, and Sanjay Sethi, MD, professors in the UB Department of Medicine, established the COPD study clinic in 1994. Over the years, with the help of the study volunteers, the two researchers have made critical discoveries about the causes of exacerbations and progression of COPD, and have developed promising vaccine candidates for pathogens involved in the disease. Their goal is to develop effective treatments for prevention of these exacerbations.

PHOTO BY NANCY PARISI

Donald Reitz

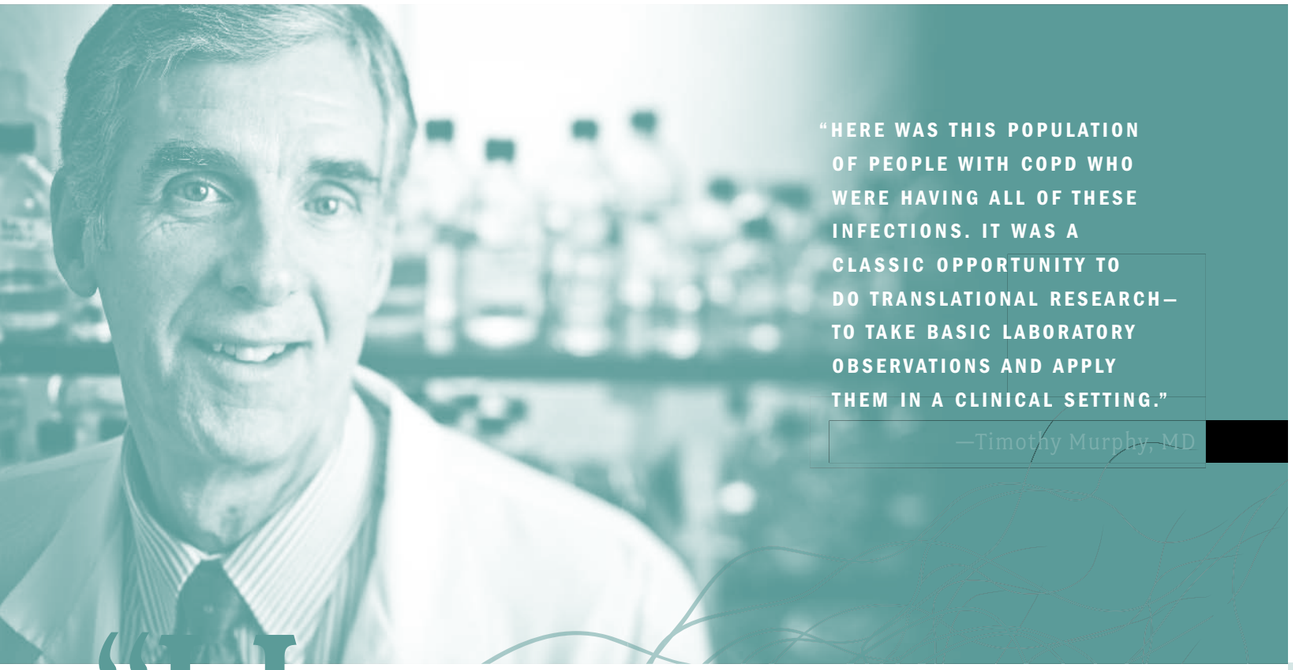
A Classic Opportunity

In May 2009, Murphy, who is a UB Distinguished Professor and former chief of the Division of Infectious Diseases, was named to head the School of Medicine and Biomedical Sciences' new Clinical and Translational Research Program. His long track record as a successful physician-scientist began in 1980, when he served a postdoctoral fellowship at Tufts–New England Medical Center in Boston and became interested in two microorganisms that have proven to be important in exacerbations of COPD.

Upon completion of his fellowship, he was recruited to Buffalo by the then-head of the UB Division of Infectious Diseases Michael Apicella, MD, who now chairs the Department of Microbiology at the University of Iowa.

By 1983 Murphy was funded by the National Institutes of Health (NIH) to conduct basic vaccine research on the bacterium *Haemophilus influenzae,* a principal pathogen in otitis media (ear infections in children), as well as in COPD and sinusitis. Several years later, when this research was progressing well, he turned his attention to *Moraxella catarrhalis,* another bacterium known to cause ear infections and bronchitis. Since that time, Murphy's research on both these bacterium has been continually funded by the NIH.

Initially, Murphy worked at Erie County Medical Center, but when the VAMC opened its new research building in 1990, he was recruited to serve as chief of its Infectious Diseases Section. There was a significant patient population with COPD at the VA, and since most of the infections that occur in COPD are caused by the two pathogens Murphy had been studying for nearly a decade, it was an opportunity that seemed tailor made.



"HERE WAS THIS POPULATION OF PEOPLE WITH COPD WHO WERE HAVING ALL OF THESE INFECTIONS. IT WAS A CLASSIC OPPORTUNITY TO DO TRANSLATIONAL RESEARCH—TO TAKE BASIC LABORATORY OBSERVATIONS AND APPLY THEM IN A CLINICAL SETTING."

—Timothy Murphy, MD

PHOTO BY DOUGLAS LEVERE

"*Here was this population* of people with COPD who were having all of these infections," Murphy relates. "It was a classic opportunity to do translational research—to take basic laboratory observations and apply them in a clinical setting."

With his basic research on the COPD-related pathogens already in place, Murphy began writing a grant proposal for funding from the VA to set up a COPD clinic to study how these pathogens were behaving in patients. In the meantime, Sethi, a postdoctoral fellow conducting research in pulmonary and critical care medicine, joined Murphy's lab at the urging of the then-chair of the UB Department of Medicine, Robert Klocke, MD, who was interested in developing collaboration between the divisions of pulmonary medicine and infectious diseases.

Klocke, editor of the *American Review of Respiratory Diseases,* the major pulmonary journal at the time, asked the two researchers, who by then were collaborating, to write a review article on what was known about the role of bacterial infection in COPD. After working together on the paper, which was published in 1992, the two investigators began to focus their research on the role of bacterial infection in COPD, specifically on exacerbations.

"In the early 1990s it wasn't really clear what bacteria were doing in COPD," says Murphy. "People thought that maybe they weren't causing the infection—that bacteria just were along for the ride. The jury was out. What people were talking about at that time was the so-called 'bacterial-load' hypothesis: that the bacteria were there all the time and when they grew in large numbers, they reached a certain tipping point and people have a serious flare-up, or exacerbation."

It's an exacerbation that gets a COPD patient's attention, Murphy continues. They experience an increase in shortness of breath, which often is accompanied by an accumulation of thick, airway-clogging mucus. Exacerbations often send patients to the emergency room, and in severe cases, they must be admitted to the hospital. Each flare-up further damages the lungs, and ultimately, exacerbations can become life-threatening.

When Murphy and Sethi reviewed the literature on exacerbations, they found the evidence supporting the bacterial-load hypothesis to be tenuous. They also found that little work had been done in this area since the 1960s and that genomics techniques that had been developed in the 1980s and '90s had not been adapted to this area of investigation.

Seizing the opportunity, Sethi and Murphy designed a study to better understand the role of bacterial infection in COPD, applied for funding and completed the proposal Murphy had earlier begun to establish a COPD Study Clinic at the Buffalo VAMC. In 1993, the U.S. Department of Veterans Affairs approved funding to establish the clinic, and the two investigators began enrolling patients a year later.

The COPD Burden

More than 12 million people in the United States currently are diagnosed with COPD, and an additional 12 million probably have the disease but don't know it, according to estimates by the National Heart, Lung and Blood Institute. The primary cause is smoking, the damage from which accumulates slowly over time as the alveoli—the tiny sacs in the lungs that collect oxygen from the air and supply it to the bloodstream—lose their elasticity and no longer can function. Breathing in, and particularly breathing out, becomes a constant struggle.

THIS OBSERVATION—THAT NEW STRAINS OF BACTERIA CAUSE THESE INFECTIONS—IS MURPHY AND SETHI'S KEY DISCOVERY, AND ONE THAT HAS *changed the scientific community's understanding of how COPD-related infections develop.* THE PAPER DESCRIBING THEIR FINDINGS WAS PUBLISHED IN THE *NEW ENGLAND JOURNAL OF MEDICINE* IN 2002 AND IS CONSIDERED A LANDMARK PAPER IN THE FIELD.

More men than women have COPD, but more women now are being diagnosed with COPD than men. (Currently, one woman is enrolled in the Buffalo clinical trial.)

"The reason the incidence of COPD is higher in women today is due to the rise in smoking among women 20 to 30 years ago; it's very predictable," says Sethi, who now heads UB's Division of Pulmonary, Critical Care and Sleep Medicine.

There also are gender differences in response to the disease. "Women actually have more symptoms," Sethi continues. "Whether that is because they are more susceptible, or because they have less capacity to spare, or their lungs react differently to smoke, that issue is not resolved. But with the same level of smoking as men, they seem to do worse. There is a lot of interest in this now."

Most people with COPD are middle-aged or older and have been symptomatic for years; however, many cases go undiagnosed and those who do get diagnosed often have lost half of their lung function by that time.

"Early in the disease, patients tend to attribute any trouble breathing to age and smoking, or their condition gets misdiagnosed as asthma," explains Sethi. "It's only later, when they experience symptoms, such as shortness of breath with exertion, and notice the progressive decrease in their ability to do things, do they realize something serious is going on."

What causes exacerbations, specifically, and how they develop are questions Murphy and Sethi expect to answer. They already have learned a great deal.

"Many people think that 50 percent of exacerbations are caused by bacterial infections, 30 percent by viral infections and 20 percent by other things," says Murphy. "Maybe air pollution, maybe allergies, maybe people run out of their medications, or maybe coexisting congestive heart failure sets it off. In fact, bacterial and viral infections account for 70 to 75 percent of all exacerbations."

In general, persons with COPD acquire exacerbation-causing infections the same way healthy people get a virus.

"They might be catching colds from grandchildren or from people around them at the supermarket, or from a colleague at work," Murphy explains. "For people who are healthy and have no lung disease, a cold is just a nuisance. They feel lousy, but they tough it out and usually their immune system eliminates the virus in a week and they are well again.

"But for people with COPD, who have compromised lung function, getting a virus sets them up for a secondary bacterial infection. So they catch viruses and bacteria the way everybody else does, but because they can't clear these pathogens, they get sicker. They are susceptible to infections by bacteria that people with healthy lungs aren't susceptible to, and an exacerbation ensues."

Critical Discoveries

Approximately 170 veterans have been involved in the Buffalo COPD clinical trial since its inception. One such participant is George Donaldson, 69, who spent 20 years in the U.S. Air Force before retiring in 1976 as a first sergeant. Donaldson had been smoking cigarettes for years by the time he enlisted, and, at that time, the military encouraged the habit. "Everybody did it," he recalls. "It was kind of a calmer-downer."

One day about seven years ago Donaldson found himself in a hospital emergency department "hacking away." He's been participating in the COPD study ever since. "I expect I'll be involved until they run out of money or I assume room temperature," he quips.

A cohort of 50 participants comes to the study clinic once a month and whenever they have an exacerbation. At each monthly visit, the study's nurse coordinator conducts a brief physical exam, discusses any problems they may be experiencing and collects a sputum specimen and a blood sample. If participants are having an exacerbation, they provide another set of samples.

To date, Murphy and Sethi's analyses of accumulated samples have yielded more than 40 papers, and their findings have altered the basic understanding of the mechanism of bacterial exacerbations in COPD.

By applying the tools of microbial genomics to their study of the sputum samples that they have isolated from clinic patients, the researchers have been able to provide "genetic fingerprints" of various bacterial strains, thereby determining which is a new strain of bacteria and which was present in a previous sample.

PHOTO BY NANCY PARISI

George Donaldson

"Before this, people would culture bacteria and count the bacteria, which is the approach that resulted in the 'bacterial-load' theory," explains Murphy.

"For example, if you look at just the results of the culture, it may appear as though a person had *H. influenzae*—one of the major bacteria involved in exacerbations—every month for 14 straight months," he continues. "That's not what's happening at all. By performing molecular typing of the bacterial strains, we showed that people were acquiring and clearing strains all the time, that there's a dynamic turnover of strains. They acquire a new strain and that is what is driving the exacerbations."

This observation—that new strains of bacteria cause these infections—is Murphy's and Sethi's key discovery, and one that has changed the scientific community's understanding of how COPD-related infections develop. The paper describing their findings was published in the *New England Journal of Medicine* in 2002 and is considered a landmark paper in the field.

Explaining how exacerbations happen, and how they recur, is an ongoing focus of the researchers' work, according to Sethi. "We've been able to show in our work, primarily with *H. influenzae,* that when patients acquire a new strain they get an inflammatory response much greater than with a previous strain, so all exacerbations seem to have an element of increased airway inflammation," he explains.

Coupled with this discovery is the collaborators' critical finding that immunity to these bacteria is strain specific. "Patients develop antibodies that can kill a particular strain of *H. influenzae,* but those antibodies are unable to kill a lot of other strains. This means that those patients remain susceptible," says Sethi, who adds that he and Murphy have developed a recurrence model based on strain differences among the bacteria.

The discovery that different bacterial strains have different disease-causing capabilities is crucial because it may help the scientists to translate their laboratory studies into a clinical application.

"If we can find some of the key genes or the key molecules in these different bacterial strains that are important in causing infection in COPD, then we can target them with vaccines and therapies," Murphy explains.

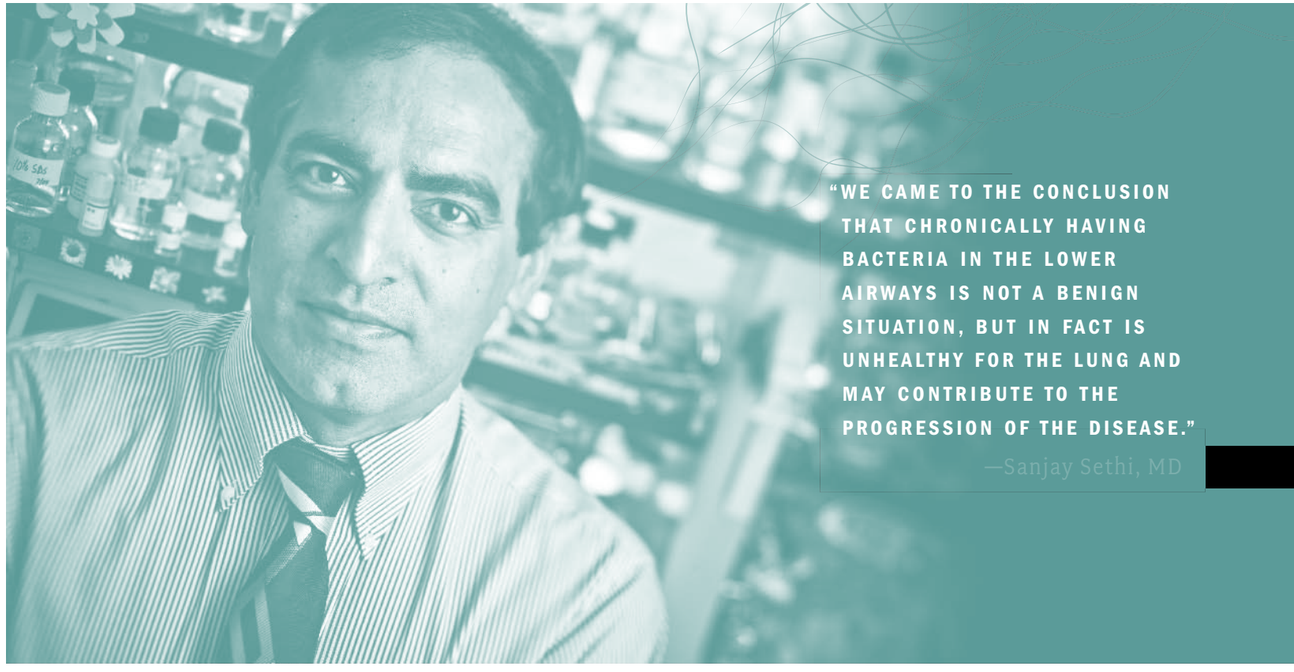


PHOTO BY DOUGLAS LEVERE

"WE CAME TO THE CONCLUSION THAT CHRONICALLY HAVING BACTERIA IN THE LOWER AIRWAYS IS NOT A BENIGN SITUATION, BUT IN FACT IS UNHEALTHY FOR THE LUNG AND MAY CONTRIBUTE TO THE PROGRESSION OF THE DISEASE."
—Sanjay Sethi, MD

Further Elucidations

In their ongoing studies that explore the role of acute bacterial and viral infection in exacerbations of COPD, Murphy and Sethi have added yet another dimension—and insight—to their work.

"With all of our clinical samples, we arrived at the idea that maybe COPD patients are chronically infected," explains Sethi, "and we have since completed studies that found that, in addition to the exacerbations and the acquisition of new strains, patients also have bacteria in the airways between the episodes."

Building on this finding, Sethi and Murphy and their collaborators have shown that even though COPD patients with these bacteria in their airways are not experiencing a clinical exacerbation, they should not necessarily be considered 'stable,' as was the case prior to their findings.

"We came to the conclusion that chronically having bacteria in the lower airways is not a benign situation, but in fact is unhealthy for the lung and may contribute to the progression of the disease," says Sethi.

Another major advancement from the longitudinal study is identifying the involvement of the "wallflower" bacteria *Moraxella catarrhalis.* Through a series of studies that looked at immune responses, inflammatory markers and acquisition of new strains, the researchers were able to show that between two to four million exacerbations of COPD a year are caused by *M. catarrhalis.*

"M. catarrhalis had been ignored, overlooked, missed for years," Murphy says. "It turns out it is the second most common cause of these exacerbations. No one had recognized that."

In the November 27, 2008, issue of the *New England Journal of Medicine,* Murphy and Sethi summarized these and other research findings in a current-concepts review article titled "Infection in the Pathogenesis and Course of Chronic Obstructive Pulmonary Disease."

Also in 2008, Murphy, Sethi and colleagues published a paper in the *American Journal of Respiratory and Critical Care Medicine* that summarized 10 years of experience investigating a bacterium known as *Pseudomonas aeruginosa,* a pathogen that causes complications in children with cystic fibrosis (CF).

"It's a deadly, devastating infection in CF," explains Murphy. "If people with CF can remain free of *Pseudomonas,* they'll live for a long time, but once they become infected with it, their clinical course goes downhill."

Murphy and Sethi had found this same bacterium in the sputum of people with COPD, but its relationship to the disease was not clear: Does it cause serious complications as it does in CF, or is it an innocent colonizer? With the more than 100 strains of *Pseudomonas* collected from their patients over time, they were able to provide new, if not definitive, answers.

"It turns out, it's complicated," Murphy says. "Sometimes *Pseudomonas* comes and goes and doesn't cause exacerbations. It's clear, however, that in a lot of cases, it does cause exacerbations, and in a small number of people, it appears to behave like it does in CF—people with COPD get it, and the organism stays there and never goes away."

This was an entirely new finding. Prior to publication of this research, no one had any idea of the role this bacterium was playing in COPD. The paper had been out for less than a year, when Murphy was invited to talk at meetings about *Pseudomonas* and write review papers. "It's really been a very important observation in terms of understanding *Pseudomonas* in COPD," he says.

Recognition and Collaboration

The monthly COPD clinic visits of Donald Reitz, George Donaldson and scores of other volunteer veterans have made possible the advancements in vaccine development, as they have provided the researchers with eight freezers full of bacterial strains, along with samples of serum and sputum.

"Having all those bacterial strains is an absolute gold mine for doing this kind of work," observes Murphy. "We have clinical information on every single one of those thousands of bacteria, so when we find a gene that looks like it might be interesting, we have a wealth of strains in which we can test that gene."

The COPD clinical study's gold mine of bacteria has attracted attention across the world. "Because we have the information, the genes and the samples, it has led to a large number of collaborations," says Sethi. "We've sent bacteria to perhaps 50 different laboratories across the world. These include academic collaborations and collaborations with companies trying to develop new diagnoses, treatments and vaccines."

One particular academic collaboration stands out. "The day after the *Pseudomonas* paper came out," relates Murphy, "a researcher in Germany emailed me, and he was thrilled because he has this absolutely state-of the-art method using microarrays to study *Pseudomonas* strains. He's used the method on strains that come from children with CF, from ICU patients, and from strains out of lakes and streams. He has all these different 'family trees' of *Pseudomonas,* but he'd never seen a set of strains from COPD.

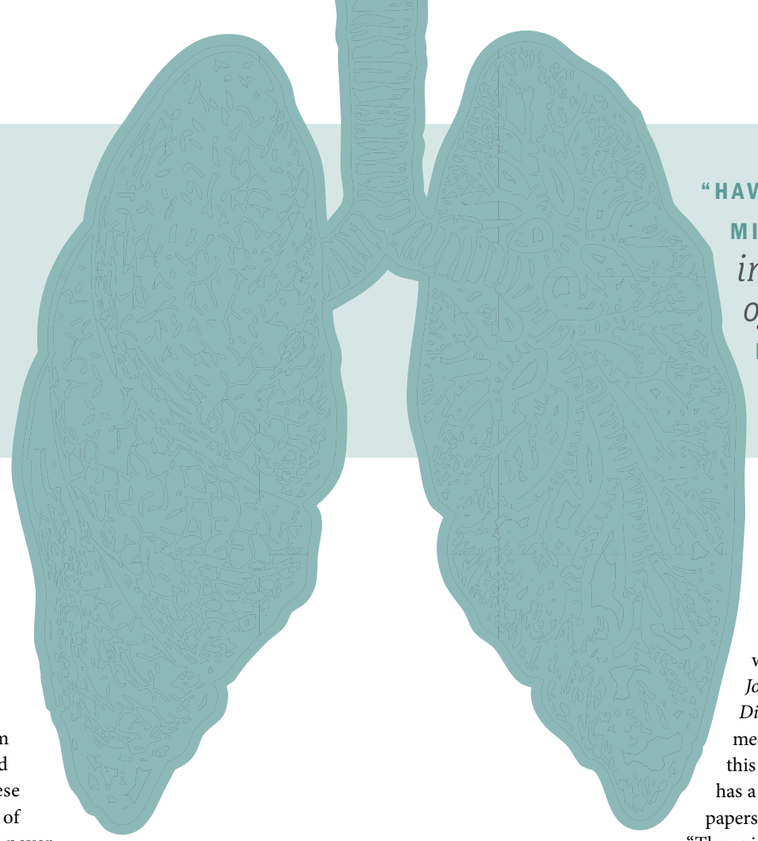
"So we sent our strains to Germany," Murphy continues. "It was a really big job. We have 150 strains of *Pseudomonas* here, and it involved producing the plates, packing them up safely and getting them through customs, but we did it. A few months later he sent his results back. He did this whole series of very cool studies that taught us a lot about *Pseudomonas* and COPD. We now have a paper on this that was just accepted for publication.

"It's almost endless, the number of collaborations that have come from this 15-year study."

Despite the research team's many successes, there have been a few setbacks along the way, one of which was especially memorable.

In 2004, Murphy and Sethi submitted to the *Journal of Infectious Diseases* a paper titled "Persistent Colonization by Haemophilus Influenzae in Chronic Obstructive Pulmonary Disease." The paper's findings, which Murphy described as "really good stuff," was based on the results of the first seven years of their clinical study.

The journal rejected it outright. Undeterred, the authors then submitted it to the *American Journal of Respiratory and Critical Care Medicine,* which accepted it immediately, acclaimed it as a classic paper and published it online in April of that year, six weeks after receiving it.



"HAVING ALL THOSE BACTERIAL STRAINS IS AN ABSOLUTE GOLD MINE FOR DOING THIS KIND OF WORK. *We have clinical information on every single one of those thousands of bacteria,* SO WHEN WE FIND A GENE THAT LOOKS LIKE IT MIGHT BE INTERESTING, WE HAVE A WEALTH OF STRAINS IN WHICH WE CAN TEST THAT GENE." —Timothy Murphy, MD

A year later, the Infectious Diseases Society of America, which publishes the *Journal of Infectious Diseases,* held its annual meeting. "Every year at this meeting, the society has a session called 'hot papers,'" recounts Murphy. "They pick 10 papers that they consider to be the most important papers from the previous year. Sure enough, in this list, rejected by their own journal, was our paper."

The Ultimate Goal

While interventions for COPD have improved, researchers still haven't been able to change the natural course of the disease, which shortens the life span.

The ultimate goal of the studies conducted by Murphy and Sethi is to understand bacterial infection in COPD so that better treatments and strategies for prevention of exacerbations can be developed. These include vaccines to prevent recurring COPD exacerbations, new antibiotics to treat exacerbations and immunomodulators that could regulate the inflammatory response to bacteria in the airways.

"Developing vaccines is one of our primary goals," says Sethi. "With the current treatments, we can prevent almost 30 to 40 percent of exacerbations, but we want to improve on that."

Murphy holds more than a dozen patents related to vaccine development. In June 2009, he met with a company to discuss designing clinical trials to test vaccines in COPD; however, he estimates that bringing a vaccine to the public is at least a decade away.

"There definitely are some promising vaccine antigens, several of which we developed," says Murphy.

Sethi adds: "In the next five years we also might see other approaches. There are people looking to see if inhaled antibiotics can make a difference in this disease. We can do it in cystic fibrosis, but the question is, can we do it in COPD? We also are looking for ways to prevent exacerbations more effectively and possibly to treat some of the chronic infections. There still is a lot of room for improvement in treatment."

Sethi is currently leading the first study of an inhaled antibiotic in COPD, the results of which are expected early next year, and he has spearheaded several multicenter international trials to assess the role of antibiotic therapy in exacerbations of COPD. He also was instrumental in developing a research tool to measure patient symptoms in COPD that has changed the way clinical studies in the discipline are conducted worldwide. In addition, he has an NIH grant that supports work to elucidate host responses in the airways in adults compared to healthy people. This work, which will lead to a better understanding of why people with COPD are more susceptible to infection, involves performing bronchoscopy on volunteers to obtain samples directly from the lower airways.

The ongoing Murphy-Sethi COPD clinical study will be critical to developing improved treatments for exacerbations of COPD.

"The longer the study goes, the more valuable it becomes," says Murphy, who has continued as co-investigator on the study since assuming his new role as head of the medical school's translational research projects. "Sanjay and I both are going strong. We're about to apply for funding for another five years. The overall goal remains the same, but now we're studying the interaction of viruses and bacteria in COPD. Not many researchers have done this. We're learning more about inflammation, and with the new technologies we can make better diagnoses.

"As long as patients struggle with this disease," he adds, "I don't see any end to our work." BP