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.

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.

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

Strain on the Lungs

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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.

Exacerbations often send patients to the emergency room, by an accumulation of thick, airway-clogging mucus. Each flare-up further damages the lungs, and ultimately, when they grow in large numbers, they reach a certain tipping point and people have a serious exacerbation-causing infection the same way everybody else does, but because they can’t clear these pathogens, they get sicker. They are susceptible to infections. They feel lousy, but they tough it out and usually their immune system eliminates the virus in a week and they are well again.

“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 mechanisms 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.

"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.

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, 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.

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 come from clinical studies in COPD that have 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 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."