

Jay Kolls and his team may soon run the table with a vaccine for pneumonia in AIDS patients and a stem cell therapy for cystic fibrosis. Kolls (left) and Chad Steele (background and right) don't get to play much pool lately because their research results are coming fast and furious.

BIG BREAKS MAY BE ON THE WAY

FOR APPROACHING DEADLY LUNG DISEASES

BY DAVID R. ELTZ

RACK 'EM UP

In the Big Easy in the late 1990s, if you worked in Jay Kolls' gene therapy lab, it helped if you shot pool. On any Wednesday after a day at the bench, Kolls would lead his researchers at Louisiana State University to a nearby pub for a debriefing. But you had to bring change. Kolls, with his deceptively boyish grin and giggle, was known for running the table all night without spending a quarter.

If the results from the LSU bench were coming fast, though, you'd find him in the lab well into the night, peering at petri dishes for the halo of a blooming virus or pondering the latest confocal microscopy images for the expression of a gene.

Even now, Kolls, a professor of pediatrics at the University of Pittsburgh since fall 2003, can as easily be found picking a classic rock tune on his Fender Stratocaster or wielding a pool cue as he can slipping on his white lab coat. And that smooth, natural transition between coolness and intensity has, for Kolls and his team, produced the perfect atmosphere for results.



PHOTOGRAPHY | CAMI MESA

He and his colleagues believe they have developed a potential vaccine for the pneumonia that kills children with AIDS, and they are likely to be the first to put bone marrow stem cells into patients with deadly cystic fibrosis.

Jay Kolls was born in 1959, the middle child of a nurse and a pediatrician. He grew up in the small town of Salisbury, Md., amidst the pungent aroma of the surrounding poultry farms.

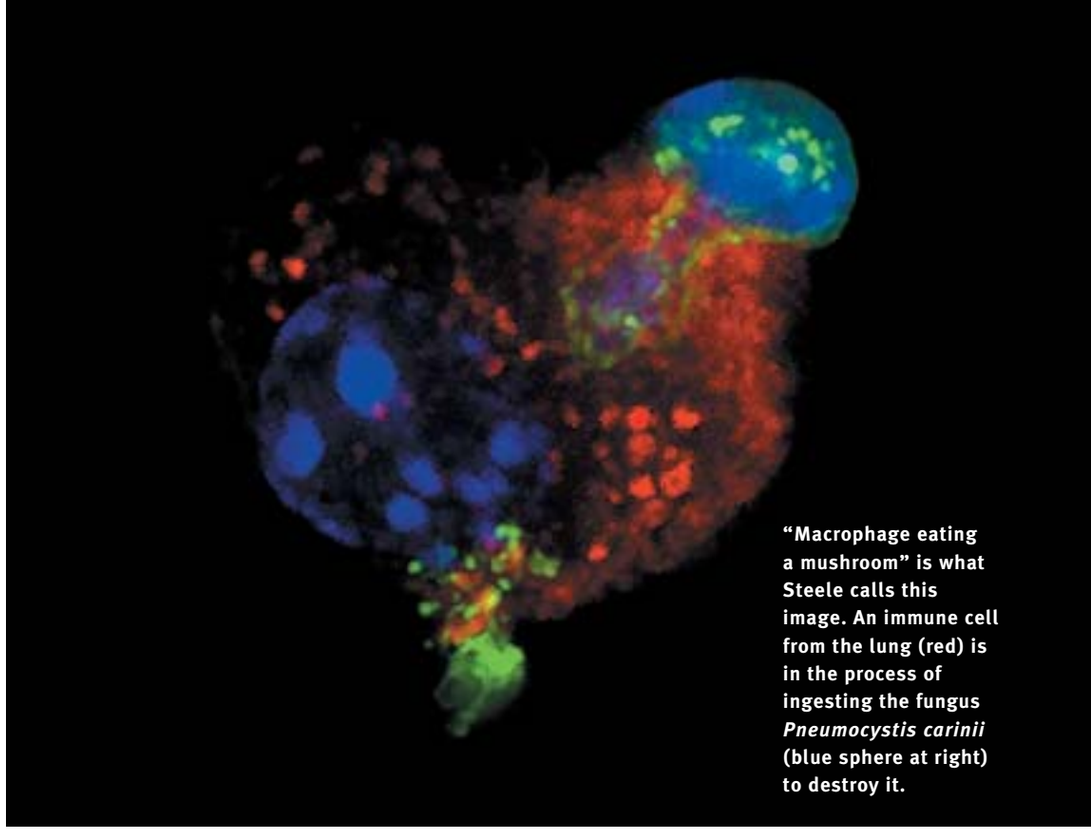
At Ursinus College, near Norristown, Pa., Kolls learned poker. (He still plays, but not well, reports one friend. He has too much fun drinking beer and laughing, says another, who still refuses to shoot pool with him.) Kolls majored in physics, liking its practicality and mathematical steadfastness.

He enrolled in medical school at the University of Maryland, where he struggled with the staid curriculum at first. *Here's the substrate. Here's the enzyme. Here's the product. Memorize it.* Finally, in clinical rotations, he felt he could be creative. At the Baltimore VA, Kolls heard of Warren Summer, a renowned pulmonologist and teacher. Summer had left Johns Hopkins to head a program at LSU, and Kolls arranged a senior elective with him at Charity Hospital in New Orleans.

Charity was a free hospital overloaded with inner-city patients, many in advanced stages of disease. There, Kolls saw one of the great urban scourges, tuberculosis. He saw mesothelioma, lung cancer caused by asbestos. (At the time, asbestos was used to insulate the hulls of ships built in New Orleans.) And Kolls saw one of the ravages of AIDS, the fungus *Pneumocystis carinii,* which causes a pneumonia.

Kolls landed an internship at LSU in pediatrics and medicine. One night, an AIDS patient suffered a respiratory arrest. Kolls and a nurse, Cynthia Cowart, intubated him. Three years later, they were married, still joking about meeting over a code.

By July 1989, Kolls had begun a dual fellowship in pediatric and adult pulmonology. He would spend the first of four years as a pediatric pulmonologist at nearby Tulane University, joining the adult pulmonology group at LSU in the second year. Kolls recalls that the gene for cystic fibrosis (CF) was discovered around then. "That was an exciting time; there was talk within minutes of the discovery of gene therapy," Kolls says. About the same time, a woman, 34, was hospitalized at Tulane. She'd previously been misdiagnosed with Wegener's granulomatosis, a rare disease of the respiratory tract. The woman had undergone heavy immunosuppression for a year, to no avail. Tests at Tulane revealed she had CF.



"Macrophage eating a mushroom" is what Steele calls this image. An immune cell from the lung (red) is in the process of ingesting the fungus *Pneumocystis carinii* (blue sphere at right) to destroy it.

The experience was a watershed moment for Kolls. In most people, the cilia attached to the epithelium in the lungs catch invading bacteria and drown the organisms in a pool of sodium chloride. In 30,000 young adults in the United States however, the cilia become impotent from fighting constant infection. Eventually, bacteria bore into the epithelium, causing chronic pulmonary problems that kill 90 percent of those with CF by a mean age of 31. Kolls was struck by the fact that symptoms of CF hadn't presented until she was 30, even though she'd obviously had the disease for years. He also was amazed that she could be immunosuppressed for so long without getting too sick. The immunology of lung disease held new fascination for him.

By 1991, Kolls headed for the bench, taking a spot in Judd Shellito's lab. Shellito had come to New Orleans from the University of California, San Francisco, having developed the first mouse model for pneumocystis pneumonia, the most common pediatric illness associated with AIDS and leukemia, particularly in sub-Saharan Africa. (About 90 percent of childhood HIV occurs in Africa; nearly 50 percent of those children will die from pneumocystis pneumonia, which is caused by the fungus *Pneumocystis carinii.*) The pneumonia occurs when a person's disease-fighting CD4 helper T cell count falls below 500. Along with a viral load test, a CD4 count is indicative of the extent of a patient's HIV infection.

Kolls became interested in a disease-fighting cytokine, tumor necrosis factor (TNF), which is produced by macrophages in response to infection. In diseases like arthritis and pneumonia, overproduction of TNF seemed to contribute to the disease. Summer, LSU's chair of pulmonology, helped Kolls win a prestigious Parker B. Francis Fellowship. Kolls moved to Dallas to work at the Howard Hughes Medical Institute in the lab of Bruce Beutler, who'd elucidated TNF's role in sepsis.

Beutler had made a transgenic mouse that weakly expressed an inhibitor of TNF. Kolls and Beutler talked about another approach: What if Kolls could make a virus that changed a mouse's genes, causing it to express the inhibitor? He started trying to make the virus in October 1992. By February, Kolls got the mixture of cells and agar, a translucent material that in a dish makes the glass look like a dirty window, just right. Ten days into culture, a telltale clearing, a halo of plaque, formed. He'd made his first virus. After another month of letting the virus grow, Kolls injected the substance into mice and challenged the mice with the bacterial disease listeria, which was thought to be dependent upon TNF. If the virus worked, the mice given the TNF inhibitor would be more susceptible to listeria. They were. In fact, his virus caused the mice to make so much TNF

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inhibitor, they died. The work proved that, as an alternative to knockout mice, you could use viral vectors to make what Kolls calls a "poor man's" transgenic mouse.

"This was really something that was quite an advance at the time," says Beutler, now a professor of internal medicine at the Scripps Research Institute in California. "The TNF gene hadn't been knocked out (in mice), and it wasn't really clear what a strong TNF blockade would do."

In July 1993, Kolls took a faculty appointment in medicine and pediatrics at LSU, continuing his work with Summer and Shellito. Summer recalls Kolls was so energized with new ideas and techniques that the pulmonology chair insisted each of LSU's lung grants have a transgenic component using Kolls' technology.

He became section chief of the pediatric pulmonology lab and director of the gene therapy program at LSU. He was also thinking about focusing his research on specific diseases. As a resident, he'd worked on pneumocystis in Shellito's lab; the disease, with its signature overproduction of TNF, and his technology seemed a perfect match. He started using his TNF receptor virus to inhibit various genes associated with the disease. But there were problems. "Everything I did made the infection worse," Kolls says. "I realized after a year that you can't go too far [in your career] by making infections worse."

Kolls was unsuccessful in getting his TNF receptor virus to help make infections better, so he switched gears. Eventually, he made two related viruses that *did* seem to work. In one ongoing project funded by the National Institutes of Health (NIH) since 1998, Kolls caused a mouse to produce interferon gamma, which helps macrophages make TNF. The interferon gamma caused T cells to change into cells that specifically helped fight lung infection. Kolls believes his virus technology caused macrophages to kill the fungus more effectively.

Another ongoing project might lead to a vaccine for pneumocystis. The CD4 helper T cell count of HIV-infected children is so depleted, they don't have an immune system strong enough to respond to a vaccine. In 1999, Kolls wondered: What if you worked around those cells? Other researchers had shown that you could genetically engineer dendritic cells in the bone marrow to tell the body to make antibody to fight infection.

Kolls discussed the idea with a postdoc, Mingquan Zheng. Zheng studied the technology and learned how to build dendritic cells from the bone marrow of mice with no CD4 cells. In the process, he engineered the cells with pneumocystis antigen, and gave the dendritic cells back to the mice—in effect, creating a cell-based vaccine using the mice's own cells. The mice developed the antibody to pneumocystis. The antibodies also offered protection in the long term; the team could challenge the mice again with pneumocystis, and they wouldn't develop infection. The researchers could even put the serum into other mice with no immune systems at all, and they too were protected against infection.

Kolls and his team set out to discover the critical antigens in pneumocystis that the mice were responding to. They've since identified two, cloned one, and left dendritic cells behind. Dendritic cell therapy, though considered a wave of the future, takes time and resources to create, meaning only the affluent will be able to afford such treatment. So Kolls' lab has been working, instead, with a DNA-based vaccine, which is much easier and less costly to grow. It's also a stable technology, not easily destroyed outside the lab. "You can ship it to Africa or wherever HIV is a major problem," Kolls says. Now he's vaccinating mice. Primate studies could be on the horizon. So might an application for investigational new drug status from the Food and Drug Administration to test efficacy in humans.

When Kolls came to Pitt in 2003, he took over pediatric pulmonology at Children's Hospital of Pittsburgh. Kolls also brought with him, from his New Orleans lab, Zheng, Chad Steele, Florencia McAllister, and Xue-Jun Zhao. Kolls sits in his office at the Rangos Research Center, still appearing boyish in his mid-40s, wearing a black polo shirt, talking about another potential blockbuster project he'll soon move to Pitt.

In the late 1990s, Kolls took note of research by others showing you could tease bone marrow stem cells to become bone culture in vitro. For about a decade, researchers had been trying to get viruses into the lungs of CF patients, only to fail because of natural barriers like cilia and sodium chloride that fight invaders. "This makes sense, evolutionarily," Kolls says. "You would not want your lung epithelium easily infected by viruses; we'd be sick all the time."

But what if you harvested a patient's own airway cells and grew them with bone marrow stem cells? Working with collaborators at LSU, Kolls' team took such cells from patients undergoing lung transplants, mixed them in culture with stem cells marked with a green fluorescent protein, and watched as the cells divided. After two weeks, they had green cells growing cilia that looked just like epithelial airway cells. What's more, before culture, the stem cells didn't express the CF gene. At the end of 14 days, they did. Work is still under way to determine if the CF gene in these new cells causes the secretion of sodium chloride, meaning the cells would be functional. So far, two experiments have shown they are functional.

Can the stem cells differentiate in a human? Kolls believes he will know soon. He's preparing for a study at Pitt in which a few patients with CF would give bone marrow samples. Kolls would extract stem cells, alter them with a normal CF gene for therapy delivery, grow them in culture, and then plant them back into the walls of patients' sinuses. (CF patients tend to have terrible sinus infections that often require surgery.) After a few months, Kolls would have them come back for a biopsy to see if the cells differentiate in the sinus wall just as they do in culture.

The FDA may ask Kolls to do animal studies to test for adverse effects. Kolls could get the technology classified as an investigational drug by September. With the FDA's blessing, he could go to the NIH to fund a clinical trial, starting in early 2005, just to see if the technology works. Then Kolls has to figure out how to deliver the therapy to the lung.

Remember the natural barriers—the cilia and sodium chloride—that protect the lung from invaders? Kolls' stem cell treatment would need safe passage around those guardians. There could be a way. When you get an infection in your lung, macrophages send signals called chemokines to attract homing molecules, which then bind to chemokine receptors on the macrophage. Kolls wonders if he can engineer the therapeutic stem cells to express these receptors. If so, maybe he could get the therapy to bind directly to macrophages and home to the lung—without getting trapped in cilia and drowning.

That would be like smacking all the solids on a pool table into separate pockets on the break. Then, again, Kolls has been known to run the table all night. ■