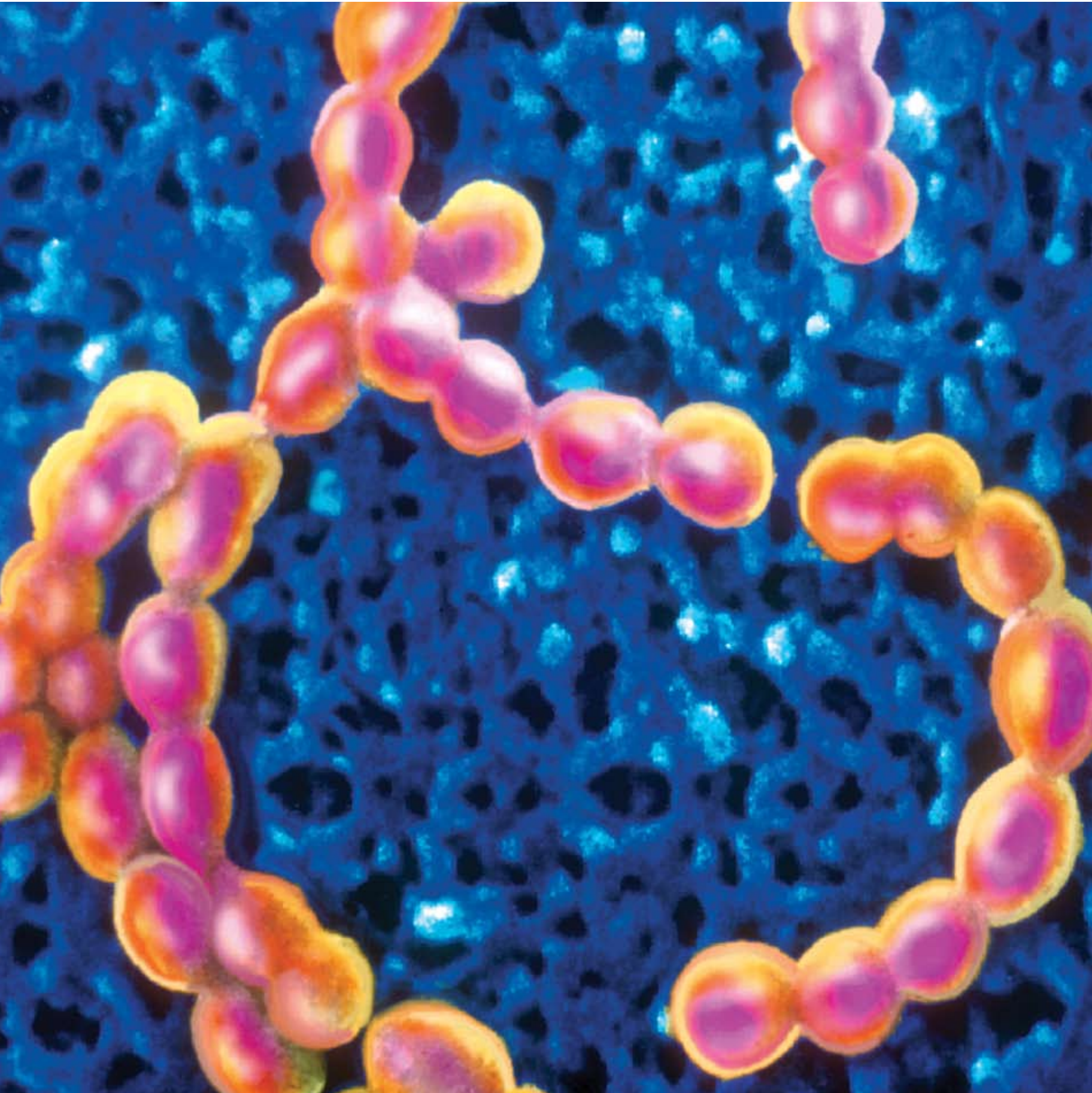


**INVESTIGATIONS**

*Explorations and revelations taking place in the medical school*



# CONFRONTING OUR OWN WORST ENEMY

TINY BEADS DESIGNED TO TAKE ON SEPSIS,  
THE OFTEN FATAL IMMUNE RESPONSE

BY REID R. FRAZIER

One afternoon, a patient comes into John Kellum's cardiothoracic ICU at UPMC Presbyterian shaking with chills. Lisa Davis, 33, is usually a healthy math teacher. But today she feels like a bus hit her. Davis (a fictional but typical case) developed a cough and fever two days ago. Now she has pneumonia.

By evening, Davis is in a hospital bed with infusion pumps delivering intravenous antibiotics and an oxygen mask pressed over her mouth and nose. Despite the antibiotics she is taking, her husband thinks she looks even worse than she did in the morning.

Inside her lungs, bacteria have been multiplying for the last few days, kicking off a full-blown battle with the immune system. Macrophages, special white blood cells that seek out pathogens, first discovered the bacteria. They sent out signals that attracted chemicals called cytokines ("cell-movers"), which in turn draw other white blood cells to the lungs. But the cytokines inflict a kind of collateral damage on Davis' body. Her brain senses the cytokines and cranks the thermostat in her body to a sweltering 103 degrees—hence her shaking chills. The cytokines also make her capillaries leak. This allows more white blood cells to enter the lung tissue in search of bacteria, but it also causes her blood pressure to drop, as fluids leak out of her blood vessels into skin and soft tissues. If her blood pressure falls too low, her organs could become deprived of oxygen. Davis is in the

throes of sepsis, the systemic inflammatory response to infection. In sepsis, the immune system causes most of the damage. It can trigger organ failure and is a rapacious killer of ICU patients. Sepsis is far from a household term, yet every surgeon and intensive care staff member is all too aware of its threat. Sepsis affects more than three-quarters of a million Americans each year and takes the lives of hundreds of thousands of them.

"No one who works in an ICU doesn't want something to treat sepsis better than what we have now, which is supportive care—basically treating the symptoms, and waiting," says Kellum.

Kellum, a University of Pittsburgh professor of critical care medicine, thinks we can do more for these patients. He'd worked with dialysis patients during his training in internal medicine and began wondering whether some form of blood filtration could remove cytokines before they initiate sepsis. Three years ago, he assembled a team of Pitt docs and bioengineers, received a \$5 million National Institutes of Health grant, and got to work.

The early result of their efforts sits on a shelf in Kellum's office in Scaife Hall and looks like something you might get if you used the pneumatic tube at a bank teller's drive-through window. It's a foot-long, clear plastic cylinder. Each end has a screw-on lid with a plug in the middle. Inside the cylinder is a whitish substance—a collection of beads—that, at first glance, resembles grated Parmesan cheese.

It's a souped-up version of a hemodialysis filter, designed to extract toxins from the blood of kidney patients.

Kellum is excited about the beads. He hopes to modify them to absorb the out-of-control cytokines that cause sepsis. "Under

a scanning electron microscope, one of the beads looks like a giant sponge. In every gram of the beads, there are 850 square meters of surface area for binding," he says.

Designing the beads is the difficult part. You don't want to filter out all cytokines—they help white blood cells know where to go to fight an infection, and you certainly want white blood cells to pass through. "Think of the cytokines like trucks in a distribution system," says William Federspiel, Pitt professor of chemical engineering, surgery, and bioengineering, whom Kellum tapped to help design the device. "We've got to design a system that will regulate which trucks we keep on the road and which we keep off."

To do that, the team will first have to conduct a traffic study of sorts. Gilles Clermont, associate professor of critical care medicine, is calculating the body's immune response in blood. Clermont wants to measure the amount and type of cytokines the blood normally carries, compared with cytokine levels in the blood of a sepsis patient. Call it "normal" versus "rush hour" traffic. When Clermont's calculations are complete, Federspiel and scientists at MedaSorb, the medical device company creating the beads, will modify the beads' structure to regulate cytokines. Take out a couple of 18-wheelers here, a dump truck there, and, hopefully, the roads will clear. Early results in animals are promising.

"Coming up with a therapy for sepsis would be a huge deal, obviously. This is a process that literally kills a quarter-million people every year, and if you could reduce that by even 10 percent, that would have a substantial impact," Kellum says.

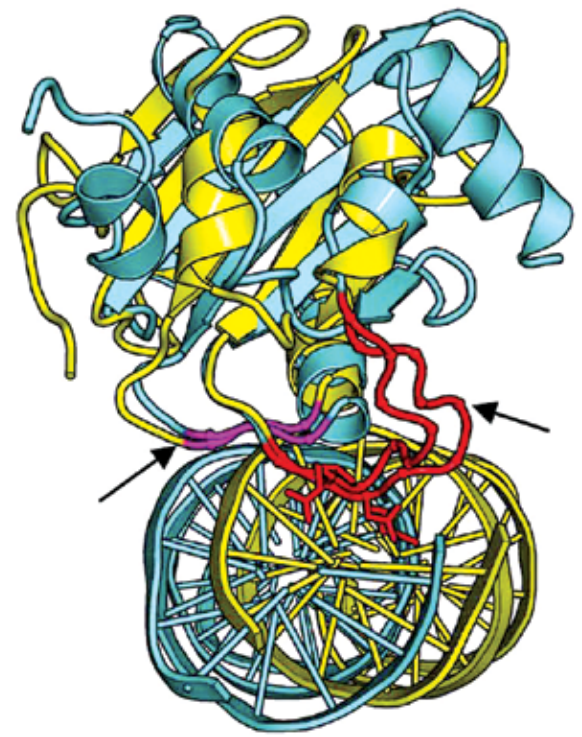
"Do I think it'll work? I wouldn't have devoted my entire academic career to it if I didn't think it had promise." ■

**Bacteria like *Streptococcus pyogenes*, shown left, can kick off sepsis, the overactive immune response. The condition kills hundreds of thousands of ICU patients each year in the United States. A team of Pitt bioengineers and physicians hopes to dampen cases of sepsis with tiny absorbent beads.**

# THE VIRUS HUNTERS

PITT RESEARCHERS BAG ANOTHER  
CANCER VIRAL PRECURSOR

BY SHARON TREGASKIS



Chang and Moore discovered a virus (MCPyV) that causes an aggressive form of skin cancer. This image shows a similar virus (SV40) as the T antigen binds to the viral DNA. Binding allows the virus to replicate.

**F**ifteen percent of human cancers owe their existence to the molecular debris field created in the wake of a viral infection. Sometimes, as in the liver cancer that emerges in response to hepatitis-induced inflammation, it's hit-and-run—no trace remains of the nucleic acids that first derailed cellular function. But sometimes, the virus leaves its tag on every tumor cell it generates. Cervical cancer carries the ghost of the human papillomavirus. Burkitt's and Hodgkin's lymphomas give a nod to the Epstein-Barr infection that precipitates each.

The concept isn't new. Scientists identified the first cancer viral precursor—murine polyomavirus (MuPyV)—in lab mice with leukemia in 1953. Finding such links could provide a critical boost to human health, refining screening, treatment, and public health initiatives. But in the half-century since MuPyV was isolated, and despite massive efforts since the first human tumor-inducing viruses were identified in the early '70s, just seven viral precursors have been definitively tied to cancer in people.

University of Pittsburgh neuropathologist and professor of pathology Yuan Chang and husband Patrick Moore, an epidemiologist who is a professor of microbiology and molecular genetics, have contributed two to the list. The scientists have devoted their careers to stalking the biomolecular cues that link a tumor to the virus that got it started.

In 1994, they bagged their first: KSHV, a herpes virus that causes Kaposi's sarcoma, the most common cancer in sub-Saharan Africa and the leading malignancy in AIDS patients. This winter, they did it again, revealing Merkel cell polyomavirus, a double-stranded ring of

DNA responsible for an aggressive form of skin cancer known as Merkel cell carcinoma (MCC). *Science* printed their findings in February. In the past 15 years, no other research group has identified a novel viral precursor to cancer.

Moore credits the couple's success to the breadth of their collaboration, joking that they have to work together every day just to feed their son breakfast.

Moore sparked the pair's focus on cancers with a link to the immune system. A healthy immune response generally fends off infections before viral DNA can rewire human cellular biochemistry to fuel tumor growth. Epidemiological analysis reveals the cancer link: Those who develop cancers sparked by viral infection have suppressed immune function, such as the elderly, people with AIDS, and transplant and cancer patients undergoing immune-suppressing treatments.

Now codirector with her husband of the Hillman Cancer Institute's KSHV lab, Chang saw MCC for the first time as a pathology resident at the University of California, San Francisco, working up a biopsy taken from an elderly man whose cancer had already metastasized. At the time, there were fewer than 500 cases of MCC diagnosed each year. In the past two decades, its incidence has tripled.

"It was a diagnostic problem, because this particular tumor has so many look-alikes ranging from small-cell lung cancer to malignant lymphoma," says Chang. "The workup to find the right diagnosis was intensive." Years later, the disease struck a medical student in a class Chang taught. Those two cases made a sharp impression. In 2002, the scientist read a report linking MCC with

immunosuppression, a common clue to a viral precursor. She was hooked.

Chang and Moore spent the next five years collecting MCC tumor samples with extensive strings of genetic material and sequencing all of it—with an eye to identifying the protein transcripts left behind by viral nucleic acids. To find them, they relied on a technique they'd developed called Digital Transcript Subtraction (DTS). Using a Macintosh computer and data available from the Human Genome Project, the pair cross-checked tumor DNA with sequences in the database, eliminating anything other researchers had already identified as human.

"That leaves us with a small group of sequences that are errors, previously unidentified, or—if we get lucky—a viral transcript," says Moore. (The strategy emerged during their inquiry into the epidemiology of squamous cell conjunctival carcinoma—a previously rare cancer now common in Uganda and long suspected of having a viral precursor because of its association with AIDS.) With DTS, Moore and Chang logged two hits: The novel technique disproved the viral precursor theory in the conjunctival carcinoma; it also revealed the nucleic acids at the root of MCC.

The next step, says Moore, is to decipher the protein structure of tumor cells. "Viruses have a vested interest in not letting their proteins degrade," he says. "If we could look at the proteins and differentiate viral from human proteins, that would be huge." ■

# A TEST FOR TOLERANCE

THE SEARCH FOR A  
GENETIC CLUE FOR SUCCESSFUL  
TRANSPLANTATION

BY JOE MIKSCH

**R**akesh Sindhi has a proposition: Give him the resources, and he'll give you a genetic test that can predict a transplant patient's odds of rejecting a new organ.

Such a tool, the University of Pittsburgh MD associate professor of surgery suggests, will reduce the incidence of rejection from 50 to 20 percent—cutting down the amount of time patients spend in the hospital and saving the health system significant expense. It also will give doctors better guidelines for prescribing antirejection drugs.

Sindhi codirects the Hillman Center for Pediatric Transplantation at Children's Hospital of Pittsburgh of UPMC. The center believes that Sindhi's optimism is well-founded, having recently given him a three-year, \$300,000 grant to pursue this research. The Hillman Strategic Award funding is in addition to a 2006 \$1.1 million National Institutes of Health grant Sindhi received for the genetic fingerprinting project.

Sindhi says current methods of predicting the likelihood of rejection are not bad but imprecise. A doctor might be able to predict that a prospective recipient is likely to reject, but not the degree of the immune response. It's all but impossible, then, to do anything but guess the level of antirejection drug needed to stave off an immune attack on the new organ.

Too much antirejection drug can be toxic to patients. Too little means the graft won't survive.

"The question remains then, how do you predict a rejecter or a nonrejecter right off

the bat?" Sindhi says. If this question can be answered, he says, antirejection medication could be tailored to individual patients. Doctors would immediately put likely rejecters on high doses of antirejection medicine and those less likely to reject on lower doses.

One way to measure the likelihood of rejection is to measure genetic predisposition. Is there a genetic mutation, or combination of mutations, that makes a transplant recipient's immune system more or less likely to recognize a new organ as a threat and go on the attack?

As genetic information is passed down from generation to generation, it's common for small mistakes to be made. Let's say a genetic message is supposed to read, "I love my mom," and ends up being transcribed as "I love mi mom." It's still pretty clear what the message is, despite the error.

"These single alphabet flaws don't alter the meaning of the code," Sindhi says. "The majority are flaws that don't really matter in [terms of] disease origin."

However, Sindhi says, these inconsequential errors sometimes point to larger sections of garbled genetic code. Perhaps the path to finding genetic variants responsible for rejection lies in these mutations.

Sindhi conducted a study of 150 children who'd undergone liver transplantations, as well as their parents. He took 500,000 "snips" (shorthand for single nucleotide polymorphisms; these are essentially revealing snippets of genetic code), looking for mutations common to those who had rejected organs and

those who tolerated transplanted organs well. He also recently began genotyping another 250 patients—all of whom had transplants after suffering from biliary atresia, a blockage in the tubes that carry bile from the liver to the intestines—and their parents and plans to replicate the process outlined above. Sindhi chose this group to examine mutations within a stable genetic population suffering from the same disease.

He is looking for variations common among those who reacted well to their new livers and those who struggled to assimilate new organs. The challenges here, Sindhi says, are significant.

"Of 50,000 genes, let's say 600 turn out to be candidates," he says.

"There are still a lot of so-called false candidates. If it's a real variation in the gene, we also need to look for associated changes in the expression of the gene."

His next step will be to look for genes whose functions are tied specifically to different types of white blood cells (which are key to rejection and tolerance). If he finds a correlation between a mutation in one of these cells and the degree of tolerance, that could mean he has identified a genetic marker.

Sindhi relates this story with the zeal of a true believer. He says he's sure it can be done. Then he pauses and says a bit wistfully, "If somebody invested \$3 million today, I could deliver a first-generation product.

"We have the patients, the technology, and the understanding. It's purely a matter of investment." ■

ELENA G. CERRI



As genetic information is passed down through generations, it's common for small mistakes to be made in transcription.