

INVESTIGATIONS

Explorations and revelations taking place in the medical school



Existing treatments for elephantiasis can leave adult worms in the lymph nodes, where they can procreate and live for years.

ELEPHANTIASIS WORM GENOME SEQUENCED

BUT HOW DOES IT SLINK AROUND THE
IMMUNE SYSTEM? | BY ERICA LLOYD

When Dutchman Jan Huygen Linschoten spent some time in Goa in the late 16th century, he noted that some locals had limbs “as thick as an elephant’s leg.” After Linschoten, medical historians find many references to lymphatic filariasis. This tropical disease is often called elephantiasis, though the afflicted typically experience both elephantiasis (thickened and hardened skin) and lymphedema (disfiguring enlarged limbs and other body parts). Infected men and women also can suffer from damaged and swollen genitalia, adding to the stigma of the disease.

The human species hosts more than 300 parasites, and the microscopic worms that cause elephantiasis have probably been living with us well before Linschoten’s travels. Bloating limbs on representations of Pharaoh Mentuhotep II from 2000 BCE suggest those who set up house along the Nile have dealt with the disease for millennia. Yet it wasn’t until 1877 that scientists realized how the parasitic infection spreads—through mosquitoes that carry the worm larvae.

Now, researchers led by the University of Pittsburgh’s Elodie Ghedin have sequenced the genome of *Brugia malayi*, a nematode that causes the disease.

Ghedin, an assistant professor of medicine in the Division of Infectious Diseases, reported on the sequencing in the Sept. 21 issue of *Science*. Her paper suggests areas of the genome that may yield new drug targets for the disease. Probably 40 million people are severely disabled by the disease, the World Health Organization estimates.

No vaccine exists for elephantiasis. Drug treatments often target the larvae or don’t kill all the adult worms—leaving procreating worms in the system that can continue to live for several years. Existing drugs also don’t alleviate the disfiguring and painful symptoms (though intense hygiene treatments help). There’s evidence that

the nematode is developing drug resistance as well, Ghedin notes.

She hopes the sequenced worm DNA also will help scientists understand the subtleties of human immunity.

Mosquitoes that carry *B. malayi* larvae in their proboscises spread the disease. When the bugs bite people, the larvae molt in the bloodstream, then wiggle their way into the lymph nodes, where they live and reproduce as adults. One worm can bear 1,000 larvae a day, which swim back to the peripheral blood. There, the larvae might be picked up by a dining mosquito, continuing the cycle.

If the immune system reacts to the parasite, the membrane of the lymph node becomes inflamed and, eventually, injured. The lymphatic system then can no longer carry out its duties—including draining lymph—resulting in the swollen, hardened limbs and other infamous symptoms of the disease.

“The parasite doesn’t feel good if you don’t feel good,” says Ghedin, explaining that *B. malayi* procreates best when it slinks beneath the immune system’s radar. Often, adult worms can keep right on reproducing without causing noticeable trouble. Most people hosting the parasite don’t get the disease, yet those same people are the most effective at spreading the infection (if they continue to come into contact with mosquitoes).

B. malayi can live for years in humans without setting off an immune response. Oddly, the worms manage to do this while cozying up within the garrisoned lymph nodes, where the body usually mobilizes its response against infection.

“We think the worms are secreting compounds to the immune system so the system ignores them and doesn’t reject them,” says Ghedin.

The National Institute of Allergy and Infectious Diseases funded the sequencing

project. Ghedin’s team was based at TIGR, the Institute for Genomic Research (now part of the J. Craig Venter Institute), in Rockville, Md. TIGR is Ghedin’s former professional home; she retains an adjunct investigator position there since joining Pitt in 2006.

The sequencing task was not straightforward, Ghedin reports. Among other difficulties, the chromosomes in *B. malayi* were tangled together and couldn’t be individually separated. This means the researchers couldn’t solve the genome chromosome by chromosome, as the sequencers of the human genome did. Instead, Ghedin’s team effectively blasted the genetic material apart, then relied on overlapping sections of DNA molecules to reassemble it, “like Legos,” Ghedin says.

“It’s still in 8,000 pieces,” she says. “But the pieces were large enough that we were able to do all sorts of analyses.”

The researchers found thousands of genes in *B. malayi* not yet identified in other organisms. Ghedin is working with Penelope Morel, Pitt associate professor of immunology, to find out whether any of these genes code proteins that allow the nematode to thrive without setting off an immune alarm. (Both investigators are affiliated with Pitt’s newly created Center for Vaccine Research.)

Such discoveries might help the millions infected with *B. malayi* as well as millions out of the worm’s reach.

“It could be huge if we could determine what proteins the worm produces that can have such a strong impact on the immune system,” says Ghedin.

“We could use them in transplantation, to prevent rejection of transplanted organs.” She adds that such proteins could also inform our understanding of autoimmune diseases, including diabetes and multiple sclerosis.

Other researchers have already hit upon promising candidate proteins. ■

AN ELEGANS SOLUTION

TINY WORM DEATHS YIELD KEY TO CELLULAR LIFE | BY JOE MIKSCH

For a biomedical scientist, getting the cover of *Cell* is akin to an emerging rock band landing the cover of *Rolling Stone*. It's a sign, in both cases, that some significant work has been done. Of course, there are some differences between finding a protein that can halt and reverse the sudden and dangerous death of cells and pumping out some edgy alternative rock. But in the realm of professional pride, that's a difference without a distinction.

The Sept. 21, 2007 coverboys, or, rather, coverscientists Gary Silverman and Cliff Luke are investigators at Magee-Womens Research Institute. About five years ago, Luke, a PhD assistant professor of pediatrics in the University of Pittsburgh School of Medicine, was attempting to dislodge a clan of tiny roundworms, *C. elegans*, from the surface of a lab dish. He squirted them with water. Many died a pretty violent death: They exploded.

The assumption shared by Luke and Silverman, an MD/PhD who is chief of newborn medicine in Pitt's Department of Pediatrics and head of the lab in which Luke works, was that the dying worms had some sort of problem regulating cell volume. In short, they thought that the worms' cells were unable to expel water, causing them to burst.

Luke and Silverman were wrong.

A couple of years of investigation have led them to conclude that the dying worms were deficient in a protein called SRP-6, a serine protease inhibitor (or serpin). The scientists had knocked out the gene for SRP-6 (pronounced *serp six*) in the exploding worms but never imagined it would have that effect.

The dying worms didn't have a volume-regulation problem. The worms died before they even had a chance to deal with the hypotonic

stress. It was a type of catastrophic, unregulated, and irreversible cell death called necrosis—the same thing that happens to human cells deprived of oxygen through a heart attack—that was decimating Luke and Silverman's worm population.

Perhaps, Luke and Silverman began to think, the lack of SRP-6 got the necrotic process started. And, if that's the case, if there's a rhyme and reason to necrosis, maybe the process isn't unregulated after all. And maybe the labels "irreversible" and "catastrophic" can be cast aside as well if the cellular death march can be reversed.

"It turned out that SRP-6 protects the lysosome," Silverman says. The lysosome is the cell's digestive center and home to powerful poisonous enzymes that are released when it is damaged. "And should the lysosome get injured and leak some of its contents, SRP-6 also plays a role in blocking some of those enzymes that are released," he adds.

The researchers say that's a powerful piece of knowledge.

"We now have a pathway we can attack therapeutically that can potentially save any cell dying by necrosis," Silverman says.

"If you can block that, and even if the lysosomes break apart—which you would think would cause catastrophic, irreversible death—if there's enough SRP in the pathway, the cell survives and the lysosome will reform." He pauses, lowers his voice almost to a whisper, and adds, "That's stunning."

Silverman and Luke, the *Cell* paper's senior and primary authors, respectively, have already embarked upon the search for a SRP-6-related therapeutic agent. In partnership with Pitt's

Drug Discovery Institute, they are screening thousands of compounds, looking for a drug that might be able to increase the presence of SRP-6 in cells that have begun to die by necrosis.

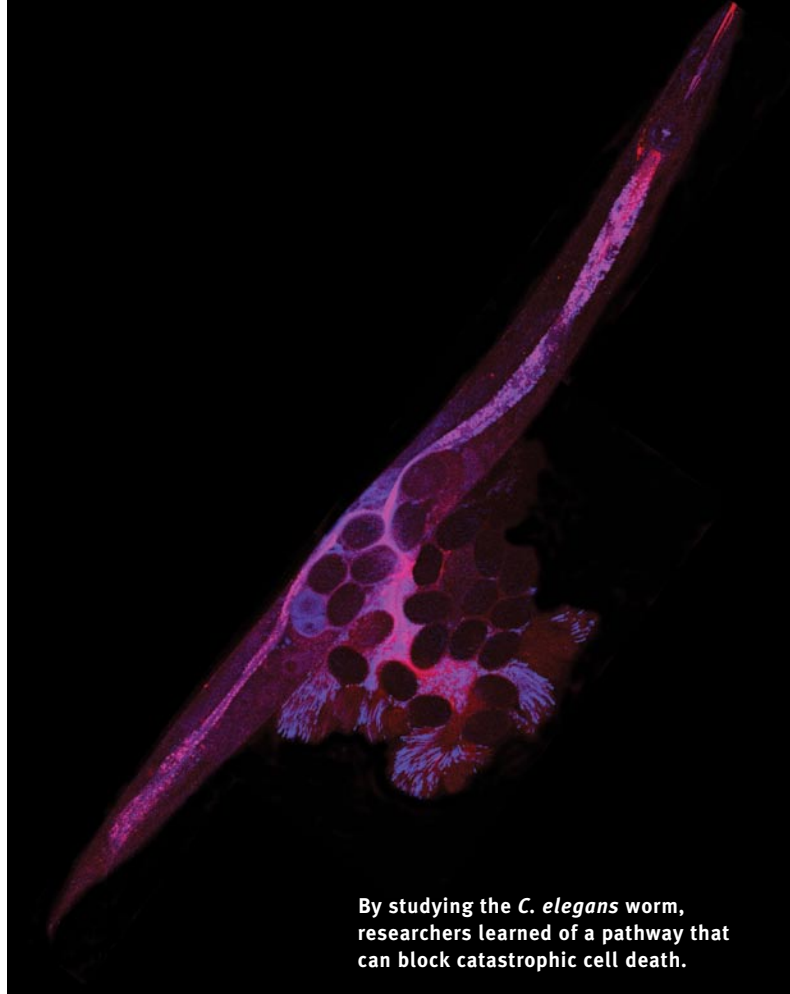
Silverman says stroke, heart attack, and various gastrointestinal disorders and neurodegenerative diseases are all potential SRP-6-related drug targets.

As an example, Silverman imagines a man stricken with a heart attack caused by a clogged blood vessel. Today, to prevent his heart from absorbing further damage, emergency room doctors are likely to rush to give him a shot of blood thinner to restore flow to the dying heart cells as quickly as possible. The cells that have already suffered a fatal insult? They're goners and aren't coming back.

"But imagine if you can get enough of a SRP-6-like drug in there," Silverman says. "You could probably protect cells from dying even if it's a very late stage of the game." As fewer cells suffer necrosis, the heart muscle could come away from the insult much healthier than it would without such a drug.

So breaking ground by defining a pathway that kills cells and often kills people, starting the search for new life-saving drugs, and landing the cover of *Cell*, that's got to be pretty cool, right?

"It is," Luke says, laughing. "It really is." ■



By studying the *C. elegans* worm, researchers learned of a pathway that can block catastrophic cell death.

COURTESY: C. LUKE

PICK YOUR POISON

CO IS GOOD FOR YOU
(IN SMALL AMOUNTS)
BY SARA GOUDARZI

GETTY IMAGES



Carbon monoxide. When we think about it, we tend to think of cars coughing up exhaust or maybe a poorly vented kerosene heater. It's a leading cause of fatal poisoning.

Yet the deadly gas is actually produced in small doses by our bodies.

And in minute amounts, carbon monoxide can be therapeutic. If your body produces more of the gas, you're likely to heal more quickly from injuries. Some researchers are hoping to one day administer it to patients in need.

"What I find interesting about carbon monoxide is that it still has a little bit of a shock value that you're using what's really thought of as a lethal gas for beneficial effects," says the University of Pittsburgh's Brian Zuckerbraun, an assistant professor of surgery.

Zuckerbraun is among a handful of researchers actively investigating the anti-inflammatory effects of carbon monoxide. He says his work is heavily influenced by research conducted by Augustine Choi and Leo Otterbein. In the mid-1990s, those scientists were investigating the beneficial effects of heme oxygenase-1 enzymes—which break down to form carbon monoxide as one of their byproducts—for preventing injury from different types of insults.

The two researchers, then at Johns Hopkins University, discovered that blocking the enzymes led to more cell damage.

"I came up with the hypothesis that carbon monoxide was the mechanism by which the enzyme heme oxygenase-1—which was known to be protective and which Dr. Choi and I had studied for years prior—was functioning," says Otterbein.

This idea became the focus of Otterbein's doctoral dissertation and, later, his research

concentration when he and Choi moved to Yale University. Shortly after they published a *Nature Medicine* paper in 2000 that showed carbon monoxide had anti-inflammatory effects in mice and in mouse cell cultures, the scientists relocated to Pitt.

At the time, Zuckerbraun, then a surgery fellow, was working on nitric oxide—another gas notorious for its toxicity and later found to be beneficial in small quantities within the body. He soon joined Choi and Otterbein's research efforts.

Now Zuckerbraun runs his own lab, with carbon monoxide as a main area of investigation. (In 2004, Otterbein left Pitt to join the faculty at Harvard Medical School. Choi recently accepted a position there as well.)

Zuckerbraun studies pulmonary hypertension, i.e., high blood pressure in the arteries supplying blood to the lungs.

By the time symptoms of the disorder present themselves, the damage is typically irreversible. Patients end up short of breath from even low levels of exertion and can't perform simple functions such as walking up the stairs.

In end-stage cases, the only treatment is lung transplantation. No therapies are available to reverse the artery thickening that causes high blood pressure.

Zuckerbraun is interested in how carbon monoxide might help those with the disorder.

He's exploring using carbon monoxide to reverse thickening of blood vessels in the lung by encouraging protective genes and restoring damaged cells to health.

The surgeon also investigates how inhaling the gas could protect against injuries of other organs and hemorrhagic shock.

"If you could deliver controlled doses of

carbon monoxide, you could potentially prevent inflammation that takes place as a result of the hemorrhage and the consequences of that," says Zuckerbraun.

In cases such as hemorrhagic or septic shock, our tissues don't get enough oxygen. When inhaled in large quantities, carbon monoxide is poisonous—entering the bloodstream where its molecules bind to oxygen-carrying hemoglobin and essentially suffocating the body by starving it of oxygen at the cellular level. Doctors have known that since the 19th-century French physiologist Claude Bernard poisoned dogs to learn about the toxic effects of carbon monoxide.

Yet Bernard had only half the story. The gas doesn't always reduce oxygen levels in tissues. In fact, in some circumstances, it can lead to increased oxygen levels, says Zuckerbraun.

And he and Otterbein suggest it's likely that carbon monoxide is helpful during shock states to limit and regulate oxygen consumption in the setting of decreased oxygen delivery to organs and cells.

Carbon monoxide also acts as a signaling molecule within the cell. "For instance, in Brian's work," says Otterbein, "nitric oxide is deficient in pulmonary hypertension."

"We need nitric oxide, driven by carbon monoxide, [to decrease the thickness of artery walls]."

"So in that case, the target of carbon monoxide is the enzyme that makes nitric oxide synthase. We believe that carbon monoxide influences that enzyme directly to make more nitric oxide."

Pick your poison. Now scientists have shown us two—nitric oxide and carbon monoxide—required to keep people healthy. ■