

## In the hunt for a Lassa Fever cure

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James Robinson (left) and Robert Garry have tracked the Lassa virus for more than a decade. (Photo by Paula Burch-Celentano)

Twelve years ago, Robert Garry first suggested that his team at Tulane University School of Medicine could unlock the secrets of the mysterious Lassa virus.

Some researchers were skeptical. “They thought it was too difficult,” Garry said.

For starters, the trip from the closest airport to Tulane’s partners at the Kenema Government Hospital in Sierra Leone took 13 hours, driving over treacherously bumpy dirt roads. Tulane would have to draw blood samples from Lassa survivors at

a lab in southern Nigeria and the hospital in Kenema, freeze the samples, then keep them frozen for another long bumpy ride and a trans-Atlantic flight to New Orleans.

Once those practical concerns were overcome, Tulane researchers were faced with a virus that science knew very little about in 2005.

“This virus was an enigma,” said Garry’s longtime colleague, Dr. James Robinson, a professor of pediatrics. “We knew it occurred and that people either died or got better.”

“Before we started, no one knew what the proteins of Lassa virus looked like,” said Garry, a professor of microbiology and immunology. “We knew little about how the immune system responded to the virus. And we didn’t know if our tests would work.”

A few years ago, the Tulane team grieved and suffered setbacks after Kenema’s hospital became ground zero for an explosive outbreak of Ebola virus, a highly contagious hemorrhagic fever whose initial symptoms look similar to Lassa in patients. Despite protective gear, 11 of the hospital’s staff were infected; several died, including the chief nurse and the doctor in charge of the Lassa fever program.

During the project’s hardest times, even Garry, the project leader for the proposal, wondered if it was possible to get past all the practical hurdles in order to develop anti-viral drugs and vaccines for Lassa. “Could we pull this off?” he thought.

But Garry knew that he had the institutional support of Tulane, because of its long-standing commitment to combating tropical illness. Garry himself had also spent much of his career unpuzzling viruses that others found difficult, most notably HIV. He and Robinson had worked with the virus since 1987, tracking it from strains taken from AIDS victims as far back as 1969 and helping to develop crucial anti-viral drugs and early-diagnosis HIV tests.

So, in many ways, Garry felt that he and his team were ready to tackle the Lassa virus. Plus, a pot of money had emerged to finance such work.

In 2001, after the attacks of 9/11 and the deadly anthrax mailings that followed shortly after, national authorities began creating a list of diseases that terrorists could easily “weaponize” and use in biological warfare. Lassa virus was on that list.

In subsequent years, the National Institutes of Health announced grants for researchers focused on certain diseases, including Lassa. So, in late 2004, to

address the U.S. government's concerns about biological weapons and to further Tulane's longtime commitment to public health, Garry started to write grant proposals for a dream team of partners. In addition to those who work with the Viral Hemorrhagic Fever Consortium out of Sierra Leone and Nigeria, Garry's team now includes researchers from Tulane working alongside scientists from Scripps Research Institute, Harvard University, Albert Einstein College of Medicine, Zolgen Labs, the Sanford Burnham Prebys Medical Discovery Institute, and the University of Texas Medical Branch at Galveston, which conducts animal studies for the project.

Through Tulane colleagues with experience in West Africa, Garry was well aware of the social toll taken by Lassa virus, which causes a deadly hemorrhagic fever. Spread by human contact through the droppings, urine or blood of a large forest mouse, Lassa is a constant threat in countries like Sierra Leone, Liberia, Nigeria and Guinea, and infects roughly 300,000 people each year.

For many, the virus is fatal, a reality made clear early on to Garry, who wrote the NIH proposal with Dr. Aniru Conteh, a Kenema doctor who contracted Lassa and died in 2004. Even now, doctors in the Kenema hospital's Lassa-fever ward see an 80 percent mortality rate in Lassa patients, most of whom are severely ill by the time they arrive from rural villages, Garry said. Pregnant women are particularly vulnerable; about 90 percent die, and miscarriage is all but certain.

Lassa virus is also disruptive within West African communities, Garry said, describing how villages often push out infected families and burn down their houses to prevent further infection. "If enough people die, an entire village will be shut down," he said.

But now, after 12 years of research, Garry's team has developed low-tech diagnostic tests—similar to disposable pregnancy tests—that can, with a finger-prick of blood, provide early identification of Lassa fever. With widespread use of the tests and strategic implementation of the vaccines and drugs that his project is also working on, Garry believes that Lassa can be eliminated entirely. "We're going to stamp this thing out," he said.

## **Lassa the cipher**

As Garry pondered work with Lassa, his first move was to the office next door.

In order for the Lassa project to succeed, Garry needed the expertise of the man in that office, James Robinson, a specialist in pediatric infections and a whiz in the lab

with Memory B cells.

For his work, Robinson used packages that arrived by air freight: big metal containers, frozen in dry ice, that look like giant thermoses that had been packed carefully with vials of white blood cells from healthy Lassa survivors.

Each of the survivors who donated blood was able to fight off Lassa fever because they'd developed antibodies to it. Those antibodies are archived in certain kinds of white blood cells called Memory B cells, which have proteins that act like keyrings, holding specific keys—antibodies—that fit perfectly onto each past invader. Each person's Memory B cells have hundreds of thousands of antibodies, for everything from a common cold to influenza to antibodies for measles made from immunizations.

To find the Lassa antibodies, Robinson added white blood cells to trays containing 96 culture wells, with a few cells in each well. Then he went through a detailed process to screen the wells and discover which wells of B-cell cultures made Lassa antibodies.

When the antibody was present, the well changed color, to blue. Wells where the color was more intense had a higher concentration of antibodies; he measured the color precisely by putting the tray into a spectrophotometer, which gave each well a digital score. The most intensely colored wells received higher scores and were deemed to contain a higher concentration of Lassa antibodies.

"If we get a positive, we shout, 'Eureka!'" Robinson said, noting that there were spans of time where no one shouted in glee.

Some weeks, Robinson's lab might have processed 20 or 40 plates that yielded only one blue well. Or none at all. "It is a type of fishing, though you have to know how to fish," said Robinson, an inveterate angler who often journeys to the Arkansas River basin with his brothers in search of bass. He sees clear parallels in his work. "You have to be able to accept failure—it doesn't keep you down as long as you make progress," he said.

After doing further analysis on a group of roughly 120 identified Lassa antibodies, Robinson deemed 16 of them "pretty amazing" because they were able to prevent an infection of cells by a Lassa pseudovirus, a mimic of Lassa virus that his team could use safely in the lab. Those 16 are the antibodies that he put forward for

further experiments, to see whether they could control the virus and be used in immunotherapeutic drugs that can treat infected patients.

The top antibodies are now being tested to see how they combat the four different strains of Lassa that the team found in West Africa. A genetic study that traced the evolution of the virus found that, while strains of it have existed in Sierra Leone for roughly 150 years, it has existed in Nigeria for about 1,000 years.

### **Moving toward a vaccine**

Before the Tulane team could use the Lassa antibodies to develop a vaccine, they needed to understand how the antibodies interacted with the virus.

All antibodies are proteins made by B cells that play a molecular Twister game each time they meet an invader. They must make contact in exactly the right way so that the invader can no longer connect to the body's host cells and infect them.

"They come together like praying hands," said Robinson, showing how the finger pads of each hand came together, similar to the way that an antibody needs to bond to an available surface to neutralize the Lassa virus, he said. To understand how the antibodies could neutralize Lassa, they needed to know how the virus made those connections.

With Lassa, the Tulane team zeroed in on a molecule on the surface of the virus called the Lassa glycoprotein precursor complex, which binds with a neutralizing antibody. Three pairs of proteins called a trimer form a tripod-like structure. Antibodies target that tripod, locking the pieces of it together, neutralizing it. At that point, the body becomes immune.

Based upon that work, Garry's group received a grant from National Institutes of Health to develop a Lassa vaccine. And in July, the NIH announced new grants worth more than \$12 million to Garry. They include two five-year grants for preclinical research—a \$5.72 million grant to evaluate a potent Lassa fever antibody drug cocktail and a \$6.32 million grant to design a vaccine based on a recently discovered key antibody target on the surface of the virus.

Already, the University of Texas facility is having great success in its tests of the immunotherapeutic drugs designed for use with infected patients. First, the scientists there tested some of Robinson's top antibodies in guinea pigs that had

been infected with Lassa. “We found that some antibodies were worthless, some pretty good, and some great,” he said. That narrowed the number to three antibodies that are now being tested in therapeutic cocktails that are given to infected monkeys.

Typically, creatures begin to die after the ninth or 10th day, Garry said. So first, the scientists gave the antibodies to the infected monkeys three days after they showed symptoms of Lassa fever. Every single monkey recovered.

In the next round of tests, the scientists waited six days before giving a dose of the antibodies. Again, all recovered. Most recently, they waited eight days. “By that time, they were very sick animals, sitting in the corner of their cages,” Garry said. Again, the antibodies worked in all the animals.

In January, Garry was heartened by an announcement from the Coalition for Epidemic Preparedness Innovation, a well-funded group supported by the Bill & Melinda Gates Foundation and the Wellcome Trust. The coalition announced that it is focusing on the development of vaccines for Lassa and two other diseases that could pose epidemic threats.

“We’re at the exciting part,” Garry said, predicting that, in less than five years, he and his partners will have developed good candidates for both a vaccine and a therapeutic treatment.

Success seems so close—and yet so far, said Garry, who feels a renewed sense of urgency every time he visits the Kenema hospital’s Lassa-fever ward. “Each time I visit, I see how we are losing patients that we will be able to save in a few years,” he said.

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