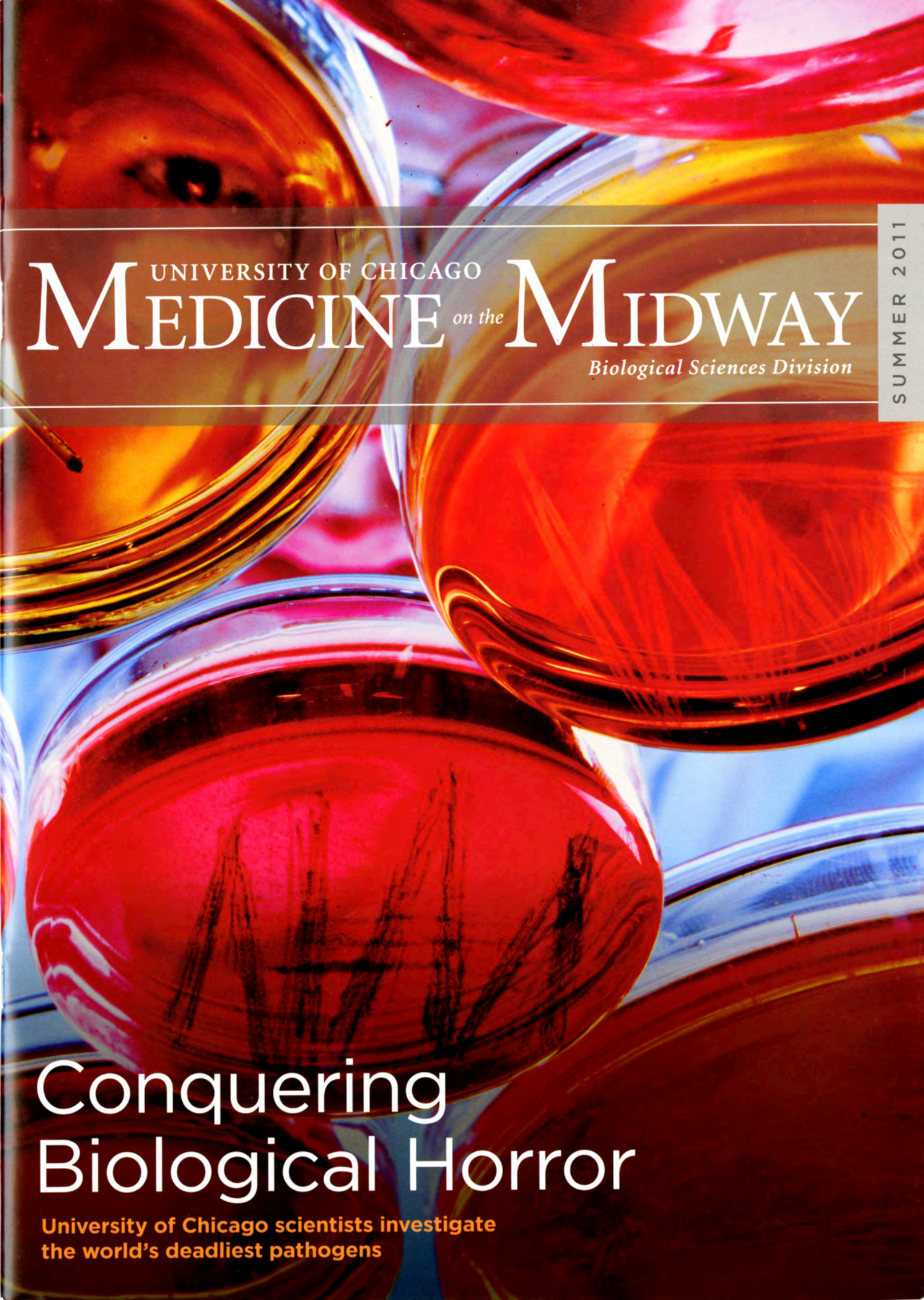

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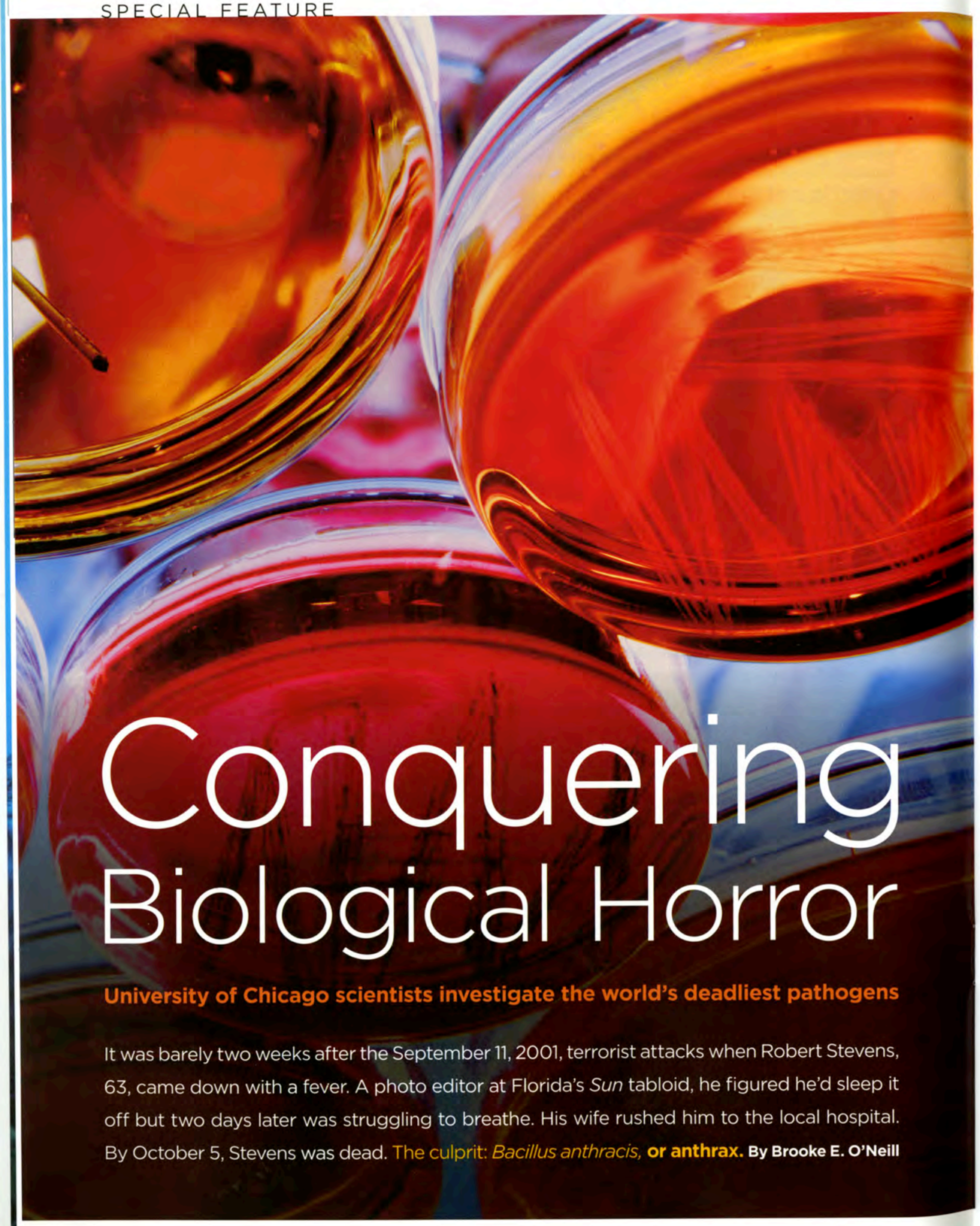
Conquering Biological Horror

University of Chicago scientists investigate the world's deadliest pathogens



Conquering Biological Horror

University of Chicago scientists investigate the world's deadliest pathogens

It was barely two weeks after the September 11, 2001, terrorist attacks when Robert Stevens, 63, came down with a fever. A photo editor at Florida's *Sun* tabloid, he figured he'd sleep it off but two days later was struggling to breathe. His wife rushed him to the local hospital. By October 5, Stevens was dead. The culprit: *Bacillus anthracis,* **or anthrax. By Brooke E. O'Neill**

By now, we know the horrific story. Envelopes were laced with lethal anthrax spores and mailed to government offices and news outlets, like the one where Stevens worked. Ultimately, the attacks killed five people and sickened 17 others, prompting the FBI to call it one of the "worst biological attacks in U.S. history."

Bioterrorism fears quickly spread. Soon University of Chicago infectious disease researcher Olaf Schneewind, MD, PhD, Louis Block Professor and chair of microbiology, was getting panicked calls from business school colleagues worried their mail might be contaminated. "I was the most popular guy on campus," recalled Schneewind, who studies pathogens such as anthrax, plague and *Staphylococcus aureus*, the so-called superbug.

Characterized by high mortality rates and capable of spawning epidemics, these dangerous microbes are part of what Schneewind calls the "biological horror cabinet." And while the 2001 incidents catapulted *B. anthracis* into the national spotlight, such bacteria are hardly a novel threat. From the estimated 25 million who succumbed to a rod-shaped bug called *Yersinia pestis* — Black Death — in the 14th century to the roughly 40,000 Americans who die of staph infections every year, infectious organisms have long exacted a devastating toll.

That's why Schneewind and his microbiology team are working against time to conquer these pathogens before a pandemic erupts. Analyzing molecular structures and dissecting some of the most deadly bacteria, they're developing new vaccines and therapies designed to tackle an ever-changing arsenal of bugs — and ward off the next outbreak.

"These diseases inflict a lot of casualties," Schneewind warned. "Our job is to stop them in their tracks."

Olaf Schneewind, MD, PhD, Louis Block Professor and chair of microbiology, studies deadly pathogens at the Howard T. Ricketts Regional Biocontainment Laboratory at Argonne National Laboratory.

"These diseases inflict a lot of casualties. Our job is to stop them in their tracks."
— Olaf Schneewind, MD, PhD

Know the Bug

To defeat a pathogen, you have to first understand how it operates. While many infectious diseases have been floating around for decades, even centuries, University of Chicago researchers are shedding new light on their inner workings.

At the heart of their efforts is the Howard T. Ricketts Regional Biocontainment Laboratory at Argonne National Laboratory, a high-security facility where federal background checks and protective Tyvek suits are the norm. The lab is located at Argonne, 25 miles southwest of Chicago (see page 15).

The Howard T. Ricketts Laboratory provides researchers a controlled environment for studying pathogens. Opened in December 2009, it houses "select agent" microbes that the Centers for Disease Control and Prevention (CDC) considers a "severe threat to public health and safety."

"These organisms are also known as bioweapons," said Schneewind. "They disseminate without an explosion — and it happens for free. Someone gets infected, transmits the disease, then it spreads."

Bioterrorism concerns aside, many of these organisms are clever enough — and common enough in nature — to wreak havoc all on their own. Anthrax spores, for example, can lie dormant in soil for years before they infect a living host, typically sheep, cattle or other livestock. *Rickettsia*, the group of bacteria responsible for typhus and Rocky Mountain spotted fever, lurk inside ticks until the pest bites an animal or human, allowing the pathogen to sneak inside and seize the host's own cell machinery to replicate itself.

"Many pathogens use variants of the same tricks because they need to accomplish the same goals: Get into the human tissue, survive there, avoid the immune response," said Juliane Bubeck-Wardenburg, MD, PhD, assistant professor of pediatrics and microbiology. "These are all things every pathogen must do successfully."

One of the trickiest — and deadliest — is *S. aureus*, the leading cause of infectious disease death in the

United States. The reason is twofold: the microbe commonly lives on humans — and can be extremely virulent when it sneaks into the bloodstream.

"When people say, 'He got an infection and died from it,' what they mean is this bug," Schneewind said.

A round bacterium that forms grapelike clusters, *S. aureus* can hang out harmlessly on the body for years. In fact, there's about a 50 percent chance you're carrying it on your skin or in your nose right now, asserted Dominique Missiakas, PhD, associate professor of microbiology. In most cases, it manifests as minor skin infections, like pimples.

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LEFT ***B. anthracis,*** **anthrax, is capable of surviving outside a host for decades.** *Getty Images*

"But every now and then," Missiakas explained, "it gets inside the bloodstream and starts replicating like crazy." Capable of infecting every part of the body — soft tissue, skin, lungs, brain, kidneys, heart — the organism gains a foothold by foiling immune cells that normally fight off such invaders.

Scottish surgeon Sir Alexander Ogston first identified the spherical microbe lurking in surgical wound infections in the 1880s. Today, the pathogen remains the most frequent cause of skin and soft tissue abscesses (pus-filled lesions) in humans.

Historically, staph infections were largely confined to hospitals, where weakened immune systems and invasive procedures make individuals more vulnerable. Open surgical wounds and medical devices like catheters can breach the body's natural barriers, creating pathways for infection. Additionally, hospital personnel may carry the microbe on their skin and without proper hygiene may transmit it to patients.

Currently, the average rate of infection for any person entering an American hospital is 4 percent. "And that's admission for any reason at all," Schneewind stressed. "Someone might come in to get a tooth removed, and they die of an *S. aureus* infection."

Over the past 50 years, increasingly virulent strains known as methicillin-resistant *S. aureus* (MRSA) have developed, rendering once-effective antibiotic treatments obsolete. The risk continues to escalate as strains resistant to vancomycin, the antibiotic of last resort, crop up.

Meanwhile, the bug has sneaked out of hospitals into communities, an alarming phenomenon first reported by a team from the University of Chicago Medical Center. Popping up in schools, locker rooms and prisons, it spreads via skin-to-skin contact, as well as transmission through clothing, towels, sheets, athletic equipment and other objects handled by infected individuals. Roughly half of all community-associated *S. aureus* strains are the insidious MRSA version.

With no preventive vaccine available on the market, "the magnitude of the problem is staggering," said Bubeck-Wardenburg, who began investigating the pathogen after seeing several previously healthy children succumb to MRSA lung infection during her pediatric clinical training at the University of Chicago.

Dissect the Machinery

That's why she and other University of Chicago researchers are investigating the bug's pathogenesis, or disease progression, at a basic molecular level. By studying the infection in mice, the team has pinpointed how the pathogen uses its devastating abscesses to survive in the body.

Normally, the body relies on immune cells to track down bugs, eliminate them and produce natural antibodies that safeguard against future infection. But *S. aureus* is a pro at hijacking the body's immune response. Missiakas describes its attack as a "play in four acts":

Act I: The microbe infiltrates the bloodstream, typically through skin cuts and wounds.

Act II: The bug sets up camp in the tissue. The body detects an invader and sends immune cells to kill it. These natural defenses are no match for *S. aureus,* which binds to immune cells and disables them.

Act III: Within four to five days of entering the body, *S. aureus* has transformed the infected area into a growing lesion. The bacteria surround themselves with a protective outer coating and multiply inside. More immune cells flock to the infected area, but are killed off and end up as pus in the abscess.

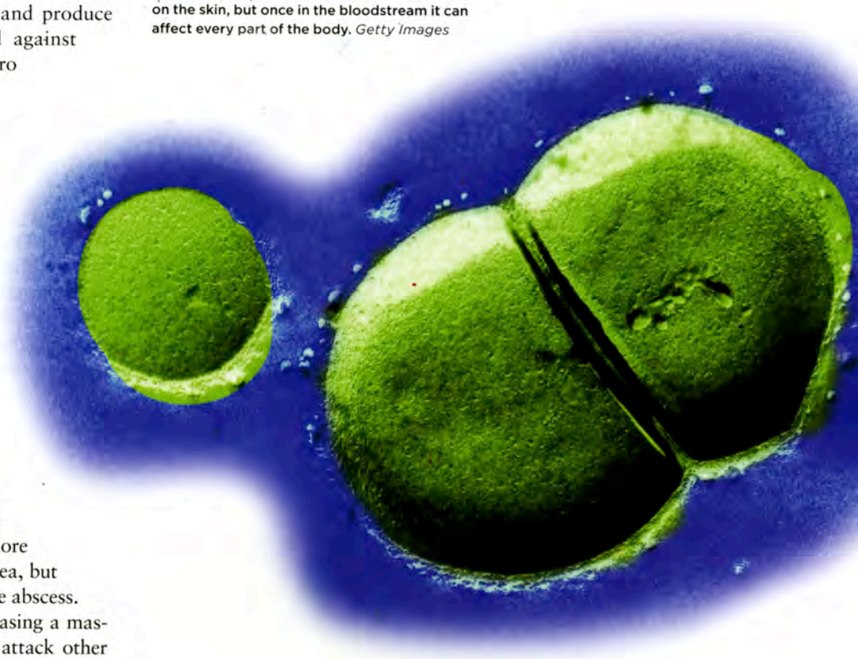
Act IV: The abscess ruptures, releasing a massive army of *S. aureus* microbes to attack other parts of the body and repeat the cycle. The fallout can be any number of life-threatening conditions, including pneumonia, a bloodstream infection known as septicemia and inflammation of heart chambers and valves.

Ultimately, roughly half of all individuals with a severe MRSA infection will die.

Those who survive do not develop immunity and infections recur in roughly 20 percent of cases. Schneewind calls *S. aureus* the "world champion of immune suppression."

Bubeck-Wardenburg agrees: "I think of it as a bug that we constantly chase."

RIGHT ***S. aureus,*** **or staph, often lives harmlessly on the skin, but once in the bloodstream it can affect every part of the body.** *Getty Images*



Find the Weakest Link

Over the past few years, the microbiology team at the University of Chicago has homed in on a target, developing a broad experimental vaccine to prevent MRSA outbreaks. Supported by a major multinational pharmaceutical company and moving toward human clinical trials, the breakthrough treatment pinpoints some of the weakest links in *S. aureus's* molecular structure.

"What we're looking for is the core activity that is required for virulence," Missiakas said. "What are the factors that are absolutely essential in this pathway to cause disease — the one step the pathogen cannot go around, regardless of strain?"

As an entry point, the researchers focused on the bacteria's cell wall. Like most human pathogens, *S. aureus* is surrounded by a thick, meshlike envelope made of amino acids and sugars. To infect its host, the bug must attack through this barrier by secreting proteins.

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Through a series of animal and in vitro (test tube) experiments, Schneewind's team identified a handful of proteins that enable *S. aureus* to undermine our immune system. One culprit is Protein A, which disables antibodies that fight infection and also kills off master cells that produce antibodies. Another is a group of proteins that cause blood to clot and form abscesses.

Once researchers identified these staph proteins as indispensable to infection, they tested whether either could be used to create a vaccine against the bug.

They used polymerase chain reaction, a standard technique for analyzing and replicating DNA sequences, to produce mutant versions of *S. aureus* that lacked the genes responsible for creating Protein A and the clotting proteins.

occurs in a population, explained Schneewind, roughly 5 percent to 10 percent of infected individuals develop a secondary pneumonia as the bacteria spreads to the lungs and replicates in the tissues. The hardy microbe evades the immune system's attempt to devour it and creates destructive lesions that ravage the airways.

Individuals with pneumonia then transmit the disease to others through air droplets emitted when coughing or sneezing. The result: explosive plague outbreak.

Three major plague pandemics, during the 6th, 14th and early 20th centuries, have caused more human fatalities combined than any other infectious disease to date.

And what if another were to hit?

"It would catch the United States by surprise," Schneewind said. In May 2000, the government ran a $3 million simulated bioterrorist attack with Y. pestis to gauge national readiness. In the hypothetical scenario, an aerosol of the bacteria was released at the Denver Performing Arts Center. Four days later, the disease had infected, by some counts, nearly 2,000 people on three continents.

That's why Schneewind and his team have developed a vaccine that targets proteins critical for infection. While various plague vaccines have existed since the late 19th century, concerns about their safety make an alternative highly desirable.

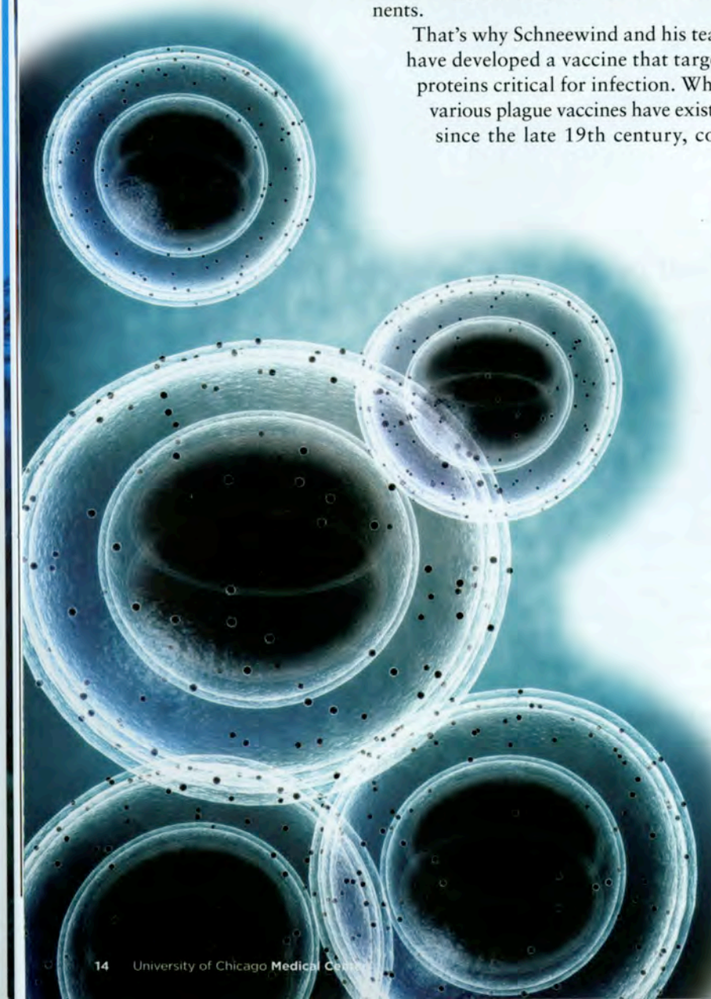
As with *S. aureus*, researchers focused on a key activity the bug must carry out to cause disease: the smuggling of toxins into cells.

At the molecular level, *Y. pestis* strikes victims by injecting its poison into cells using a syringe-like mechanism. Embedded along the bacterial wall, this complex system of needles allows the pathogen to secrete proteins directly into the host cell. Once inside, the proteins cripple the immune response by preventing phagocytosis, the body's normal process of engulfing and eliminating foreign microbes.

To counter the bug's sneaky ways, the microbiologists homed in on a protein factor called LcrV that sits at the tip of the secretion needle and helps transport toxins across the membranes. Essential for infection, it also prompts the host to produce antibodies, making it a key source of protection against plague.

Inoculating mice with different variations of this antibody, the researchers isolated one version that conferred 100 percent immunity against plague. When they later inserted the same variation into samples of plague-infected human blood, it killed off much of the bacteria.

By disabling a fundamental infection mechanism, the vaccine enables immune cells to work their magic. The promising treatment is currently undergoing human clinical trials.



BELOW **Methicillin-resistant *S. aureus* (MRSA) has rendered once-effective antibiotic treatments obsolete.** *Getty Images*

Stay Vigilant

Whether mobilizing against natural threats like MRSA, bioweapons like anthrax or future plague outbreaks, University of Chicago microbiologists remain on guard against the unforeseeable. "One has to be ready for the next thing there is to fight," Missiakas said. "We never know where it'll come from."

Yet unlike ordinary folks who fret about apocalyptic scenarios, she and her colleagues forge ahead, unraveling the proteins and molecular behaviors that make these pathogens so dangerous. As they inch closer to stamping out disease, they stay focused on the task at hand.

"One could work endlessly and have nightmares about the next move of a bug," Missiakas said. "That's why, ultimately, I prefer to be rational." ■

How to Work with Dangerous Bugs

A behind-the-scenes look at how University of Chicago microbiologists handle lethal organisms

By Brooke E. O'Neill

"You going in?" lab director Howard Shuman asked a tall man in green scrubs. Researcher Sean Crosson, PhD, assistant professor of biochemistry and molecular biology, nodded.

"We're doing an array experiment in a few minutes," he said, heading toward his lab at Howard T. Ricketts Regional Biocontainment Laboratory.

Located at Argonne National Laboratory, 25 miles southwest of Chicago, the Ricketts Laboratory offers University of Chicago scientists one of the nation's most secure, state-of-the-art facilities to study dangerous pathogens.

From the outside, it looks like any suburban office park. Yet housed inside the Howard T. Ricketts Laboratory are bacteria that cause some of nature's most insidious diseases: anthrax, plague and other microbes the Centers for Disease Control and Prevention (CDC) deems significant threats to public health and safety. Once these bugs infiltrate the body, all have high mortality rates.

Named after University of Chicago microbiologist Howard Taylor Ricketts — he discovered *Rickettsia*, the bacteria that cause typhus, and died during a 1910 outbreak in Mexico City — the lab builds on Chicago's history of infectious disease investigation. Federally funded by the National Institute of Allergy and Infectious Diseases, the Ricketts Laboratory went "hot" with live pathogens in December 2009.

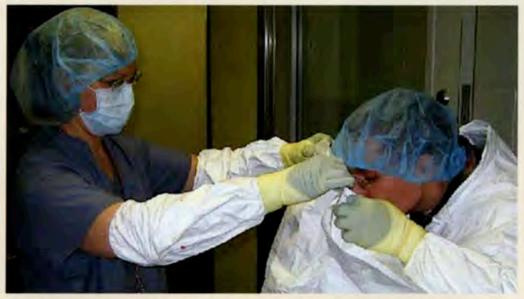
Before Crosson can enter his workspace, he'll pass through a biometric fingerprint scanner, suit up in a protective Tyvek suit and put on a respirator, an astronaut-type helmet to shield his head and face.

As he works, multiple security cameras monitor his every move. Come end of day, nothing can leave the lab, and his protective clothing is decontaminated while he takes a mandatory shower.

Inside the lab space awaits Crosson's bug, *Brucella abortus*.

Primarily an agricultural organism, the bacterium jumps from animals to humans through contaminated food or dairy. Extremely difficult to kill, it causes flulike symptoms and often leaves victims with chronic joint pain, a risk Crosson and his colleagues face on a daily basis.

Not surprisingly, the notion of a lab filled with lethal bacteria can conjure nightmarish doomsday visions, said Joseph Kanabrocki, PhD, CBSP, assistant dean for biosafety and associate professor of microbiology. "Some people imagine that the minute you walk into the building, you're walking into a cloud of anthrax."



Employees at the Ricketts Laboratory must go through a personal protective equipment training. The equipment is required for entry. *Photo by John C. Bivona, RBP*

The reality isn't quite so alarming. For starters, the quantity of live biological agent at the facility is extremely small. "Even in our worst case scenario, we don't have a lot of material stored here," said Kanabrocki, who oversees biosafety protocols. "We grow it as we need it."

All pathogens studied at the Ricketts Laboratory must have at least one available antibiotic therapy. As a Biosafety Level 3 facility — the second-highest of four risk levels designated by the CDC — the lab is cleared to house organisms that cause severe to fatal disease, but not agents that have no treatment.

To handle their microbes safely, researchers undergo extensive training led by Kanabrocki, a member of the National Science Advisory Board for Biosecurity.

"To understand what the risks are for a particular experiment, you have to understand the bug," he explained. "Are there going to be infections that involve the use of needles? Is the pathogen transmitted via the aerosol route?"

Not just anyone can work with such pathogens. "Everyone who works here has been vetted by the government and given a security clearance," Kanabrocki said.

Being located at Argonne adds yet another layer of security. Owned by the U.S. Department of Energy, the national laboratory was founded by the University of Chicago in the 1940s to house nuclear chain reaction research. A perimeter fence surrounds the grounds, and no one can enter without a gate permit.

Inside the Ricketts Laboratory itself, any photos or description of how corridors and rooms connect are strictly forbidden. In addition to the extra security, Argonne also puts some of the world's most powerful genetic sequencing and X-ray technologies right at University of Chicago researchers' fingertips.

"They do things at Argonne that cannot be done anywhere else," director Shuman said. One example is analyzing bacterial structure using Argonne's Advanced Photon Source, which produces the Western Hemisphere's most brilliant X-rays. Such collaborations put scientists one step closer to finding cures for some of our worst diseases.

With bacteria forever evolving into more powerful enemies that outwit our best treatments, those efforts couldn't come at a more critical time.

After all, Kanabrocki said, "infectious diseases aren't going away." ■

The Howard T. Ricketts Regional Biocontainment Laboratory is located at Argonne National Laboratory, 25 miles southwest of Chicago. *Photo by Jennifer Crotty*