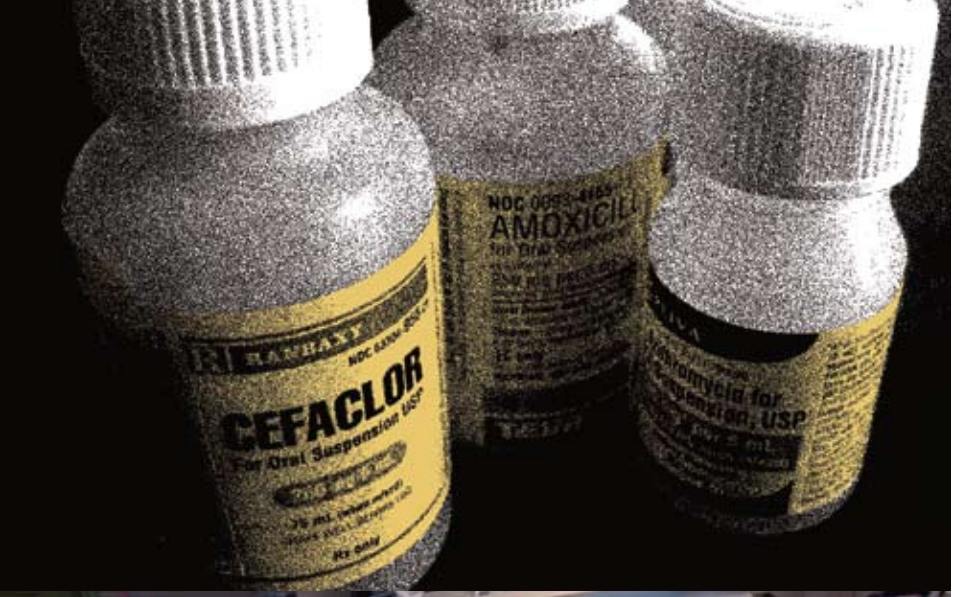
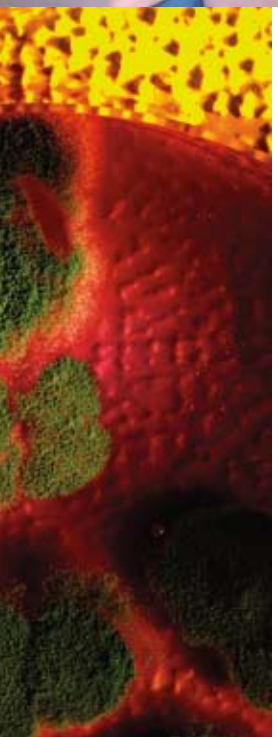
SEEKING NEW DRUGS FOR BAD BUGS

By Sue Pondrom

One of the biggest threats facing the world today is the growing prevalence of antibiotic-resistant infectious diseases. With the advent of antibiotics, many experts believed bacteria would no longer pose a threat to humans. However, with the rise of antibiotic-resistant bacteria, it is clear that better treatments and therapies are essential to tackling these evolving diseases.

Just one of these antibiotic-resistant bacteria—methicillin-resistant Staphylococcus aureus (MRSA)—kills nearly 20,000 Americans each year while seriously infecting nearly 100,000. Another bacterial infection, group A Streptococcus (GAS), causes relatively mild illnesses such as strep throat or severe, life-threatening infections such as the "flesh-eating bacteria" necrotizing fasciitis. According to the Centers for Disease Control and Prevention, serious forms of GAS infect nearly 12,000 people each year and kill more than 500.





Nizet (center) and UC San Diego postdoctoral fellows Fabiano Pinheiro da Silva, M.D., Ph.D., and Shauna McGillivray, Ph.D., analyze a gel blot of immune system proteins responding to a bacterial infection.

"Traditionally, medical treatment of infectious disease has been very one sided . . . The focus has been to kill the bug, kill the bug! But, as these crafty pathogens have evolved to resist our immune system and the best antibiotics, I think we have to start thinking outside the box for the next generation of effective infectious disease treatments."

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Robust Washington Redskins defensive tackle Brandon Noble contracted MRSA twice. Though not life threatening, it almost cost him his legs and his football career. Not so fortunate was 21-year-old college football player Ricky Lannetti of Williamsport, Pennsylvania. What his family initially thought was the flu turned deadly within one week of the infection's onset.

Hollywood actress Alicia Cole was in top physical shape when she entered the hospital for a routine treatment of uterine fibroids. However, within hours of her surgery, Alicia developed necrotizing fasciitis and barely survived the vast open sores she developed on her abdomen.

These are startling cases, but they are not at all rare. Fortunately, a cross-disciplinary team of UC San Diego physician-scientists, marine chemists, cell biologists, and pharmacologists are using novel approaches to develop new therapeutic approaches for Staph and Strep infections, particularly those strains that have learned to evade treatment.

"Traditionally, medical treatment of infectious disease has been very one-sided," says Victor Nizet, M.D., Chief of the Division of Pediatric Pharmacology and Drug Discovery at the UC San Diego School of Medicine and Skaggs School of Pharmacy and Pharmaceutical Sciences. "The focus has been to kill the bug, kill the bug! But, as these crafty pathogens have evolved to resist our immune system and the best antibiotics, I think we have to start thinking outside the box for the next generation of effective infectious disease treatments."

For this reason, Nizet and his colleagues from Health Sciences, the Scripps Institution of Oceanography (SIO), and UC San Diego's Biological Sciences are attacking MRSA, GAS, and other leading bacterial pathogens with three innovative approaches.

MOLECULES IN THE MUD

In recent decades, antibiotics that were promising 40 years ago have increasingly become ineffective against resistant strains of bacterial infection. The majority of these antibiotics were derived from soil bacteria as pharmaceutical scientists roamed the planet, digging up microbes in the dirt. Drug companies soon realized they were not going to find any new antimicrobial compounds in the soil, so many ceased production of new antibiotics developed from this source.

"When this former treasure trove of natural antibiotics dried up, we needed to open our eyes to new environmental sources,"

Nizet says. For that reason, he collaborated with William Fenical, Ph.D., Director of the UC San Diego Center for Marine Biotechnology and Biomedicine at SIO.

Fenical had already discovered several potential anti-cancer agents from microbes present in sediment from the ocean floor.

"We are very pleased with our anti-cancer work, but we began to realize there was almost no effort to discover new antibiotics in the ocean. What is curious is that no one had thought to examine the ocean, despite the fact that it's 70 percent of the earth's surface," says Fenical.

Exploring the ocean bottom for the past 10 years, Fenical's team at SIO has collected sediment samples from as deep as 5,000 meters (15,000 feet) to shallow areas near the Scripps pier in La Jolla. Earlier this year, Fenical and Nizet joined forces in a successful $2 million grant application to the National Institutes of Health to support efforts in discovering new ocean-based antibiotic agents.

"We've already discovered seven different structural classes of compounds unlike any observed before," says Fenical. One of the most promising chemical compounds they have identified has shown initial effectiveness against MRSA and is undergoing extensive testing by Nizet.

The potential of the sea is amazing. "In a drop of mud the size of a sugar cube, there are one billion one-celled creatures, any one of which could contain a novel compound with the potential to combat diseases," Fenical notes. "This means we need to sample broadly and in replicate numbers—and that's what we are doing." Currently Fenical and his team are confident and have already seen enough positive results to know their research efforts are working.

How long until a new family of antibiotics from the sea is available to treat patients? Nizet says it could be as soon as five to seven years, "if we're lucky in our research and development. And, we will need to collaborate with local biotechnology and pharmaceutical companies to help us prepare a product suitable for human clinical trials."

DISARMING THE VIRULENCE FACTOR

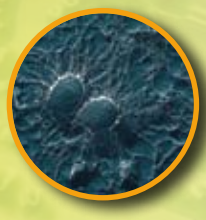
As a medical student at Stanford in the 1980s, Nizet's curiosity in infectious disease was piqued with the availability of new genetic tools that helped researchers answer questions at the mechanistic level. While treating patients as a pediatric resident at Harvard, he became particularly interested in bacterial pathogens such as Staph and Strep.

"It always struck me as fascinating that leading bacterial pathogens such as Staph and Strep are encountered day-in and day-out by thousands of individuals without producing disease or causing mild infections," says Nizet. "Yet, in certain cases, these same bugs can cause a serious and even life-threatening infection in an otherwise healthy person or child."

A near tragic patient example was 14-month-old Bryce Smith of Santee, California. The healthy toddler caught a cold just before Christmas. When his condition gradually worsened, his parents took Bryce to Rady Children's Hospital San Diego where Nizet and his colleagues diagnosed him with MRSA. During the next two weeks, the child's condition deteriorated and he was close to death several times. Doctors tried to force air into Bryce's lungs, but told his parents it was like trying to pump air into a brick. For six weeks, Bryce didn't wake up. Finally, his condition improved and after two months in the hospital, he was able to return home. Today, Bryce is a happy, active four-year-old, but permanent right-lung damage occasionally leaves him breathless.

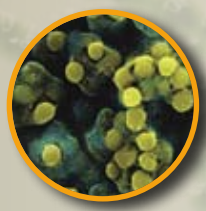
Bryce's story is similar to other patient stories Nizet has experienced. It is one of the primary reasons he has focused his research on developing new therapies for pathogens such as Staph and Strep. One of the novel approaches he and his colleagues are undertaking is the identification of specific

The "S" Bugs



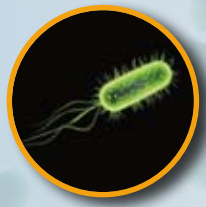
Staphylococcus aureus (Staph)

According to the National Institute of Allergy and Infectious Diseases, one-third of the world population has Staphylococcus aureus bacteria on their bodies at any given time, primarily in the nose and on the skin. It can be present without causing an active infection. In many United States communities, the drug-resistant form of methicillin-resistant Staphylococcus aureus (MRSA) can represent up to 30 to 50 percent of disease components. MRSA is categorized according to where the infection was acquired: hospital-acquired MRSA or community-associated MRSA.



Group A Streptococcus (GAS)

GAS infections can range from mild skin infections or sore throats to severe, life-threatening conditions. Health experts estimate that more than 600 million mild to moderate infections occur each year, including strep throat, impetigo (a skin infection), and scarlet fever. The more severe forms of GAS, including bacteremia (bacteria in the blood), toxic shock syndrome, and necrotizing fasciitis (commonly known as flesh-eating bacteria), account for more than half-a-million cases each year. About 30 percent of those who develop necrotizing fasciitis die from the disease.



Group B Streptococcus (GBS)

GBS is a type of bacteria that causes illness in newborn babies, pregnant women, the elderly, and adults with other illnesses such as diabetes or liver disease. It is the most common cause of life-threatening infections in newborns, including sepsis (blood infection) and meningitis (infection of the fluid and lining around the brain).

Streptococcus pneumoniae

This form of strep is the most common cause of community-acquired pneumonia, bacterial meningitis, bacteremia, and otitis media in the world. S pneumoniae infection is also an important cause of sinusitis, septic arthritis, osteomyelitis, peritonitis, and endocarditis.

According to UC San Diego's Victor Nizet, M.D., "these bugs share a lot of similarities, starting with their round shape and thick cell walls. We are fortunate that we can use many of the same genetic approaches to study them in the laboratory."

components of infectious microorganisms that cause disease—known as virulence factors.

"When we see disease, it's because a pathogen such as Strep or Staph has virulence or infectious factors that differentiate it from the hundreds of millions of good citizen bacteria that sit in our body every day, causing no problems and actually making a contribution to our normal, healthy system," Nizet says. "In this new treatment concept, we aim to disarm these pathogens, to render them harmless rather than kill them directly."

An advantage of this approach is that most powerful antibiotics used today are indiscriminate and kill not only the bad bacteria but also the good microorganisms. Virulence factor-based therapy selectively targets only the strain of bacteria responsible for disease in the patient. And Nizet says because this approach does not place a "life-or-death" selective pressure on the pathogen, it may lessen the development of antibiotic resistance.

During the last several years, Nizet's lab has made several important discoveries about such virulence factors. Investigating the characteristic yellow-orange color of Staphylococcus aureus ("aureus" is Latin for "golden"), his team proved for the first time in 2005 that the golden pigment contains antioxidants that shield Staph from neutrophils, an important type of white blood cell that is essential to the body's normal immune defense against invading microbes.

In February 2008, a multi-institutional effort led by Nizet and his colleagues uncovered a completely new treatment strategy for MRSA, the most serious form of Staph. They found that a compound called BPH-652—originally designed to lower cholesterol—blocks a key enzyme that can weaken Staph's defenses and allow the body's immune cells to prevail against the infection. Through the UCSD Technology Transfer Office, Nizet has partnered with pharmaceutical chemists and business development officers to move BPH-652 toward human clinical trials for difficult Staph infections, including MRSA.

In another form of Strep, group A Streptococcus (GAS), the Nizet team discovered in 2006 that this strain releases an enzyme that prevents neutrophils from capturing and destroying bacteria. The following year, the team pinpointed an event 30 years ago that led to the development of the deadly strain of flesh-eating bacteria that is familiar today. The culprit is a mutated strain of Strep called the M1T1 clone.

Then earlier this year, Nizet showed

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that flesh-eating bacteria use a digestive enzyme protease known as SpyCEP (Strep pyogenes cell envelope protease) to attack the body's immune system. This protease degrades key immune defense molecules, making white blood cells slower and weaker so that infection spreads out of control.

"In these studies, we're learning which genes are necessary and sufficient for producing disease," Nizet says. "In animal models, if we see that the loss of the virulence factor leads to decreased disease, then pharmacological approaches to inhibit the virulence factor will offer hope for effective new therapies."

A BOOST FOR THE IMMUNE SYSTEM

UC San Diego researchers are also investigating the body's natural immune response to infection in an effort to boost naturally occurring defense mechanisms. Several recent discoveries are pertinent to Staph and Strep.

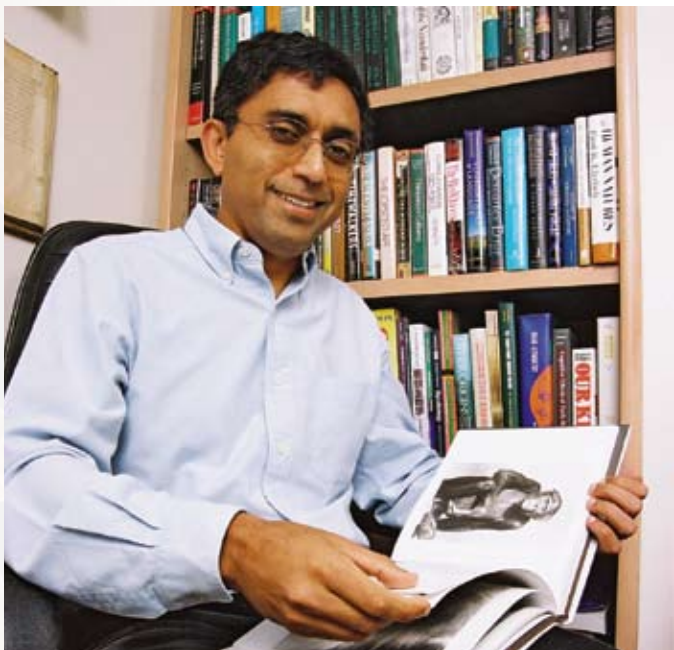
Richard Gallo, M.D., Ph.D., Chief of the Division of Dermatology at UC San Diego and Veterans Affairs San Diego Healthcare System, has found that natural antibiotics, including one called cathelicidin, provide the first line of defense against invading bacteria and keep many fast-moving infections at bay until the immune system can mount a full-blown attack. Cathelicidins are found in various tissues in all mammals, including skin, lungs, intestines, and circulating white blood cells. Last year, Gallo's team reported the body's natural supply of vitamin D3 controls the production of cathelicidins, and that a deficiency in vitamin D3 may compromise the immune response to infection. Recently, the Gallo team determined that oral supplements of vitamin D3 resulted in a significant increase in cathelicidin expression in patients.

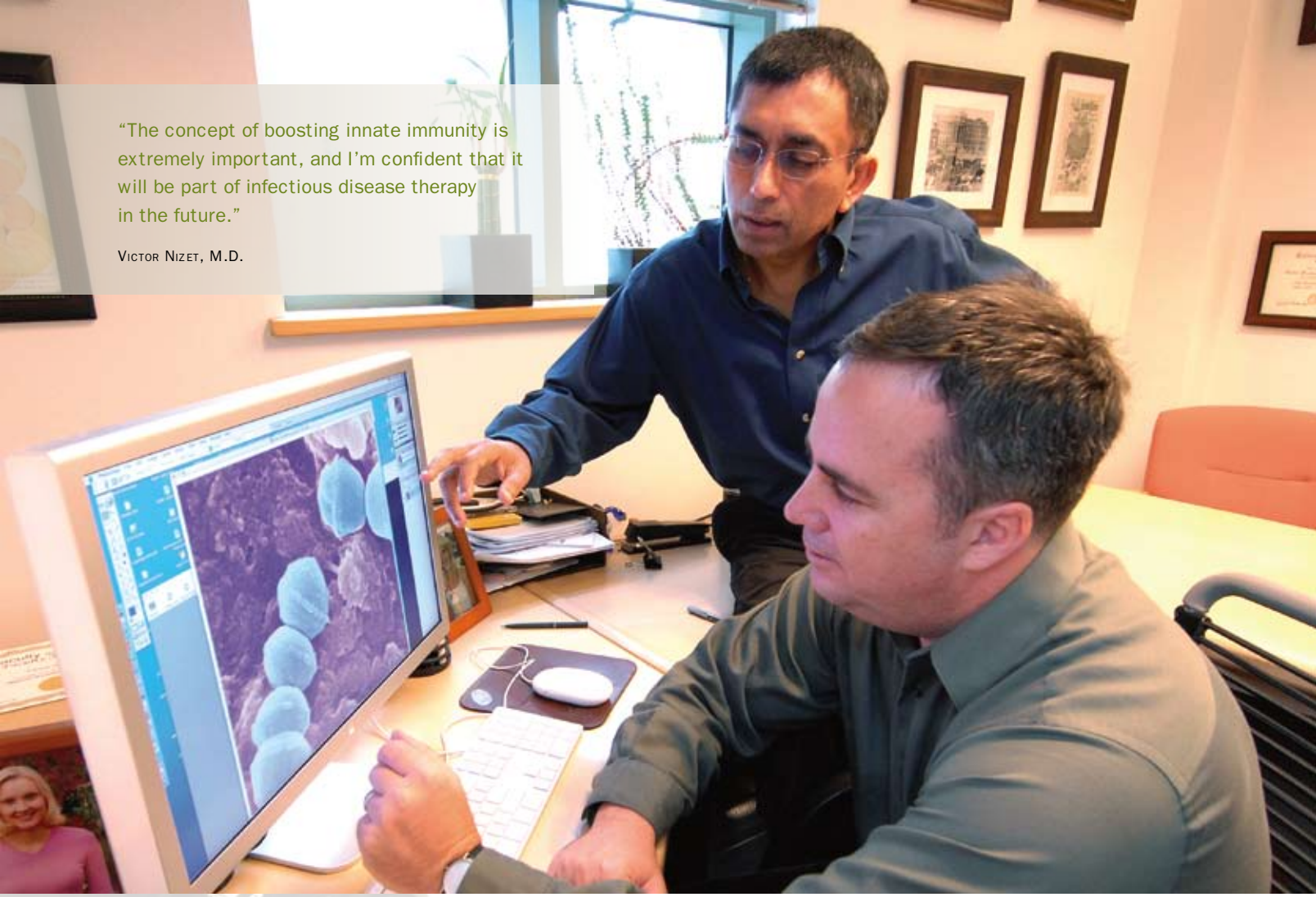
"Here at UC San Diego, we've been able to determine how natural antibiotics are essential for protection against infection," says Gallo. "We also know now that some patients are susceptible to infection because they do not produce these antibodies in sufficient quantities."

Across campus from Nizet's lab, researcher Randall S. Johnson, Ph.D., Professor of Biology, has shown that low oxygen levels found at the site of bacterial infection activate a protein called HIF-1 that stimulates white blood cells to release antimicrobial compounds that kill bacteria. Both Johnson and Nizet have determined that treating white blood cells with chemicals to stimulate HIF-1 activity could be used to boost the bacterial-killing ability of white blood cells.

Recent research led by Michael Karin, Ph.D., Professor of Pharmacology, has shown that a molecular on-off switch called NF kappa B (NF-_B) is essential for HIF-1 to respond to bacteria and low oxygen. "NF-_B provides the link to explain how HIF-1 is activated in response to bacteria and why both proteins are so critical to innate immune function," says Karin.

Ajit Varki, M.D., Co-Director of the UC San Diego Glycobiology Research and Training Center, studies glycans, the natural sugars that coat all cells in mammalian bodies. His research is providing a better understanding of the immune system and what causes it to fail.





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Another researcher Nizet collaborates with is Ajit Varki, M.D., Co-Director of the UC San Diego Glycobiology Research and Training Center, who studies glycans, the natural sugars that coat all cells in mammalian bodies. The two labs have investigated the phenomenon in which certain bacterial pathogens coat their surfaces with a thick capsule made of glycans similar to those found on the surface of human cells. This process tricks the human immune system into thinking the bacterial cell is a natural part of the body.

"We call this molecular mimicry," Varki says. "Group B Strep (GBS), for example, coats itself with a sialic acid-containing glycan very similar to a glycan that coats human cells."

Nizet says these studies increase understanding of the immune system and what causes it to fail. "They also provide us with insight into how we might develop vaccines to join the battle against invading pathogens when our natural defenses don't get the job done.

"The concept of boosting innate immunity is extremely important, and I'm confident that it will be part of infectious disease therapy in the future," Nizet says. "It's also the most challenging because the immune system is so complex and represents millions of years of evolution in response to a broad range of environmental and infectious challenges. Our challenge as physicians and scientists is to improve upon Mother Nature where immunity has failed and infection has taken hold."

THE BUG-SQUASHING TEAM

"We're fortunate that the environment at UC San Diego fosters collaboration," Nizet says. "Finding a way to stop these serious infections requires a cross-disciplinary approach. With different perspectives, we can be innovative in exploring new areas no one else has tried. I think that's where new discoveries will be found, especially if you work on something as complex as infectious disease."