This issue of Endeavor magazine features the work of three researchers at The Scripps Research Institute (TSRI) whose research attempts to unravel the mysteries of the immune system. Immunology, the focus of the institute's oldest department, is a field of study relevant to many major diseases—either their causes or their potential cures.

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"Binary" Enzyme Demonstrates Darwinian Evolution at its Simplest

TSRI Research Associate John S. Reader, D.Phil., and Professor Gerald F. Joyce, M.D., Ph.D., have succeeded in creating an enzyme based on a "binary" genetic code—one containing only two different subunits.

This research demonstrates that Darwinian evolution can occur in a genetic system with only two bases, and it also supports a theory in the field that an early form of life on earth may have been restricted to two bases.

"Nobody will ever top this because binary systems are the most reduced form of information processing," says Joyce. "Two different subunits are the absolute minimum number you need for Darwinian evolution."


Expanding the Genetic Code

Scientists at TSRI report the synthesis of a form of the bacterium *Escherichia coli* with a genetic code that uses 21 basic amino acid building blocks to synthesize proteins—instead of the 20 found in nature.

This is the first time that anyone has created a completely autonomous organism that uses 21 amino acids and has the metabolic machinery to build those amino acids.

"We now have the opportunity to ask whether a 21-amino acid form of life has an evolutionary advantage over life with 20 amino acids," says the report's lead author Professor Peter G. Schultz, Ph.D., who is Scripps Family Chair.

"We have effectively removed a billion-year constraint on our ability to manipulate the structure and function of proteins," he says.

In addition to demonstrating that life is possible with additional amino acids, the work enables scientists to chemically manipulate the proteins that an organism produces within the organism itself. This gives scientists a powerful tool for research, from determining molecular structures to creating molecular medicines.

TSRI Scientists Discover a New Approach for Treating "Misfolding Diseases"

Professor Jeffery W. Kelly, Ph.D., and his colleagues in the Department of Chemistry and The Skaggs Institute for Chemical Biology at TSRI have demonstrated a new approach for treating "amyloid" diseases—particularly transthyretin amyloid diseases, which are similar to Parkinson's and Alzheimer's.

These amyloid diseases are caused by proteins misfolding into a structure that leads them to cluster together, forming microscopic fibril plaques made up of hundreds of these misfolded proteins. The plaques deposit in internal organs and interfere with normal function, sometimes lethally.

These scientists have discovered a new approach to treating these diseases, which involves small molecules that can inhibit the formation of fibrils and stabilize the normal "fold" of transthyretin. This method, researchers say, could provide a new treatment for these diseases.

In recent study, Kelly, who is Lita Annenberg Hazen Professor of Chemistry in The Skaggs Institute for Chemical Biology and TSRI's vice president for academic affairs, and his colleagues demonstrate the efficacy of using small molecules to stabilize the normal "fold" of transthyretin. Using this method, researchers were able to inhibit the formation of fibrils.

"I'm very excited about pursuing these potential therapeutic opportunities," says Kelly.


TSRI Scientists Lerner and Schultz Win Top German Prize

TSRI President Richard A. Lerner, M.D., and Professor Peter G. Schultz, Ph.D., both members of TSRI's Skaggs Institute for Chemical Biology, were recently awarded the Paul Ehrlich and Ludwig Darmstaedter Prize, one of the most renowned prizes in the Federal Republic of Germany in the field of medicine.

Lerner, who is Lita Annenberg Hazen Professor of Immunology and Cecil H. and Ida M. Green Chair in Chemistry, and Schultz, who is Scripps Family Chair, were selected for their achievements in connection with the development of catalytic antibodies. These molecules combine the enormous diversity of antibodies with the catalytic properties of enzymes.

$24-Million Grant Funds Encyclopedia of the Innate Immune System

The National Institute of Allergy and Infectious Diseases has awarded a multi-year, $24-million grant to a group of researchers at TSRI, the Institute for Systems Biology, and The Rockefeller University.

The group's task is to create an "encyclopedia" of innate immunity—a comprehensive and detailed picture of this ancient, essential first line of defense against bacterial and fungal diseases that is muddled by humans, fruit flies, and all creatures in between.

The funds will go towards discovering new ways to study the immune system in living tissue in real time and to provide materials and information to the scientific community at large. Knowledge generated could help scientists develop treatments for septic shock, certain autoimmune disorders, and diseases caused by potential agents of bioterrorism.

Richard A. Lerner, M.D., and Peter G. Schultz, Ph.D., have won the prestigious Paul Ehrlich and Ludwig Darmstaedter Prize.
Arghyrios Theofilopoulos Examines Lupus
A Medical Doctor in the Lab

There comes a day in the early careers of many professionals—a singular defining moment that both shows their strengths and sets the stage for the rest of their careers. Think North Carolina Freshman Michael Jordan’s winning jumper in the waning seconds of the 1982 NCAA championship game.

For Professor Argyrios Theofilopoulos, M.D., of the Department of Immunology at The Scripps Research Institute (TSRI), that event occurred when he was a young doctor in the Greek army—fulfilling his mandatory service after completing medical school in the late 1960s.

This was a transitional time for Theofilopoulos. A few years earlier, he had graduated at the top of his medical school class at the University of Athens, and a few years later, he would be leaving Greece to come to the United States and begin a lifelong career as an immunologist. But in 1968, Theofilopoulos was an army doctor stationed near the Bulgarian and Turkish borders.

“I was taking care of not only soldiers, but also people in remote villages,” says Theofilopoulos, recalling how he would make his rounds—sometimes in a jeep and sometimes on the back of a horse—in all sorts of weather and difficult circumstances, often until late in the evening. “I was the only doctor in this area.”

It was on one of these trips that Theofilopoulos was called to a village where a plea had been made to help an old man who was laid out, about to die, in his small home.

“The old man was bringing me chickens and nuts and other gifts, and my fame exploded to all the surrounding villages.”

When Theofilopoulos arrived, the old man’s blood pressure was very low, and after examining him, Theofilopoulos found a hemorrhage in the back of the man’s nose, which, if left unchecked, would surely lead to the man’s death. Stopping the bleeding might well save the man’s life, but that was easier said than done 100 miles from nowhere in the remote border region between Greece and Bulgaria.

Theofilopoulos had practically no medical supplies. “I had nothing to work with,” he says.

He did, however, have a box stuffed with some random medical surplus donated by the United States Army. He quickly opened the box, dumped its contents out on a table, rifled through, and found a urethral catheter. This thin tube was hope.

Theofilopoulos grabbed the urethral catheter, pushed it up the man’s nostril, and pulled it out through the man’s mouth. He then attached a cotton ball to the end of the catheter, pulled it back up the nostril, pushed it behind the pharynx where the hemorrhage was, and stopped the bleeding. Outside the tiny building, the villagers and the soldiers cheered, and Theofilopoulos became something of a hero in that region.

“After that,” says Theofilopoulos, “The old man was bringing me chickens and nuts and other gifts, and my fame exploded to all the surrounding villages.”

AFTER THE ARMY, A CAREER IN RESEARCH

Although he eventually gave up clinical practice to focus on basic science, Theofilopoulos’s experience as a clinician has driven his subsequent career, where he has endeavored for many years to address some of the basic concepts of autoimmune diseases. Throughout this distinguished career, he has never forgotten that moment, decades ago, in that small village.

“I have always had great concern for the patients,” he says. “This has been the guiding force in my life.”

As a researcher, he has sought answers that could alleviate suffering—a journey that began in 1970, soon after he was discharged from the Greek army.

Theofilopoulos came first to the University of Texas Southwestern Medical School in Dallas, on a Rotary Fellowship to study rheumatic diseases in the laboratory of Professor Morris Ziff. It was the first time...
Theofilopoulos had ever been outside of Greece.

In 1972, he felt ready to move on and had a desire to expand his knowledge in immunology, so he came to Scripps to work as a postdoctoral fellow under the guidance of world-renowned immunopathologist Frank Dixon, who was director of the institute and chair of the Department of Experimental Pathology.

Initially, Theofilopoulos worked on issues related to how the immune system dealt with a rare tropical disease, dengue hemorrhagic fever. In the process, he developed a variety of systems for identifying molecules known as immune complexes from blood samples, including one that became a standard test in clinical laboratories. Theofilopoulos was invited to stay and continue work at the institute.

Theofilopoulos settled down, both in La Jolla and at TSRI, and raised a family while he pursued basic research. His wife, Ellie, obtained a master's degree in psychology and is involved in cultural events and volunteer work. His three children, now grown, were all born and raised in La Jolla. Aliki, his daughter and the oldest, is an animator. Dimitri, the middle child, is just finishing law school, and his youngest son, Andreas, is a computer programmer and was recently married.

Soon after Theofilopoulos began to put down roots at TSRI, he turned his attention to a disease known as lupus that he and Dixon had been interested in for some time. For the next two decades, he contributed an extraordinary amount to our knowledge of this disease—work for which he has received a number of scientific awards and honors. These include, most recently, two honorary doctorates from the University of Athens, Theofilopoulos's alma mater, and the University of Patras, in the town where he grew up.

**ONCE THOUGHT TO BE FROM WOLF BITE**

Lupus is a chronic, inflammatory autoimmune disease caused by multiple genetic, environmental, and other factors, most of which are unknown. It is a complicated disease that affects women ten times more often than men, and it can appear a thousand different ways in a thousand different people. The Lupus Foundation of America estimates that approximately 1.4 million Americans have a form of lupus, a disease that ranges widely case by case, has a long list of symptoms, and affects a wide variety of tissues—especially the skin, joints, blood, and kidneys.

The diversity of symptoms means that lupus is often misdiagnosed. In fact, even the name "lupus," which means "wolf" in Latin, is a mischaracterization of sorts. The word lupus was first used in the Middle Ages to describe a chronic rash on the skin. The name may have been chosen because the rash on the skin resembled the effects of a bite from one of these wild animals. Or, some believe the name arises from the fact that the rash was common about the cheeks, giving lupus victims a werewolf-like appearance.

Lupus was rigorously described and defined as a medical condition in the early 1800s, but it was not until the latter half of that century that real progress was made in defining the full clinical spectrum of this disease. The first positive step was when doctors recognized that the disease could be systemic and could cause damage to the kidneys and other internal organs distinct from and sometimes in the absence of its defining rashes.

Today we know that the disease is not the bite of a Canis lupus, but the bite of a person's own immune system.

Lupus occurs when a person's own B cells are directed against "self" molecules. B cells are one of the immune system's front-line fighters and are responsible for producing antibodies that target infected cells or foreign pathogens in the bloodstream and help the body control and clear infections.
But in lupus, these antibodies target the body's own molecules instead. For instance, many people who have lupus produce an antibody that targets red blood cells, which are a vital oxygen-transporting component of blood. The antibodies coat the red blood cells, which are then taken up and destroyed by macrophages. This can lead to a deficit of red blood cells and anemia. Since a person's own immune system causes lupus, it is categorized as one of the "autoimmune" diseases.

In the early years of his research, Theofilopoulos worked with a mouse model discovered in New Zealand that develops symptoms resembling lupus. Theofilopoulos spent several years analyzing this model, describing lupus's basic characteristics. He wrote some of the first detailed descriptions of the molecular and cellular characteristics of the disease, including its relationship to T cells, B cells, organs like the thymus, and antibodies.

"Then," says Theofilopoulos, "when molecular cloning began, we started defining the structural characteristics of the autoantibodies and other immune system-related genes that were implicated in lupus."

He and his colleagues sequenced a monoclonal anti-DNA antibody that is a major factor in lupus, and he discovered that the genes that encode these pathogenic antibodies are not very different from those encoding regular antibodies against foreign antigens. He also cloned and characterized several T cell receptor genes and studied the mechanism by which self-reactive T cells are eliminated in mice and humans with systemic autoimmunity.

Theofilopoulos. "Knowing one of them may not be sufficient to change the disease process. Yet some genes may have a greater effect than others, and if we can identify them, we may be able to intervene." Evidence in mouse models, he adds, indicates that deleting chromosomal segments that carry such genes significantly reduces the severity of the disease.

In addition, Theofilopoulos is interested in identifying specific "effector" genes and molecules that act in concert with other genes and contribute to the pathogenesis of the disease. Once identified, he is looking for ways to block them.

“For lupus, there is considerable variation—possibly hundreds of phenotypes. This makes it difficult to find one answer and one panacea.”

For instance, Theofilopoulos has identified one possible target for therapy, an inhibitor of cyclin-dependent kinases that is overexpressed in T cells during lupus that may be responsible for one symptom of advanced lupus—a flood of helper T cells, a type of immune cell resistant to proliferation and apoptosis (programmed cell death). Targeting this inhibitor is likely to lead to the elimination of these helper T cells and a reduction in the severity of the disease.

Other types of genes that have been found to contribute to lupus are those encoding for Type 1 and Type 2 interferons, pro-inflammatory molecules referred to as IFN-α/β and IFN-γ, respectively. Based on these findings, Theofilopoulos has reported using cDNA that encodes the receptor for IFN-γ to block the activity of the IFN-γ and cure lupus in animal models.

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Forty-One Years and Counting

Professor Charles G. Cochrane calls himself “the Last of the Mohicans.” Not because he’s friends with Daniel Day Lewis or because he lives off the land with his adopted companion, Hawkeye, but because he is the last of a breed—the last active original member of the Department of Immunology at The Scripps Research Institute (TSRI).

“The department goes all the way back to the beginning of Scripps,” says Professor Richard Ulevitch, who is presently chair of the department and who started his career working in Cochrane’s laboratory in the early 1970s. Of course, Ulevitch adds, when it all started over 41 years ago, both the department and the institute had different names.

That year was 1961, and a group of young immunologists led by Frank Dixon—among them, Cochrane—had come from Pittsburgh to La Jolla to start the Department of Experimental Pathology at what was then called Scripps Clinic and Research Foundation (SCRF). SCRF had been formed a few years earlier from the Scripps Metabolic Clinic, established in 1924 with a gift from Ellen Browning Scripps.

“When we first came from Pittsburgh, the idea was to focus on immunology and do that so well that we would grow in strength,” says Cochrane. “We were down on Prospect Street in La Jolla next to the old Scripps Hospital.”

The five occupied the brand-new (and unfinished) Timken–Sturgis Research Laboratories Building, where they were shortly joined by two other departments—Microbiology and Biochemistry. For a while, all three would occupy the Timken–Sturgis building, sharing an amphitheater, a library, and other support facilities.

“In those early days,” recalls Professor Thomas Edgington, who came to the department in 1965, “you knew everybody.”

“We ate lunch in the lunchroom with the doctors in Scripps Clinic, and we would chat about things in the lab and clinic,” says Cochrane. Things soon grew beyond that small dining room.

DEEP ROOTS AND A LASTING LEGACY

“We laid down a basic foundation in the institute that promoted maximal freedom for the scientists to pursue their individual interests unfettered by committees and regulations,” says Cochrane. “Then we all went to work to put the institute on the map, through publications and presentations at national and international meetings. We were looking at a future that was undetermined—a horizon beyond our scope of imagination.”

Jonas Salk, also from the University of Pittsburgh, arrived in La Jolla shortly thereafter, and while The Salk Institute was being built, he worked in the Timken–Sturgis building, too, borrowing space from the SCRF experimental pathologists. And in the early 1960s, the newly designated University of California, San Diego School of Medicine campus had only a few faculty members (located temporarily at the oceanography institute) and no undergraduates.

“When we came from Pittsburgh, the idea was to focus on immunology and do that so well that we would grow in strength.”

“We would meet with all the members of Scripps, Salk, and the UC School of Medicine in one room to discuss research projects and journal articles of common interest,” says Cochrane. “We should have taken pictures, given all the growth that followed in the three institutions.”

TSRI, of course, has grown from the original handful of researchers to almost 3,000 employees overall, with a staff of nearly 300 full-time investigators, nearly 1,000 research associates and graduate students, and a total of over 1,500 technical and support staff.

The Department of Experimental Pathology underwent many changes as well. In the early 1970s, it split into three separate departments—cellular immunology, molecular immunology, and immunopathology. Of the three, one became today’s Department of Immunology.
In the early days, all the members of Scripps, The Salk Institute, and the University of California at San Diego School of Medicine would meet in one room of a Scripps Clinic and Research Foundation building to discuss research projects.

Later, the separate departments of Cell Biology and Molecular Biology were founded.

Today's Department of Immunology has 90 investigators, senior research associates, and guest scientists, along with hundreds of postdoctoral fellows and technicians, a handful of graduate students, and dozens of other support staff. The focus has broadened significantly in terms of the number of diseases that are studied and the approaches used — members of the department have always sought to put all new technologies to work as they are developed.

“It is gratifying to know that a therapeutic coming from Scripps will help millions of infants around the world who currently have no treatment.”

“If you go around and ask your colleagues, you can find experts in almost any topic,” says Professor Argyris Theoiloopoulos, who arrived at TSRI in the early 1970s.

“But the bottom line is still the same,” says Ulevitch, “understanding how the immune system functions in the setting of various diseases.”

A UNIFIED DEPARTMENT OF DIVERSE INTERESTS

Today, as 41-plus years ago, TSRI's immunology department seeks to understand the major human diseases in which the immune system plays a role. These encompass all the major categories of diseases, including pathogenic viral infections, like Ebola or HIV; autoimmune diseases, like diabetes or lupus; diseases that involve the innate immune system, like bacterial-induced septic shock; and diseases where immune-based therapy can possibly make a big difference, like cancer.

Many major diseases involve the immune system either directly or indirectly. Many, in fact, result directly from some action or inaction on the part of the immune system. Our bodies depend on our immune systems to fight off common pathogenic infections on a daily basis and maintain our health. Many diseases, like diabetes and lupus, are the direct result of our immune system's autoimmune actions.

And even in diseases, like certain cancers, that are not caused by our immune system, immune cells may hold the secrets to the cure. Significantly, cells of the immune system have the ability to kill tumor cells and some investigators at TSRI are attempting to harness this ability in cancer patients through immunotherapies — drugs that help the body's immune cells target and destroy tumors.

TSRI investigators in the department seek to extend the understanding of human immunology to the molecular level so that enough information can be assembled to enable drug development and the creation of new therapeutic approaches.

“We have good interactions with our colleagues and it's a stimulating environment,” says Professor Ralph Reisfeld, who arrived at TSRI in the mid-1960s. "I've received a lot of help from our colleagues. This is very important.”

A TREATMENT FOR DAMAGED LUNGS

Cochrane has developed a synthetic pulmonary surfactant that has been undergoing clinical trials since 1996 and is currently in the final Phase III clinical trials for two indications. It is called KL4-Surfactant, named for the synthetic peptide, KL4, that mimics Surfactant Protein B. One of these indications is for pre-term
infants who are born prior to the time their natural lung surfactant is made in the lungs.

The synthetic surfactant is administered directly into the lungs of the babies shortly after birth and within hours, their lung function approaches normalcy. Babies are being treated this way in Russia, Europe, and the Americas.

Current treatments for pre-term infants involve using surfactants derived from chopped cow or pig lungs, but these are expensive and they cannot be produced in quantities sufficient for pre-term infants world-wide, let alone for adults. "Synthetic surfactant is the only hope for infants in developing countries", says Cochrane. "It is gratifying to know that a therapeutic coming from Scripps will help millions of infants around the world who currently have no treatment."

There is a similar condition of collapsed lungs in adults known as acute lung injury, or respiratory distress syndrome, with any number of causes, such as trauma, smoke inhalation or sepsis.

"There is no approved therapy and about 30 to 40 percent of these mostly young people die from it each year in this country—about 50,000 in total," says Cochrane. "A Phase II trial is currently underway in which the injured lungs are being washed with dilute KL4-Surfactant to remove inflammatory exudate as they are being re-expanded."

Results so far are encouraging. But Cochrane believes the pulmonary inflammation requires, in addition, inhibitors of specific effectors of the injury. Current studies are directed toward this goal so that these inhibitors can be incorporated into the surfactant.

This research may also lead to an aerosol treatment for acute asthma, a possibility that Cochrane and his group are currently investigating. Clinical trials should begin this year.

A Lasting Legacy

One afternoon last summer found Professor Cochrane busy at work doing part of his daily routine—entering numbers into a calculator from a page in a well-worn notebook with numbered pages. Data from the week's experiments, he says, explaining that in recent years he has reduced his laboratory from over 50 members to just four, including long-time associates Sue Revak and Zenaida Oades.

"It's like I'm a postdoc again," he says.

And while Cochrane may also call himself "the last of the Mohicans," there is at least one major difference between him and the tragic figure of Chingachooky in James Fenimore Cooper's famous novel. Cochrane's tribe—in the broadest sense, his department, laboratory, and the laboratories of those whom he has mentored—has grown in the last 40-odd years. Grown and flourished.

Over the years, Cochrane, like all the investigators in the Department of Immunology, has kept one tradition alive—the training of postdoctoral fellows. Soon after it came into existence over 40 years ago, TSRI began training postdocs, and young M.D. and Ph.D. graduates have been burning the midnight oil (and burners) in the department's laboratory for several generations now. Through the years TSRI has trained thousands of postdoctoral researchers. In fact, a lot of important immunologists have trained at Scripps.

"This is a distinguished place to train in immunology," said Professor Donald Mosier in 2001 as he took a break from putting the paperwork together for the renewal proposal of the training grant he administrates.

This grant currently provides eight slots that give salary and other support for postdoctoral fellows. Established when the institute was founded in 1961, it is the oldest continuously funded postdoctoral training grant in the country and one of several training grants in the Immunology Department at TSRI.

"The institution should look upon the training of postdocs with great pride," says Cochrane, who knows whereof he speaks. He has been training them longer than any other active investigator at TSRI. "They come here and learn without limits and when they leave they have done extraordinarily well. That's always a pleasure to see."
A Sense of Wonder

He calls it basic research. Gary Bokoch, Ph.D., a professor and immunologist/cell biologist at The Scripps Research Institute (TSRI), uses the term "basic" to describe his work with Rho GTPases, a family of proteins that regulate various processes that all cells, including white blood cells, need to function.

Basic the research may be, obscure it is not. When activated by chemical signals like hormones or cytokines, GTPases prompt white blood cells to protect the human body against invading pathogens. Without GTPases, our defense against invading microorganisms wouldn’t function or would be in shambles.

GTPases, in fact, are indispensable to the regulation of almost any type of complex cell function. Acting as molecular switches in a variety of physiological systems, they control such key processes as changes in the cell's cytoskeleton, cell growth, and cell death.

Still, GTPases are not a subject you’re likely to stumble across at your next dinner party. Like anything in science, discussing these proteins can sound as simple as a spring day or as complex as an explanation of the precise function of neutrinos. Bokoch often thinks of this research topic both ways, usually with a touch of amazement in his voice.

"These proteins are basically switches. When they bind to [guanine nucleotide] GTP, they’re active and things happen. When they bind to GDP [another guanine nucleotide], they become inactive. When they’re active, they regulate very fundamental events like enzyme activity. But because there are so many of these GTPases acting on so many fundamental processes—more than 20 members of the Rho family have been identified so far—the operation and regulation of the system can quickly become very, very complex."

A lot like explaining neutrinos, come to think of it.

A NEW WORLD

Bokoch first worked with these unique and elegantly simple proteins when conducting postgraduate work with Alfred Gilman, a future Nobel Prize winner and one of the men responsible for their discovery. Gilman, along with fellow researcher Elliott Ross, was a co-discoverer of the G protein—another name for GTPases due to their binding of and regulation by nucleotides GTP and GDP—purifying the first one in 1980. For his work on the discovery of the G protein and its roles in hormonal signaling, Gilman shared the Nobel Prize in Medicine or Physiology with Martin Rodbell in 1994.

Bokoch had pursued his graduate work at Vanderbilt University in Tennessee studying leukocytes on what he calls the "functional level." This involved research into intracellular signaling, a field the university was famous for worldwide. Gilman had taken a novel approach to understanding signaling—duplicating the actions of these signal-transducing proteins in the laboratory instead of trying to observe them in vivo.

It was that scientific activism that brought Bokoch to the project.

"Leukocytes are amazing cells in that they’re almost like independent creatures."

Bokoch signed up with Gilman at the University of Texas in Dallas as a postdoctoral fellow in 1981, only a year after Gilman's discovery of the first G protein, and worked with him until 1985. By this time, Bokoch was married to Janet Nicola from his hometown of Erie, Pennsylvania (the couple now has two children). During his postdoctoral period, Bokoch identified and purified the second known G protein, whose discovery suggested that there might be larger numbers of these signaling entities than anyone suspected.

"It opened up a new world for me, discovering these proteins, and trying to understand how they worked in a laboratory setting," Bokoch says. "I wanted to do the same thing with leukocytes [another name for white blood cells], so we could finally understand how they..."
worked at a biochemical and molecular level. Leukocytes are amazing cells in that they're almost like independent creatures. They move around, they engulf other cells—they eat them, really—and they respond rapidly to things that you do to their environment. Yet it was always clear to me that these activities were ultimately the result of highly organized, yet astonishingly adaptive, signaling processes."

On the other hand, he says, white blood cells are somewhat delicate. Their lifetime is measured in hours, so that the body must produce millions of them each day to provide the necessary level of immune protection. What they lack in lifespan, they make up in ingenuity. Smaller than most cells (one of the reasons that they're so difficult to work with in the laboratory), they are flexible enough to squeeze through the spaces between other cells. Like tiny mutant warriors, they distort themselves, slip through cell layers, and pop up on other side, ready to attack the next pathogen they meet.

About this time, Bokoch came across a series of papers from a group headed by Professor Charles Cochrane, M.D., at TSRI. It turned out that the TSRI researchers were also interested in the biochemical qualities of leukocytes, the very topic that Bokoch was hungry to study.

"They were advertising for somebody to help with their work," he recalls. "I was fascinated by what they were doing, and that was the thing that brought me here." Bokoch had intended to stay on the East Coast, but the chance to expand his studies of GTPases and leukocytes at TSRI proved to be stronger than the pull of geography.

**COMPLEX AND FASCINATING**

When Bokoch first went to work at TSRI, his main interest was in the big GTPases that receive signals on the cell surface. But Bokoch, along with other researchers, began to recognize that there were other, smaller types of GTPases, although little was known about their biochemistry or their function. His work led to the discovery in 1991 of the first known function for a small GTPase—regulation of the formation of toxic oxygen metabolites (oxidants) by white blood cells.

Since then, his laboratory has focused on understanding the complexities of cellular regulation by the Rho group of small GTPases, as well as continuing studies on leukocytes, especially their ability to chemotax and to generate oxidants to kill microorganisms. Curiously enough, one form of these oxidants is hydrogen peroxide, and another is related to household bleach.

"It's a complex and fascinating defense system," he says. "A number of proteins have to assemble to make the functional oxidase, but the most critical component is a small GTP-binding protein called Rac. Since we realized that oxidants contribute to inflammatory responses, it became apparent that these small GTPases might someday have an impact on the treatment of certain inflammatory diseases including arthritis, toxic shock, atherosclerosis—even heart attack and myocardial infarction/reperfusion injury.

"The thing I like most about Scripps is its tremendous academic environment... Collaboration is encouraged, and there's a real sense of camaraderie..."

"That would be a significant benefit of our research, since [a large number] of patients who see a doctor are being treated for problems related to inflammation. Also, the recent realization that oxidants function as signaling molecules in non-leukocytic cells suggests our studies may have implications for other diseases as well."

It soon became apparent to Bokoch and his colleagues that these small GTPases were involved in other cell processes, including the regulation of the cell cytoskeleton, which determines the ability of cells to move, and to change shape and function. In the case of cytoskeletal regulation by Rho GTPases, one of the primary components is a kinase with the name of PAK. Kinases are enzymes that transfer certain chemical groups between molecules.

The PAK enzyme, in conjunction with other proteins, allows the cellular cytoskeleton to modify its
shape—to shape shift, in other words, like a fictional superhero. The implication of this for such critical processes as inflammation, angiogenesis, nervous system development, and cancer metastasis is another area where Bokoch’s GTPase research connects with the therapeutically significant.

His laboratory’s intensive studies of how Rho GTPases regulate the intricate machinery of the cell cytoskeleton, Bokoch explains, also provide insights into the innate immune response against bacterial and viral infection.

“I’m constantly amazed by these incredibly intricate biological systems. How they evolved is just stunning to me.”

If Bokoch is changing the way medicine views the immune system, he’s also part of a revolution in technology that allows him and his colleagues to understand the mechanisms of cell signaling by watching them in real time.

“It became clear very quickly [a few years ago] that an important aspect of cell signaling is where and when it takes place within the cells,” he says. “[Until recently,] we had no good ways to observe that. At Scripps, several people were developing fluorescent indicators to look at signaling in real time, and we’ve tried to incorporate that into our work. In a paper published this year with our TSRI colleague Klaus Hahn, [Ph.D.], we look at the actions of Rac in human leukocytes with a fluorescent probe. We can see Rac turned on at very specific places within the cell and can observe how the dynamics change constantly during a complex process such as chemotaxis.”

FROM SPIDERMAN TO SCIENCE

A fan of X-Man comics where one of the more attractive mutants is also a shape shifter, Bokoch also has a ragged edition of the first Spiderman comic. Like Spiderman—a.k.a. Peter Parker—Gary Bokoch’s life has involved some transformations.

Born on the hardworking shores of Lake Erie, Bokoch’s father was a policeman, his grandfather a miner who spent his life in the coal mines of western Pennsylvania. Bokoch started out catching snakes and frogs in the nearby woods and began his life-long habit of reading science fiction, a habit that got him thinking about actual science.

He built science projects in his basement, Van de Graaff generators, and an electrical carbon arc furnace—a contraption that produces an electrical arc between two carbon rods that he used to melt nails and, on more than one occasion, short out the entire electrical system of his parents’ house. Like others of his generation, he was fascinated by the ongoing space program and kept a file of newspaper clippings.

“As I got deeper into the idea that I might want to be involved in science, I also had a couple of high school biology teachers who made me excited about biology,” he remembers. “One even had a Ph.D., unusual for a high school teacher.”

From there it was a short leap to college chemistry and biology, and trying to figure out a way to combine the two, which he accomplished with a Ph.D. in pharmacology. In the end, he arrived at TSRI, an institution that would seem custom made for anyone interested in seeing how that combination of sciences might actually work.

“The thing that I like most about Scripps,” he says, “is its tremendous academic environment. There’s interesting work going on, and since everybody is excited about their work, we all look for ways to interact with each other. Collaboration is encouraged, and there’s a real sense of camaraderie, especially within the department. Because so many biological systems depend on GTPases, we connect with a lot of people.”

As a scientist, Bokoch has managed to hang onto his own sense of surprise and wonder about what he does, in and out of the lab.

“I’m constantly amazed by these incredibly intricate biological systems. How they evolved is just stunning to me. If you try to rationalize how things got that way, you can only come away with a sense of awe, not to mention a certain feeling of humility.”
A research team led by Stephen P. Mayfield, Ph.D., showed that antibodies can be expressed in algae, suggesting a fast, cost-effective way to make human therapeutic proteins. (Image of Chlamydomonas—courtesy of the U.S. Environmental Protection Agency.)

A group of scientists at TSRI have used algae to express an antibody that targets herpes virus. This antibody could potentially be an ingredient in an anti-herpes topical cream or other anti-herpes treatments, but more importantly the algae expression technology that the TSRI team used could facilitate production of any number of human antibodies and other proteins on a massive scale.

This is a fast, new, effective way to make human therapeutic proteins," says TSRI Associate Professor Stephen P. Mayfield, Ph.D., who conducted the research with Research Associate Scott E. Franklin, Ph.D., and TSRI President Richard A. Lerner, M.D., who is Lita Annenberg Hazen Professor of Immunochemistry and Cecil H. and Ida M. Green Chair in Chemistry. Their study appeared in the January 21, 2003 issue of the journal Proceedings of the National Academy of Sciences.

Significantly, the researchers were able to produce the antibody at a much lower cost than has been achieved in the past. In fact, they say they can now make antibodies, soluble receptors, and other proteins so much more cheaply that an entire new class of therapeutics may become accessible.

"You can’t make [a drug] if the time and expense is such that you have to sell that drug for hundreds of thousands of dollars," says Mayfield. "This has to be the way we make drugs in the future."

FROM POND SCUM TO PHARMACY SHELF

Also called immunoglobulins, antibodies are proteins produced by immune cells that are designed to recognize a wide range of foreign pathogens. After a bacterium, virus, or other pathogen enters the bloodstream, antibodies target antigens—proteins, carbohydrate molecules, and other pieces of the pathogen—specific to that foreign invader. These antibodies then alert the immune system to the presence of the invaders and attract lethal "effector" immune cells to the site of infection.

"This is a fast, new, effective way to make human therapeutic proteins."

Antibodies can also be useful as therapeutics for a number of human diseases ranging from rheumatoid arthritis to leukemia. Likewise, there are many other human proteins that could potentially be used as drugs.

In fact, there may be over 200 proteins that could
potentially be new anti-cancer, anti-inflammatory, anti-arthritis compounds, says Mayfield. As an example, an anti-IgE antibody, termed Omalizumab, has already shown great efficacy in human clinical trials for the treatment of allergic rhinitis and asthma. Unfortunately, the costs of producing the antibody, coupled with the relatively small amounts that can be produced with current technologies, have severely limited its availability.

In cases where scientists want to make an abundance of proteins, they often turn to the simplest expression system—bacteria. However, this does not work for large, complicated proteins like antibodies because bacteria do not have the machinery to assemble them into the correct structure. So large proteins are usually produced through an expensive and complicated process involving the fermentation of mammalian cells.

Algae may offer a cheaper and easier way to produce the proteins. Since algae grow naturally and use carbon dioxide from the air as a carbon source and sunlight as an energy source, whole ponds—tens of thousands of liters—of the algae can be grown once they are modified to produce the protein of interest.

“The scale on which you can grow these algae is enormous,” notes Franklin.

Modifying the algae to produce proteins entails inserting a gene into the genome of the chloroplast, the organelles within the alga cell that converts sunlight and carbon dioxide into plant matter. The algae then express and assemble the antibodies within the chloroplasts, which can later be purified, intact.

Now that the researchers have established the fundamental technology, they are looking at applying it to any number of proteins and receptors.

“We think we can now put in pretty much any gene that we want and have it express,” says Mayfield.

Theofilopoulos continues from page 5

Similar beneficial effects have been observed in lupus mice that lack the IFN-α/β receptor.

Use of recombinant receptors for IFN-α/β and IFN-γ should provide a better means to reduce the severity of the disease.

NEW TREATMENTS NEEDED

However sophisticated and promising all this might sound, it does not mean that a cure is anywhere near. In fact, one single cure may not be possible because of the heterogeneous nature of the disease.

"For lupus, there is considerable variation—possibly hundreds of phenotypes," says Theofilopoulos. "This makes it difficult to find one answer and one panacea."

Instead of viewing maladies like lupus as one disease, one might be able to identify a sub-group of patients with a particular genetic defect that contributes to their particular form of the disease.

"Then we would be much better off designing treat-
ments," says Theofilopoulos. Knowledge of the subtypes of the disease might enable doctors to direct specific treatments to specific individuals.

No significant new treatments for lupus have been found since the 1950s, when doctors began using corticosteroids to treat it. Prior to that, at the turn of the 20th century, doctors discovered the effectiveness of anti-inflammatory and anti-malaria drugs. Although a few other treatments have been developed in the last 50 years, corticosteroids, anti-inflammatory, and anti-malarials are still the main therapies for the disease today, despite a high incidence of side effects.

The hope is that instead of giving these non-specific drugs, one could design drugs to treat a specific form of lupus. Theofilopoulos believes that this is the future, and his will to realize it is what drives him forward.

"I have a strong desire to make a breakthrough to help somebody—even a single individual," he says. "Then I will feel like I have accomplished something."

AUTOIMMUNITY AND CANCER

His work in autoimmunity has also recently led Theofilopoulos and his colleagues Wolfgang Dummer, M.D., Andreas Niethammer, M.D., and Ralph Reisfeld, Ph.D., to suggest a new, potentially more effective way to battle cancer—hit the immune system with cancer vaccines or cancer cells when it’s down and it will bounce back swinging harder than ever against those cancer cells.

The technique involves administering an injection of fresh immune cells to replace the ones that die immediately after chemotherapy or irradiation. An injection of cancer cells at the same time serves as a form of "immunotherapy," which induces a person’s immune system to attack existing colonies of those cancer cells.

In the technique, the fresh immune cells immediately begin to multiply through a mechanism involving recognition of self-molecules and, because they see the cancer cells, they are rapidly and preferentially activated to kill them.

"In this case, autoimmunity can be beneficial to the patient, and the treatment has utility on the basis of its simplicity," says Theofilopoulos.
Bricks and Mortar for Immunology

Most of the 60 lead immunologists at The Scripps Research Institute (TSRI) conduct their research in the institute's Immunology Building, on the southern end of campus. Now, due to the terms of the original lease, TSRI has the one-time opportunity to purchase the building—for below current market value.

Most of our fundraising efforts are currently focused on raising money to buy the Immunology Building,” says John Diekman, Ph.D., who chairs the TSRI Board of Trustees Development Committee. “Land is no longer available on the Torrey Pines Mesa, which makes acquiring the building an important priority.”

The goal is to raise a minimum of $16 million in private funds, which would enable the institute to commit to the purchase.

While in general it is a difficult time for raising money—the stock market’s fluctuations have affected discretionary funds and individual wealth—members of the development team remain optimistic. They are making their case for the long-term medical potential of work that goes on within the Immunology Building’s walls, emphasizing that research in this field could lead to treatments for some of the world’s major killers—including cancer, HIV, diabetes, and septic shock.

“Last year, 64 million people suffered from cancer, HIV, diabetes, and septic shock,” notes Cary W. Colwell, president of the Scripps Foundation for Medicine and Science. “Breakthroughs in immunology could affect so many lives.”

The Immunology Building has housed some of the world’s leading researchers over the last 23 years. Now, the institute has the opportunity to ensure that the building will continue to provide a home to the innovative work aimed at unlocking the secrets of the human immune system and its potential to alleviate suffering.

Diekman acknowledges that the success of the fundraising effort all comes down to people. “We need to find and connect with those forward-looking individuals with the vision, passion, and means to make the purchase of the Immunology Building possible,” he says.